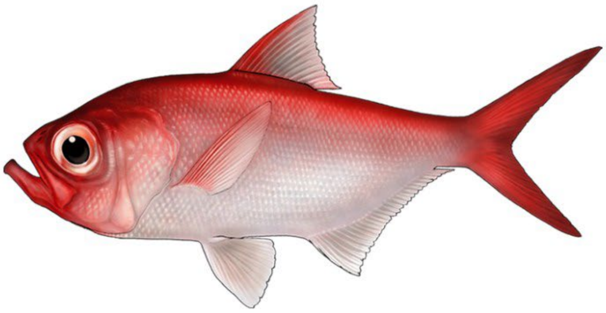
Age, growth, and lifespan investigations of Splendid Alfonsino (*Beryx splendens*) of the Indian Ocean using bomb radiocarbon dating



Project Code: SER2022-BYS2:

CONTRACT FOR THE PROVISION OF SCIENTIFIC SERVICES

BOMB RADIOCARBON AGEING OF ALFONSINO (*BERYX SPLENDENS*)

by

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Scientific Inquiries and Innovations

for

SIOFA

Southern Indian Ocean Fisheries Agreement

Le Port, La Réunion

Funded by the European Union

October 2023 (Version 1.4)

1. Executive summary

Otoliths of splendid alfonsino (*Beryx splendens*) are primarily aged using whole otoliths by viewing the distal surface with reflected or transmitted light. Growth zone structure in this view is well defined and maximum age estimates cover 10–25 years from studies across the world. Its congener, the red bream (*B. decadactylus*), is also aged in this manner in numerous studies with similar longevity estimates, but the use of thin-sectioned otoliths revealed fine-scale growth zone structure that led to estimates of 50–70 years. These estimates were supported with bomb radiocarbon (^{14}C) dating to at least 49 years — a revelation in that the lifespan of red bream increased by a factor of 3–5 times. Splendid alfonsino were recently investigated using thin-sectioned otoliths and an age reading protocol that agreed with whole otolith ages was derived. To investigate the validity of splendid alfonsino age estimates using whole and thin-sectioned otoliths, twelve otoliths were selected based on whole otolith age estimates (4–25 years) and otolith mass covering smallest to the most massive otoliths available (0.160–0.714 g) to assist with assessing maximum age. Measurements of ^{14}C from the earliest otolith growth — extracted as core material from within the first year of growth with a micromilling machine — revealed a pattern through time that can be attributed to the full bomb-produced ^{14}C curve. Hence, the most massive otoliths were clearly pre-bomb (hatch years prior to ~1958) leading to calculated ages of at least 50 years for fish originally aged 19–25 years from whole otolith age reading. The most massive otolith used in this study was further investigated in the transverse section finer growth zones and was estimated to be 61 years old, an age that can account for the bomb ^{14}C alignments in time. A detailed investigation of splendid alfonsino otoliths for fine-scale growth zone structure in transversely sectioned otoliths is recommended to elucidate an age reading protocol through ontogeny that would provide accurate growth characteristics for this long-lived species.

2. Introduction

This project was set forth by the SIOFA Scientific Committee as a pilot study to test the validity of otolith age reading estimates for splendid alfonsino (*Beryx splendens*) using bomb radiocarbon dating under Project Code SER2022-BYS2. Tasks of this project were to perform a literature review to summarize current knowledge, and acquire the appropriate specimens that would most likely lead to success in an application of this age validation method. The findings of this work and requisite recommendations for alfonsino age estimates from bomb radiocarbon dating of otoliths are submitted here as a final report.

Recent reviews of deep-sea fishes indicate that age estimates for splendid alfonsino are up to 10–25 years depending on the location and specimen availability from numerous deep-water locations across tropical and subtropical oceans of the world (Shotton 2016, McMillan et al. 2022). This maximum age range is similar to what was described initially for its congener the red bream (*B. decadactylus*) from numerous studies world-wide, as well, with ages of up to 18 years (Shotton 2016). The common thread between these species is the method of age estimation and the limited forms of age validation applied to date — every study that could be located used whole otolith age reading and the estimates of age were in some cases validated in a limited manner using methods that are only effective for the earliest and most rapid growth (i.e., daily increment counting, marginal growth observations, and length frequency analysis). These methods confirm to a limited extent that the early growth of these species is rapid with a massive nuclear region of the otolith that can be attributed to the first 1–3 years of growth for splendid alfonsino, specifically (Massey and Horn 1990, Isidro 1996, Lehodey and Grandperrin 1996, Taniuchi et al 2004). Despite more than 45 years of research on this species (Ikenouye 1969), no approach that can accurately address maximum age has been applied — only one age validation study on red bream from the western North Atlantic was successful with addressing issues of longevity with bomb ^{14}C dating (Freiss and Sedberry 2011).

The age validation study of red bream using bomb ^{14}C dating is one of the first to investigate transverse sectioning of otoliths over the use of whole otoliths for age reading of alfonsino species. Otolith sections revealed fairly well-defined growth zone structure that exhibits a fine scale that is not visible in whole otoliths past a certain otolith size or age, at which point the otolith begins to thicken in the sagittal plane on the proximal side (Freiss and Sedberry 2011). The findings were not surprising in the context of other deepwater fishes that were initially aged using whole otoliths and were followed by investigations of the transverse plane (both break-and-burn and thin sectioning) that revealed a strong departure toward much greater ages. The seminal publication on Pacific ocean perch (*Sebastes alutus*) of the northeastern

Pacific Ocean demonstrated that whole otoliths could be aged to mid-20s in years but that age reading in the transverse plane of the same otoliths led to significantly greater ages up to 72 years, an increase to longevity of more than 50 years (Beamish et al. 1979). The section-based ages for this species were later validated with bomb ^{14}C dating to more than 40 years (Kastelle et al. 2008). Similarly, otoliths of red bream were noted to thicken in a similar manner and growth zone counting to 69 years was tested for validity with bomb ^{14}C dating — ages up to 49 years and the age reading protocol were well-supported.

One of the recommendations from a recent SIOFA age estimation project for splendid alfonsino was that direct validation should be explored through use of bomb-produced radiocarbon (Krusic-Golub and Robertson 2020). This method can provide the weight of evidence to support the current longevity estimates based on whole otolith age reading or suggest an alternate scenario where maximum ages could be much greater than previously reported. While some initial work was completed to compare age estimates between whole and sectioned otoliths by the Central Ageing Facility (CAF; Anonymous 2008), the fish available for the recent SIOFA age estimation study had a greater proportion of large specimens (Krusic-Golub and Robertson 2020) — 32% were >40 cm FL compared to 8% for the earlier method comparison. The most significant factor was that the largest otolith for the SIOFA specimens was 78% more massive than the largest otolith used in the CAF study (0.7319 g *cf.* 0.4110 g). Hence, it is reasonable to expect that the maximum estimated age would be greater than previously reported because otolith mass is often useful as a rough proxy for age.

Bomb ^{14}C dating relies on a regional time-specific marker of the marine environment that was created by atmospheric testing of thermonuclear devices in the 1950s and 1960s. This signal manifests itself in carbonates of skeletal and non-skeletal biogenic structures of marine organisms as a departure from naturally occurring ^{14}C levels in the late 1950s to a peak in tropical surface waters that is attenuated and phase lagged by ~10 years relative to the atmospheric signature. Use of this environmental ^{14}C signal as a temporal reference in marine fishes covers 30 years of research by establishing valid bomb-produced ^{14}C timelines with either known-age otoliths or dated hermatypic corals and then using the reference chronology to test estimates of age by hatch date comparisons. Because oceanography is a factor in the distribution of bomb-produced ^{14}C across the world ocean, variations in the timing and strength of the signal must be accounted for in the experimental design by considering the early life history location of the juvenile fish for the species under consideration. For many deep-water fishes, the early life history is in the mixed layer of the sea surface and hence an application of this method is often effective because the timely surface bomb ^{14}C signal is taken to adult depths. Hence, the goal of this preliminary study was to use a series of otoliths from the deep-dwelling splendid alfonsino of the southern Indian Ocean to test the accuracy of whole otolith age reading using bomb ^{14}C dating.

3. Methods

Otoliths from collections made by the Cook Islands and Australia under the SIOFA program were selected for the study based on a steady increase in otolith mass from the youngest to oldest fish. Because the approach was to use the post-peak bomb ^{14}C decline as a reference chronology, given a maximum age of approximately 25 years, a single collection year was the focus to create a relationship of increasing ^{14}C levels in concert with increasing age as the calculated hatch years dated back toward the bomb-produced ^{14}C peak in the 1970s and 1980s. The approach is similar to successful studies of tuna age and growth where the alignment of measured ^{14}C values through time agrees with the rate of environmental ^{14}C decrease (Ishihara et al. 2017, Andrews et al. 2020).

The earliest otolith growth was extracted with a micromilling machine (Figure 1). The sampling location was centred on the nucleus by examining whole and sectioned otoliths of progressively larger fish (e.g., Krusic-Golub and Robertson 2020). A view of the transverse plane revealed some deposition on the distal otolith surface that needed to be removed to avoid contamination from the inclusion of more recently formed material because the mill extraction proceeded from this direction. This was accomplished by hand grinding the surface of the otolith, to lesser or greater extent depending on the thickness and typically otolith mass, until the radial structure of the first few years of growth was visible (e.g., Andrews et al. 2012). A Brasseler carbide 0.5 mm bur was used on a New Wave micromilling machine to tap out the centre of the otolith to a depth of 0.3 mm. The extraction pattern on the micromill was determined as 3 mm long by 2 mm wide to match visible differences in radial growth dimensions — the extraction was estimated as much less than the first year of growth based on previous daily increment studies and personally observed residual increments from some cored otoliths. The mass extracted was ~1–2 mg $CaCO_3$.

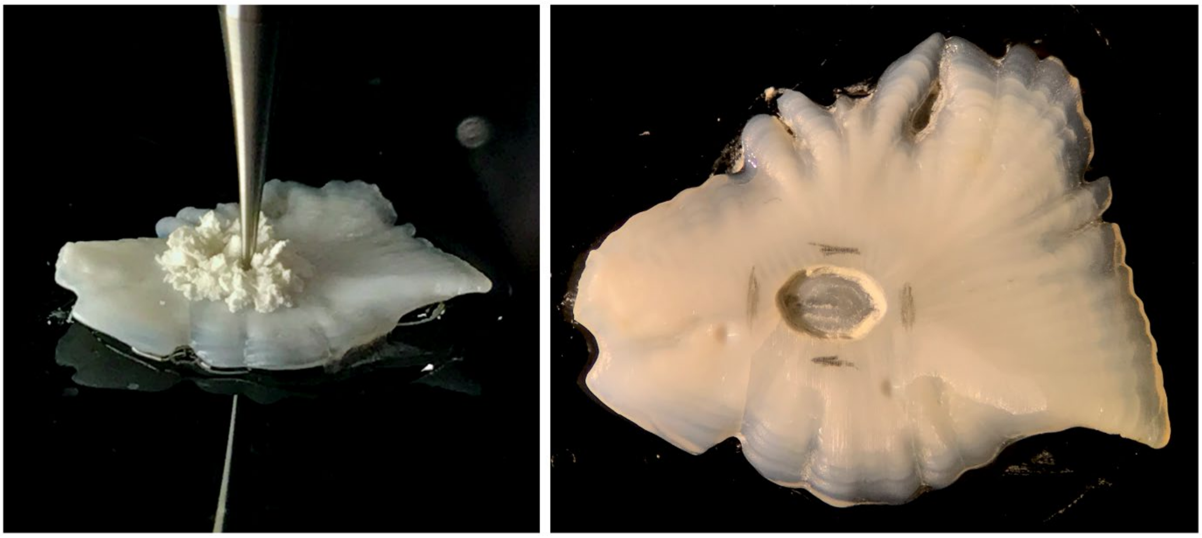

Figure 1. An extraction of a splendid alfonsino otolith with a Brasseler 0.5 mm carbide bur on a New Wave micromilling machine (left) and the end result as a cored otolith (right). The distal surface was initially prepared by hand grinding to just shy of the nuclear region to avoid the inclusion of more recently deposited carbonate. The colloidal powder extracted to the surface of the otolith (right) was collected and analysed for ^{14}C in the Laboratory of Ion Beam Physics at ETH Zürich, Switzerland.

Gas-AMS

The calcium carbonate samples were analysed by gas-accelerator mass spectrometry (AMS) for carbon isotopes using the Mini Carbon Dating System (MICADAS; Synal et al., 2007) in the Laboratory of Ion Beam Physics at ETH Zürich, Switzerland (Wacker et al. 2013), using a time-efficient approach for small samples that excludes the graphitisation step. The samples were placed in septum-sealed vials, after which ambient CO_2 was replaced with Helium. Sample CO_2 was subsequently generated with 80% phosphoric acid. In contrast to conventional graphite AMS analysis where liberated CO_2 is reduced to graphite and measurements are performed on solid targets, the CO_2 gas is concentrated by means of a zeolite trap and transferred with a helium gas carrier into a syringe on the gas interface system. This approach is cheaper, faster, and allows analysis of smaller sample masses (~10–50 µg C). Fossil and modern reference materials (IAEA-C1; Rozanski 1991) and an in-house coral standard (CSTD, nominal $F^{14}C$ value 0.9447 ± 0.0002, G. Dos Santos, pers. comm.) were analysed in concert with the samples. Data evaluation was performed with the "*Beautiful AMS Tool of Switzerland*" software (BATS), an analysis routine that functions as a reliable data reduction tool (Wacker et al., 2010). All radiocarbon results were reported as fraction modern ($F^{14}C$), relative to the activity ratio of the modern reference standard material Oxalic Acid II as described in Stuiver (1983), which corresponds to the fractionation-corrected sample activity (Reimer et al., 2004), and as decay-corrected $\Delta^{14}C$ (Stuiver and Polach 1977).

Reference chronology

A series of bomb ^{14}C chronologies from coral and otoliths were assembled for the Indian Ocean to provide a temporal reference for measured ^{14}C values from otoliths of splendid alfonsino (Figure 2). These records cover a wide range of geographical locations, but few are complete for the post-peak decline. Only two from the northern Indian Ocean, a region affected by upwelling in the west and terrigenous influxes in the east, provide highly variable references into the 2010s (Raj et al. 2021). All coral records exhibit a nearly synchronous bomb-produced ^{14}C rise in the late 1950s. Because these chronologies may not represent the lower thermocline waters that some early growth phases of splendid alfonsino are likely to inhabit, two deep water records that can be used as a proxy for this habitat were considered. The red steenbras (*Petrus rupestris*) chronology of South Africa is from juvenile and well-defined otolith sections and they reflect a bomb ^{14}C signal that is strongly affected by upwelling of deep ^{14}C-depleted waters on the Agulhas Banks (Andrews et al. 2018). The closest midwater chronology is a deep-water teleost of the southern Pacific Ocean from ocean perch (*Helicolenus barathri*) as a proxy for depth-depleted ^{14}C (Grammer et al. 2015). Each may reflect the levels encountered by the midwater juvenile stage of splendid alfonsino and the latter may be the most applicable.

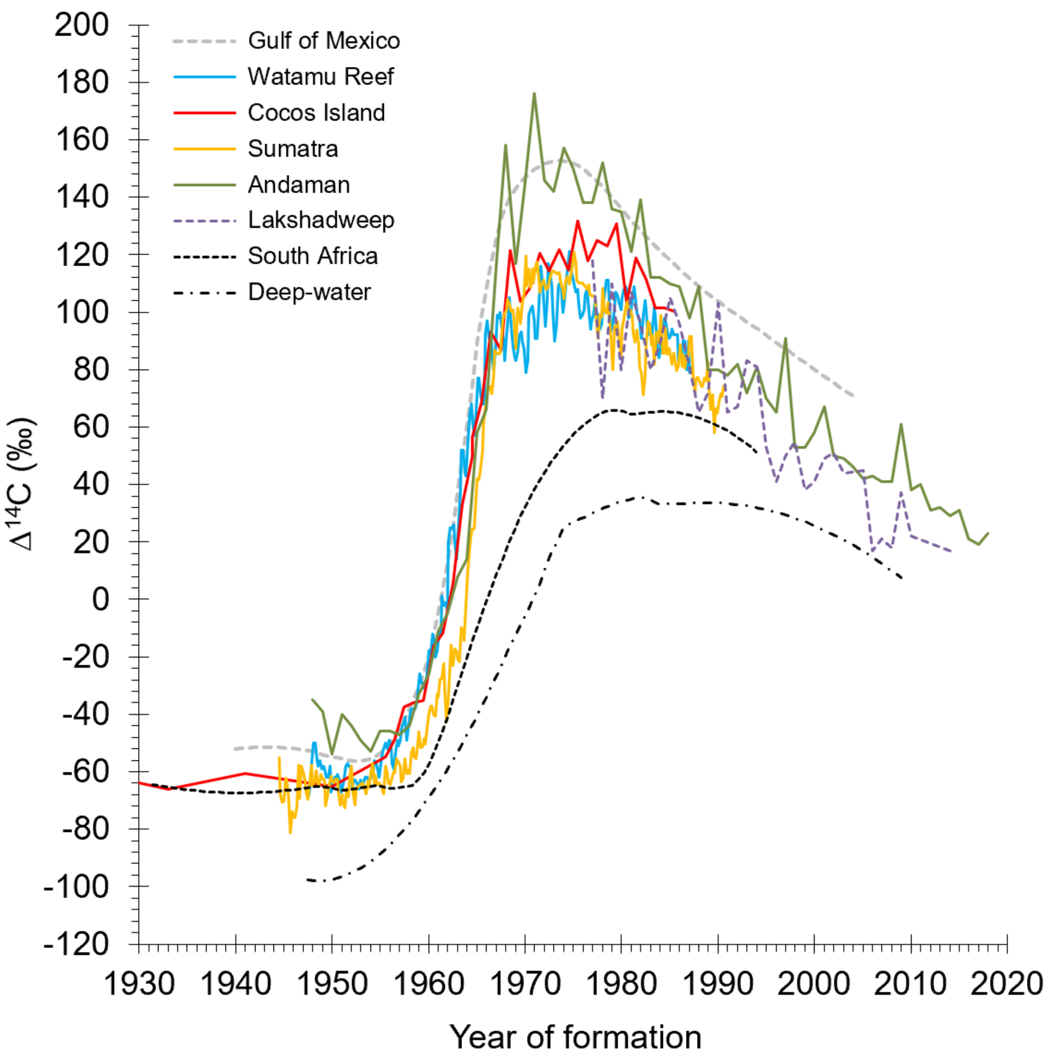

Figure 2. Plot of all known coral and otolith ^{14}C data from the tropical and subtropical Indian Ocean that can be used as temporal references for bomb-produced ^{14}C, with a North Atlantic record for a distant comparison. Coral core ^{14}C data cover much of the Indian Ocean (Watamu Reef, Kenya (Grumet et al. 2002), Northern Andaman and Lakshadweep (Raj et al. 2021), Sumatra (Grumet et al. (2004), Langkai Island (Fallon and Guilderson 2008), Lombok Straight (Guilderson et al. 2009), and Cocos (Keeling) Islands (Hua et al. 2004, 2005)). Included as otolith records are red steenbras (*Petrus rupestris*) from South Africa (Andrews et al. 2018) and ocean perch (*Helicolenus barathri*) from the southern Pacific Ocean (Grammer et al. 2015).

Date alignments

Alignment of the measured ^{14}C values from the aged and weighed otoliths were initially placed at the calculated hatch date from age reading of each whole otolith. These values were then assessed for alignment with the reference chronologies and adjustments were made based on what is expected for the bomb ^{14}C signal under various environmental conditions. These alignments were not made based on anything more than an assumption of fits to the chronological references with consideration for the shallower depths that the youngest phase of splendid alfonsino is likely to have occupied and the effects of deep water.

4. Results and Discussion

The calculated hatch years for measured ^{14}C values from aged whole splendid alfonsino otoliths did not align with the bomb ^{14}C reference chronologies in the manner expected and cannot be explained by environmental conditions (Figure 3). If the estimated ages of 4 to 25 years from whole otolith age reading were accurate, the measured ^{14}C levels would have progressed toward greater ^{14}C values as age increased, and the hatch years approached, the peak bomb ^{14}C period. Specifically, the calculated hatch year of 1982 for the 25-year-old fish would have approached or reached peak levels, depending on the appropriate reference chronology, and the younger fish would have followed the post-peak decline up to the most recent hatch year — no fish in the time series would be expected to reach pre-bomb values. However, the results did not follow this expectation and as a result, each otolith specimen was classified as forming during a particular period associated with the bomb ^{14}C timeline (pre-bomb, rise, peak, and post-peak decline) based on the measured ^{14}C value and otolith mass as a rough proxy for age (Table 1). The most telling ^{14}C measurements were from three specimens that were clearly related to the pre-bomb period with years of formation that would be prior to the late 1950s and early 1960s based on the bomb ^{14}C chronologies (Figure 3). These fish were three of the four most massive otoliths (up to 0.714 g) with one of the four having an elevated ^{14}C level and expected to be associated with the bomb ^{14}C rise period. The second most important observation was a specimen with an intermediate otolith mass (0.380 g) that reached an elevated peak ^{14}C level, effectively creating a division between more massive and less massive otoliths as hatch years associated with either the rise or post-peak decline periods. Two specimens were more massive than the peak-related fish; hence, they were expected to be older resulting in estimated hatch years during the ^{14}C rise period. Similarly, the third most massive otolith was elevated from pre-bomb and expected to be on the ^{14}C rise. On the lower mass side of the peak period, the measured ^{14}C values were expected to be in the post-peak decline period. These measurements decreased in concert with otolith mass, as expected with decreasing age and estimated hatch years during this period.

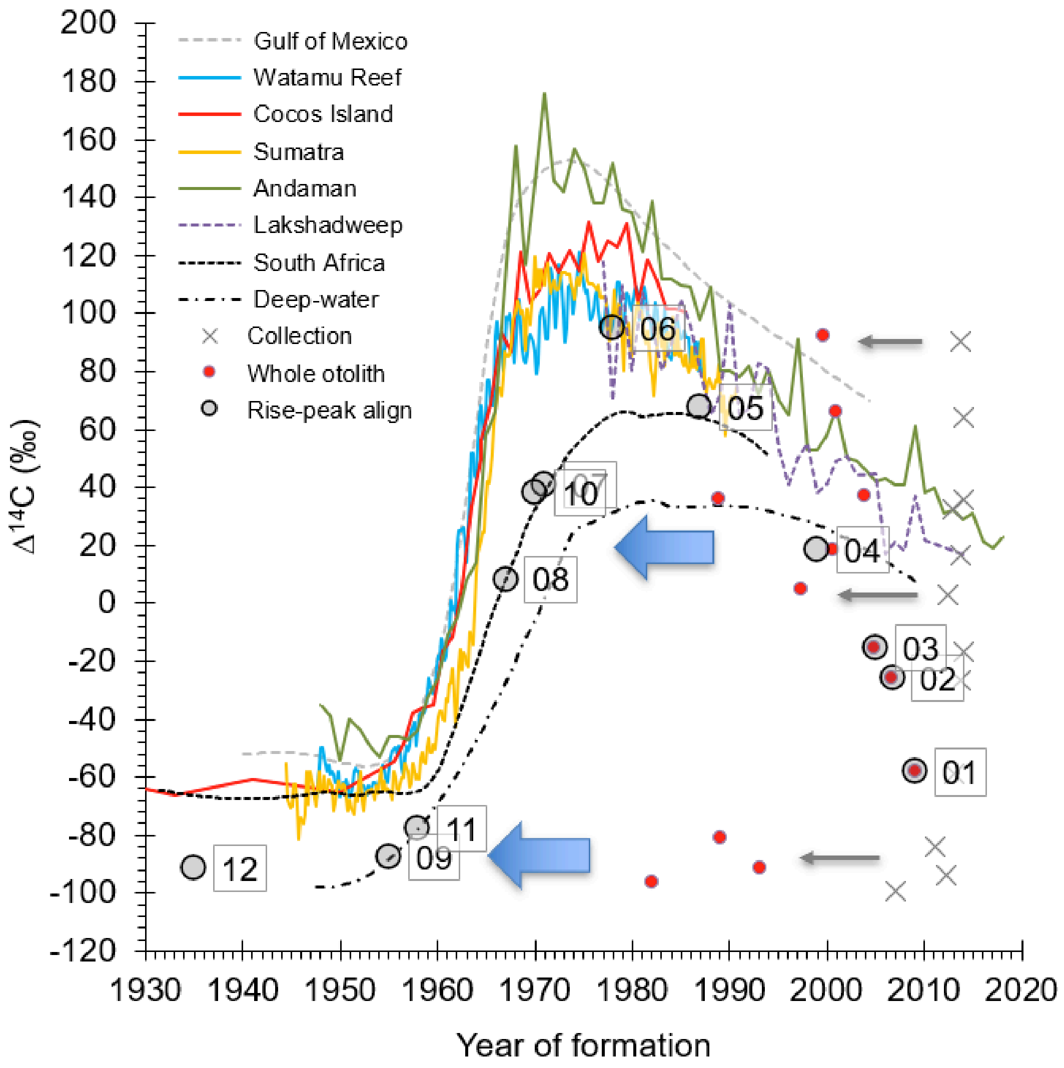

Figure 3. Reassessment and realignment for an older-age scenario for splendid alfonsino that would be supported by measured ^{14}C levels in the series of otoliths of increasing mass (grey circles with sample numbers). The initial age estimates from whole otoliths create a pattern that cannot be explained by any known bomb ^{14}C reference chronology (red circles) — the misalignment with the various chronologies is an indication that whole otolith ages were not accurate. Keystone alignments are pre-bomb levels that must be shifted back in time to before the rise period and the peak period measurement shift to within the reference chronologies. Age increases were roughly based on otolith mass and consequently leads to older ages for smaller otoliths. Minimum age for some fish was at least 50 years based on the deep-water reference chronology — the lifespan of splendid alfonsino is consequently at least 2 times greater than can be accounted for with whole otolith age reading.

These rough temporal approximations (pre-bomb, rise, peak, and post-peak decline) for the measured ^{14}C values led to the use of a series of hypothetical dates that would be expected if otolith mass was a reasonable proxy for age (Table 1) — these dates were based on nothing more than a succession of oldest to youngest fish that cover the complete bomb ^{14}C signal (Figure 3). The pre-bomb fish were likely more than 50 years old, and the most massive otolith could have been a few decades older based on other studies where pre-bomb otoliths continued to increase in otolith mass, like with large grouper species (Cook et al. 2009, Andrews et al. 2011, 2013, 2019). For the rise period, typically the most diagnostic for age, there is some variation that is not understood for the ^{14}C levels taken up by the earliest growth of splendid alfonsino otoliths. Some deep-water species exhibit a timely ^{14}C rise from core material of otoliths because juveniles are associated with the mixed layer before settling out to greater depths that are ^{14}C-deficient, like yelloweye rockfish (*Sebastes ruberrimus*) and black cardinalfish (*Epigonus telescopus*) that live to depths of up to 500–1000 m as adults but have mixed layer larvae and juvenile phases (Kerr et al. 2002, Tracey et al. 2017). Other deepwater fishes, like ocean perch (*Helicolenus barathri*) and orange roughy (*Hoplostethus atlanticus*), are known to maintain greater depths than the thermocline through ontogeny and have been used to show how the deepwater ^{14}C signal is manifested through time, exhibiting a lagged and attenuated signal (Grammer et al. 2015).

Table 1. Specimen numbers with fish collection year, fork length, and otolith mass with the initial estimated age and calculated hatch year (year of formation, YF1). The results from radiocarbon (^{14}C) measurements are listed as fraction modern ($F^{14}C$) and decay corrected $\Delta^{14}C$ and measurement error (SD). Ages were adjusted through time for alignments to an expected bomb ^{14}C scenario that approximated regional reference chronologies. Initial ages were increased after an age of 10 years (assuming whole otolith are accurate for younger ages) to approximate years of formation during the decline, peak, rise, and pre-bomb periods (YF2) — these age estimates are NOT based on anything more than placement in time from a rough otolith-mass relationship and are intended ONLY as guidance for otolith section age reading.

Lab #	FAS #	Collect	FL cm	Mass (g)	Age (YF1)	$F^{14}C$	$\Delta^{14}C$	Age (YF2)	Align	Age+
BYX-001	87-003	2013	26	0.160	4 (2009)	0.9485	–58.7 ±10.1	4 (2009)	Decline	0
BYX-002	86-052	2013	34	0.218	7 (2006)	0.9809	–26.6 ±9.7	7 (2006)	Decline	0
BYX-003	86-092	2014	37	0.278	9 (2005)	0.9910	–16.7 ±9.9	9 (2005)	Decline	0
BYX-004	86-056	2013	48	0.330	13 (2000)	1.0243	16.4 ± 8.1	15 (1999)	Decline	2
BYX-005	86-101	2014	41	0.338	13 (2001)	1.0725	64.2 ±8.9	27 (1987)	Decline	9
BYX-006	86-063	2013	45	0.380	14 (1999)	1.0987	90.2 ±9.6	36 (1978)	Peak	22
BYX-007	86-102	2014	44	0.386	10 (2004)	1.0436	35.6 ±7.1	43 (1971)	Rise	33
BYX-008	86-021	2012	47	0.426	15 (1997)	1.0104	2.8 ±11.4	45 (1967)	Rise	30
BYX-009	86-010	2012	49	0.434	19 (1993)	0.9130	–93.9 ±10.2	57 (1955)	Pre-B	38
BYX-010	87-001	2012	54	0.589	24 (1988)	1.0405	32.6 ±7.5	43 (1970)	Rise	31
BYX-011	83-314	2011	53.5	0.612	22 (1989)	0.9230	–83.8 ±6.9	53 (1958)	Pre-B	31
BYX-012	83-324	2007	49.4	0.714	25 (1982)	0.9069	–99.4 ±8.2	72 (1935)*	Pre-B	47

* Later investigated the otolith section and counted to an age of 61 years (Figure 4).

In the case of splendid alfonsino, it has been documented as residing within, or at the lower end, of the mixed layer with epipelagic eggs in its early life history. This benthopelagic species exhibits a similar water column life history than species like black cardinalfish and ocean perch. Thus, the pattern of ^{14}C measurements through time may be a mix of near surface to deeper ^{14}C-depleted levels. With this in mind, a comparison of the hypothetical pattern derived for splendid alfonsino in this study tends to follow this line of thinking — ^{14}C levels in the pre-bomb period are lower and similar to the ocean perch chronology but ^{14}C values also cover what can be considered more timely alignments for the peak period. It is unclear why there is a rapid decrease of environmental ^{14}C during the post-peak decline period to levels that reach pre-bomb far sooner than other records. It is possible that these young fish recruited to a ^{14}C-depleted water mass that is either depth or upwelling related.

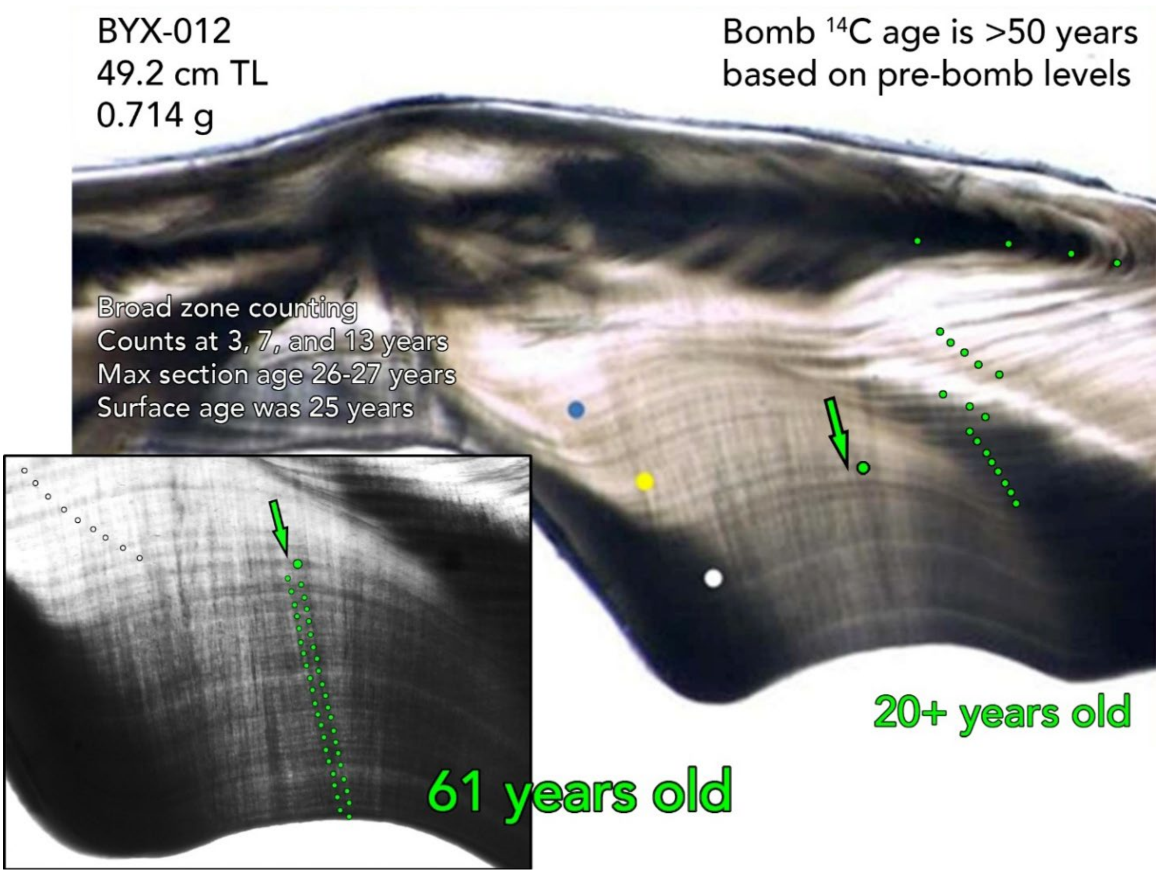

Figure 4. Splendid alfonsino otolith section image that displays two ways to count in the transverse plane of the largest otolith used in this study (0.714 g, BYX-012). One method conforms to the estimates that are visible in the whole otolith view for age reading, effectively a broader band counting interpretation that blends finer growth structure deemed subannual (blue, yellow, and white dots; Krusic-Golub and Robertson 2020). The finer counting scenario (small green dots) begins with an approximation of the first 1-3 years of growth (quantified with daily increments in other studies) and then follows a counting scenario that leads to greater ages that were not well defined in this view but became visible at greater magnification (green arrows are the 20-year tie points between section and inset). This age reading protocol (green dots) accounts for the bomb ^{14}C minimum age of >50 years for an estimated age of 61 years. Section image rendered was from figure 12 of Krusic-Golub and Robertson (2020) with follow-up magnified inset provided by K. Krusic-Golub.

Because daily increments and observed early growth of splendid alfonsino support the first few years of annual growth zone counting, it is likely that whole otoliths are useful for smaller fish with low mass otoliths. This was assumed to be the case in this study for the lower mass otoliths up to an original whole otolith age of approximately 10 years based on personal microscopic observations of the whole otolith surface and experience with other fishes that were later revealed to live much longer once the otolith was sectioned. Hence, there is an age or otolith mass limit beyond which otolith sections must be used for accurate age estimates. With this consideration, an otolith section image that was analysed for whole vs. section age reading was reassessed with the aim of finding a scenario that could explain greater ages (Figure 4). A transverse section of the most massive otolith used in this study that was originally aged to 25–27 years using surface ages and section ages that met the criteria of whole otolith age reading (Krusic-Golub and Robertson 2020) was available for this reassessment. For the age reading criteria, a broader growth zone structure was described that agreed with whole otolith age. However, an alternate interpretation is possible to an age of 20+ years at some distance from the margin of the otolith section, and further inspection for fine-scale growth zones revealed an age reading protocol that would explain hatch years in the pre-bomb period and an age estimate of 61 years (Figure 4). In a study of a deep-water snapper, a similar but opposite situation occurred in which early growth was split into finer growth zones that were consistent with other deep-water snapper studies. However, the fine growth age reading was not accurate based on bomb ^{14}C dating and the problem would not have been obvious if the calculated hatch years were not tested (Andrews and Scofield 2021). The findings for splendid alfonsino in this study are similar to those of red bream in the western North Atlantic where pre-bomb and bomb ^{14}C levels revealed ages were much greater than could be accounted for with whole otolith age reading and that lifespan exceeds 49 years (Freiss and Sedberry 2011).

Further study of bomb ^{14}C results for splendid alfonsino in the southern Indian Ocean, as well as other fisheries where this species has been captured for decades, is warranted to answer questions of age and temporal alignments. It is recommended that an additional 20–40 specimens of similar size and otolith mass classes be added to the existing data that was from just 12 individuals. In addition, use of younger fish that were collected over a series of collection years may help with understanding a proper alignment of hatch years to the post-peak decline. Furthermore, other regions should be considered because otolith archives may be available from numerous sources across the distribution of this species.

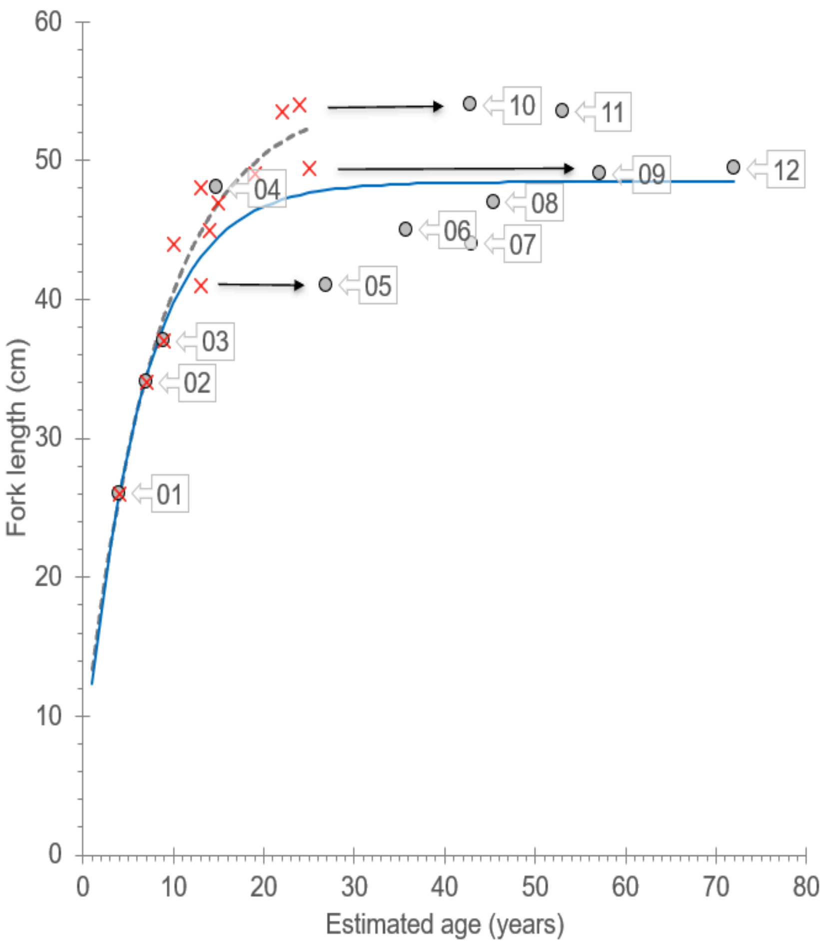

Figure 5. Estimated age-at-length for splendid alfonsino for otoliths used in this study from two scenarios — original whole otolith age estimates (red X) and greater ages (grey circle with sample number) supported by ^{14}C. Revised ages are from rough placement at potential hatch years relative to what is expected from a species that exhibits the full bomb ^{14}C signal (pre-bomb to post-peak, See Figure 2). Old ages are NOT from section age reading and are simply an illustration of what may come from a deeper investigation of thin-sectioned otoliths.

5. Conclusions

The otoliths used in this study revealed that whole otolith age reading for splendid alfonsino underestimated age and that transverse sections must be investigated. As a first look at the effect of these changes to estimates of age-at-length, this set of otoliths was fitted with von Bertalanffy growth functions for each scenario to demonstrate how the life history of splendid alfonsino could change (Figure 5). No parameter values are provided because the figure is for illustrative purposes only — it is apparent that a full set of smallest to largest fish with least to most massive otoliths should be investigated for thin section age reading using an interpretation of fine-scale growth zone structure that may be similar to red bream (Freiss and Sedberry 2011). Revision of otolith age reading to this long-lived scenario using transverse sections for splendid alfonsino may explain, or create the opportunity to resolve, issues with the (1) loss of numerous otolith specimens deemed unreadable (e.g., Taniuchi et al. 2004, Brouwer et al. 2020), (2) conflicting growth parameter results across its distribution (e.g., Santamaria et al. 2006, Kozlov 2014), and (3) complicated assessments of population dynamics and important life history parameters like natural mortality (e.g., Wiff et al. 2012, Shotton 2014).

6. Recommendations

- Investigate a series of 100–200 small to large alfonsino otoliths (low to high otolith mass) for an age reading scenario using a finer increment structure that is similar to what is exhibited here (Figure 4).
- Take a subset of 20–40 of the aged otoliths (young to old) that exhibit a high confidence in the age estimate — derived from the revised age reading protocol — for extraction of otolith cores and measurement of ^{14}C to refine the initial findings of this study.
- Run an additional series of young alfonsino otoliths (collected over several decades, if possible) that may be used as temporal references in the development of the full bomb ^{14}C signal through time.
- Utilize the validated age reading protocol and age estimates from the 100-200 fish to generate an accurate von Bertalanffy growth function with an estimate of natural mortality.

7. Acknowledgements

Kyne Krusic-Golub provided the otoliths used in this study, with the permission of associated stake holders in the Cook Islands and Australia, and provided a new section image that could be evaluated for finer growth zone structure. Dr. Negar Haghipour processed the extracted otolith samples on the MICADAS at ETH Zürich in the Laboratory of Ion Beam Physics. This project was funded by SIOFA and the final report was generated with the assistance of the SIOFA Scientific Committee advisory panel.

8. References

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Terms of Reference (ToR) for the provision of scientific services to SIOFA Scientific Committee

Project title: Bomb radiocarbon ageing of alfonsino (*Beryx splendens*)

Project Code: SER2022-BYS2

INTRODUCTION

SIOFA CMM2020/01 (paragraph 6a) requires the SIOFA Scientific Committee to provide advice to the Meeting of the Parties on the status of stocks of deep-sea fishery resources, including alfonsino (*Beryx splendens*). In 2020, the SIOFA Scientific Committee (SC3) conducted the first alfonsino stock assessments in the SIOFA region and provided to the Meeting of Parties on the stock status and sustainable yields. In 2023, the assessment for alfonsino will be updated.

This document describes the project Terms of Reference (ToR), milestones, and administrative matters for a consultancy to undertake a pilot study to validate ageing of alfonsino using bomb radiocarbon analyses of alfonsino otoliths. Alfonsino otoliths from the SIOFA Area are likely to be available for fish of suitable age or time period that could be used to evaluate a bomb radiocarbon signature. However, if not, then some samples from the same species in the Pacific region may be used.

Once appointed, the Consultant should direct any questions and clarifications to the SIOFA Science Officer (Marco Milardi, marco.milardi@siofa.org) who will coordinate the project and its interactions with the project advisory panel, the relevant SC HoDs and the SIOFA Scientific Committee Chair, as appropriate.

1. TERMS OF REFERENCE

The project objective and tasks are described as below. The Consultant shall undertake these tasks and consult with the project coordinator, to ensure that the project objectives are met.

A project advisory panel consisting of the SIOFA Scientific Committee Chair, selected members of the SIOFA Scientific Committee, and the SIOFA Secretariat will meet periodically with the consultant to assist the consultant access and interpret reports, data, and to provide advice on relevant analyses or data interpretation for the project.

1.1 Overall objectives

Objective 1: Provide advice to the SIOFA Scientific Committee on the validity of ageing of alfonsino (*Beryx splendens*) in the SIOFA Area.

1.1.1 Task 1: Literature review

Review the general scientific literature and other relevant information sources, including alfonsino in other areas, to summarise information that may assist in the validation of alfonsino ageing, and if available, any information on ageing validation using bomb radiocarbon methods for alfonsino or similar species.

1.1.2 Task 2: Bomb radiocarbon validation of ageing for up to ten otoliths from the Indian Ocean region

Undertake bomb radiocarbon age validation using supplied alfonsino otoliths sampled from the Indian Ocean region that were sampled from the earliest time period where otoliths are available (likely to be later than the mid-2000's), using appropriately selected otoliths and ages.

1.1.3 Task 3: Recommendations to the Scientific Committee

Provide advice to the SIOFA Scientific Committee on the validity of standard methods of reading alfonsino otoliths from the investigation using bomb radiocarbon ageing.

1.2 Reporting requirements

1. Provide updates and engage with the project advisory panel that will assist the consultant access and interpret reports, data, and to provide advice on relevant analyses or data interpretation for the project.

2. Provide a draft report detailing the methods, outcomes of reviews, conclusions, and recommendations to the SIOFA project advisory panel for review by 31 January 2022. [dates revised]

3. Update the draft report in (2) by considering any comments and advice from the project advisory panel and submit this report to SIOFA Secretariat for submission to the SIOFA Scientific Committee meeting in 2023 by 15 February 2023. [dates revised]

4. Present the draft report in (3) to the SIOFA Scientific Committee to its meeting in March 2023 by videoconference. [dates revised]

5. Provide an amended final report to the SIOFA Secretariat, considering any comments made at the SIOFA Scientific Committee meeting in March 2023, by 15 April 2023. [dates revised]

6. Provide all the information collected to the SIOFA Secretariat (including that sourced from the Secretariat) before the final payment of the contract is made to the consultant. Such information includes electronic data files, analysis codes, biological samples, and other relevant data if applicable.

1.3 Confidentiality and distribution of project outcomes

The Consultant shall not release confidential data provided for conducting this study to any persons nor any organisations, other than SIOFA Secretariat. The consultant shall delete all the confidential data after the completion of the contract. Any arrangements for ownership, storage, or disposal of physical samples shall be agreed by SIOFA as a part of the contract.

All Intellectual Property generated as a part of this contract shall become the property of SIOFA unless otherwise excluded in the proposal and agreed by SIOFA in the contract.

All reports and presentations will be reviewed by the SIOFA Secretariat prior to any form of further distribution. The Consultant will revise the report according to comments received from the review process before the report or presentation is accepted as a submission against the requirements in the Terms of Reference.

1.4 Relevant SIOFA information

1. SIOFA data (provided by the SIOFA Secretariat upon request)
2. SIOFA reports:
    a. SIOFA SC reports. Scientific Committee Meeting | SIOFA (apsoi.org)
    b. SIOFA technical and scientific reports (public reports available from apsoi.org, and restricted reports available from the SIOFA Secretariat to the project consultant)

WORK PLAN AND PAYMENT SCHEDULE

The funds for this project are budgeted under General Objective 1 of the SIOFA/EU Grant Agreement SI2837681 - Scientific Work Support, for a total allocated budget of 5000 euro (including all costs and including any travel related expenses).

The consultant shall follow the timeline described in Table 1 below.

Table 1: Timeline for payments, milestones, and report submission

Milestone	Date	Activities
Initiation of contract	1 December 2022	First instalment payment (30% of the total contract sum)
Delivery of draft report	15 February 2023 (revised to 31 September 2023)	Submission of draft report to SC8
Delivery of final report	15 April 2023 (revised to 15 October 2023)	Submission of final report and project information to SIOFA. Final instalment payment (70% of the total contract sum) on acceptance of the final report and the submission of project information

SUBMISSION OF APPLICATIONS

The applicants should have appropriate experience and knowledge of developing stock structure hypotheses and preferably on the stock dynamics and life cycle of alfonsino. The applicants should submit a proposal to the project coordinator (SIOFA Science Officer - Marco Milardi, marco.milardi@siofa.org) containing the following items:

1. A current CV that summarises the applicant(s) relevant educational background and professional experience,
2. A brief proposal (indicatively 1-2 pages) outlining the proposed methods and analyses, including a description of how the objectives of the ToRs will be achieved,
3. Any proposed exclusions to the intellectual property clause,
4. The proposed consultancy price (including all consultant expenses and project related costs), noting that the available budget for this work is a maximum of €8,333,
5. Identification of any project risks and associated mitigation and management required to successfully complete the project,
6. A statement that identifies any perceived, potential, or actual conflicts of interest of the applicant(s), including those described in paragraph 4 of the SIOFA recruitment procedure (see Box 1), and
7. Any additional relevant information the applicant(s) wish to submit.

Only applications received before 12 AM (9 AM UTC) on Monday the 12th of December, Reunion Island time, will be considered in the following selection process.

EVALUATION CRITERIA FOR THE SELECTION OF CANDIDATES

The selection criteria will be developed by the evaluation panel along with the project manager, the Secretariat, and the Chairpersons of the relevant subsidiary bodies. The criteria may include following items:

1. Adequate submission of information to allow the panel to evaluate the candidate,
2. Evaluation of the proposal from the candidate, including the proposed contract price,
3. Ability to undertake and complete the analyses or work required in the ToR,
4. The candidate's agreement with confidentiality provisions required for the project,
5. Acceptable conflict of interest statement,
6. Agreement with the data submission and intellectual property terms required in this ToR, and
7. Financial and resourcing considerations.

CONFLICTS OF INTEREST. PARAGRAPH 4 OF SIOFA'S RECRUITMENT PROCEDURE

To ensure that situations relating to potential and actual conflict of interests are avoided, persons falling into the following categories may not normally be considered for SIOFA consultancy: (i). any person designated as a designated representative or alternate representative of a CCP to the Meeting of Parties (MOP) as per Rule 3.1 of the Rules of Procedure, and to the SC and any other subsidiary bodies of the MOP, as per Rule 21.3 of the Rules of Procedure; (ii). Any person fulfilling the function of Chair or Vice-Chair of the MOP or Chair or Vice-Chair of a SIOFA subsidiary body or working group; (iii). Any person acting as a member of a delegation involved in the SIOFA decision-making process resulting in recommendations and/or approval for the SIOFA work requiring the engagement of a consultant; and (iv). Individuals who were SIOFA Secretariat staff members at the time when the recommendations and/or approval for the SIOFA works were adopted or who are members of immediate family (e.g., spouse or partner, father, mother, son, daughter, brother, or sister) of any Secretariat staff member or of the persons identified in 4 (i), (ii), and (iii).

CONTACTS

Project Coordinator – SIOFA Science Officer (Marco Milardi, marco.milardi@siofa.org)

Administration – SIOFA Executive Secretary (Thierry Clot, thierry.clot@siofa.org)