

# Rapid elemental profiling for species identification of marlin

Patrick J. Burke <sup>1,\*</sup>, Kyle Zawada<sup>1</sup>, Tristan Guillemain<sup>1</sup>, Julian Pepperell <sup>2</sup>, Jane E. Williamson<sup>1,3</sup>

<sup>1</sup>School of Natural Sciences, Macquarie University, Sydney, NSW, Australia, 2113

<sup>2</sup>Pepperell Research and Consulting Pty Ltd, Noosaville, QLD, Australia, 4566

<sup>3</sup>Sydney Institute of Marine Science, Mosman, NSW, Australia, 2088

\*Corresponding author. School of Natural Sciences, Macquarie University, 14 Eastern Rd, Macquarie Park, NSW, 2113, Australia. E-mail: Patrick.b711@gmail.com

## Abstract

Marine ecosystems are increasingly threatened by overfishing and human-induced pressures that compromise stock assessments through species misidentification. In this study, we evaluate the efficacy of portable X-ray fluorescence (pXRF) as a rapid, non-destructive tool for species identification in fisheries monitoring, using marlin (Istiophoridae) as a model system. Anal fin spine samples from black, blue, and striped marlin were collected at recreational tournaments and processed for elemental analysis. Multivariate analyses, including permutational multivariate analysis of variance, non-metric multidimensional scaling, and similarity percentage analysis, revealed significant interspecies differences in elemental composition. Notably, blue marlin exhibited distinct elemental profiles—potentially reflecting unique habitat use and trophic interactions—compared to black and striped marlin. Classification using canonical analysis of principal coordinates achieved correct classification rates of 68.0% for concentration data and 81.3% for raw spectral counts, while machine learning achieved classification rates of  $51.5\% \pm 1.73\%$  for concentration data and  $62.6\% \pm 1.44\%$  for raw spectral counts—each substantially exceeding the null hypothesis expectation of 33% accuracy for random guessing among three classes. These results underscore the ability of pXRF to discriminate species based on their elemental signatures and highlight the potential of pXRF as a cost-effective, field-deployable complement to traditional genetic methods in improving fisheries monitoring and conservation strategies.

**Keywords:** portable X-ray fluorescence; fisheries monitoring; Australia; machine learning; billfish; elemental fingerprint

## Introduction

Marine ecosystems are facing unprecedented challenges under current human-induced pressures, leading to significant declines in biodiversity and ecological integrity (Storch et al. 2022). These declines pose not only ecological but also socioeconomic risks, as marine biodiversity underpins food security, livelihoods, and climate resilience globally (FAO 2024). Among these pressures, overfishing is a substantial contributor to biodiversity declines in the oceans, with an estimated 37.7% of fish stocks now fished at unsustainable levels (FAO 2024). The status of many stocks, particularly pelagic species and those caught in the high seas remain poorly understood (McWhinnie 2009, Campana 2016). This status is further complicated in instances where species are likely to be misidentified or misreported, masking the true numbers of individual species being caught and leading to inaccurate assessments of stock health (Williams et al. 2018). In some instances, species within the same genus or family exhibit remarkable resemblances in external morphology, making accurate identification solely based on visual inspection a daunting task, particularly after truncating or post-catch processing of fish (Williams et al. 2018). This inherent similarity underscores the necessity for more sophisticated and reliable identification techniques for effective fisheries monitoring and accurate stock assessments.

Mislabelling of seafood, intentional or incidental, is widespread across international markets with 30% of seafood

estimated to be misidentified or mislabelled (Pardo et al. 2016, Cundy et al. 2023, Ahles et al. 2025). Current fisheries monitoring relies heavily on genetic techniques to identify fish when morphological traits are unable to discern species (Teletchea 2009, Myun Park et al. 2020). While effective, these genetic methods have limitations: they are time-consuming, costly, and require specialized laboratory facilities and expertise. These constraints reduce their utility as rapid deterrents or forensic tools in real-time monitoring scenarios. This gap underscores the need to expand the forensic conservation toolbox for fisheries monitoring, complementing and enhancing existing genetic approaches with faster, more accessible techniques.

One promising alternative is elemental profiling using portable X-ray fluorescence (pXRF), an analytical technique that is rapidly emerging as a tool in wildlife forensics (Budhachat et al. 2016, Nganvongpanit et al. 2016, Brandis et al. 2024). pXRF offers a rapid, non-destructive approach to measuring elemental concentrations in tissues, generating elemental profiles within minutes (Brandis et al. 2023, 2024). Elemental profiles vary not only among species but also within them, reflecting differences in diet and environmental exposure that shape each organism's unique elemental signature (Duarte et al. 2022). In the context of fisheries monitoring, pXRF offers the unique opportunity to equip researchers and regulatory bodies with an 'elemental fingerprint' that could identify species, based on the elemental composition of wildlife spec-

imens. This elemental fingerprint can be combined with classification algorithms to identify species and provenance via consistent yet subtle differences in elemental composition of inert tissues and has shown promise in the terrestrial realm (Benedet et al. 2021, Gil-Delgado et al. 2025).

The marlins (Istiophoridae)—a charismatic and economically valuable group of species in recreational and commercial fisheries—exemplify the identification challenges faced in fisheries management. In Australia, black (*Istiompax indica*), blue (*Makaira nigricans*), and striped (*Kajikia audax*) marlin represent a valuable sector of recreational fisheries (Kalish et al. 2002, Bromhead et al. 2004). While black and blue marlin are no-take species in commercial fisheries, striped marlin are a targeted species in the longline fisheries and are currently considered overfished in the southwestern Pacific (Ducharme-Barth et al. 2019, Butler et al. 2023). Distinguishing between these species can be challenging due to their morphological similarities, especially at younger life stages or following truncating and post-catch processing (Williams et al. 2018, Guillemin et al. 2025). Even in cases by trained fisheries observers, errors up to 77% in identification have been observed due to similarities between black and blue marlin (Williams et al. 2018). Such high rates of error have resulted in instances of no-take species being landed and sold in commercial markets (NSW Department of Primary Industries 2021), complicating fisheries management and obscuring our understanding of species-specific catch rates. Despite the morphological similarities among these three sympatric marlin species, they have unique ecologies, use different habitats and exploit different prey species (Guillemin et al. 2022).

Thus, there is a clear need for efficient, cost-effective monitoring tools that can reliably identify marlin landings and support sustainable fisheries management. To address these challenges, this study evaluates the efficacy of elemental profiling using pXRF as a tool for species identification in eastern Australian marlin. We hypothesize that (i) different marlin species possess distinct elemental profiles and that (ii) elemental profiling of the first anal fin spines alone can reliably identify these species. Collectively, these hypotheses aim to validate elemental profiling as a rapid, accessible, and complementary technique for fisheries monitoring and species conservation.

## Methods

### Sample collection and preparation

Samples of black, blue, and striped marlin were opportunistically obtained from catch associated with game fishing tournaments conducted from 2005 to 2024. All tournaments were organized by clubs affiliated with the New South Wales Game Fishing Association and took place along the New South Wales coastline. For each sampled fish, sex and ‘short length’ (measured from the tip of the lower jaw to the fork of the tail, the standard-length measurement in Istiophorid research) were recorded when available. Fish were weighed whole by the tournament weighmaster before any sample extractions. The largest anal fin spines were removed using a knife, transported to the laboratory, and stored at  $-20^{\circ}\text{C}$ . In the laboratory, anal fin spines were manually cleaned by carefully removing all adherent muscle tissue, skin, and connective tissue. This process was performed using a sterile scalpel and a clean cloth, ensuring meticulous removal of any extraneous biological material until only clean bone remained. Once thoroughly cleaned, the spines were placed



**Figure 1.** Illustration of the three different sampling areas for pXRF on a cleaned and dried marlin anal fin spine. Locations include one condyles width from the base (C1), the length of three condyles width from the base (C3), and the tip of the spine.

in a drying oven set at a constant temperature of  $50^{\circ}\text{C}$ . Morphometric measurements, including condyle width and widths at three distinct intervals, were recorded to assess site variation and determine infinite thickness (overpenetration of the X-rays) (Fig. 1). A total of 25 spines from each species were processed and analysed (Table 1).

### pXRF data collection

An Olympus Vanta M-Series pXRF instrument [4-watt X-ray tube with rhodium (Rh) anode, 8–50 keV, silicon drift detector], with three beam energies (10, 40, and 50 keV) was used to analyse all samples. The GeoChem3 method that uses a fundamental parameters algorithm to correct for inter-element effects was used for each scan. Calibration checks were conducted regularly using onboard calibration software, with a detector calibration check performed using a milled quartz ( $\text{SiO}_2$ ) blank.

The pXRF instrument provided two output data types: raw beam spectra across 2048 keV bands and elemental concentrations as percentages for 42 elements, calculated via Olympus’ onboard algorithms. To identify the optimal sampling location on the anal spine and to assess infinite thickness, a subset of samples was analysed in three different locations: the length of one condyles width from the base (C1), the length of three condyles width from the base (C3), and the tip (Fig. 1). Each spine was analysed once using a 30-s beam time at 10, 40, and 50 keV, totalling 90 s per location with a backing of  $\text{SiO}_2$  to test for infinite thickness. Upon identifying the ideal location, all spines were analysed and compared using only one sampling location and one sampling interval.

To ensure that reported elements analysed were actually present in the samples and not an artifact of using non-biological algorithms on biological samples, we checked for diagnostic peaks in the beam spectra data using 90 s scans per beam (total 270 s scanning time) for each species group. We excluded any elements that did not match their  $K\alpha 1$  and  $K\beta 2$  peaks (Brandis et al. 2024). This approach allowed us to remove false positives from the concentration data. Ninety-second beam times were chosen to provide the greatest possibility of detecting the element if present. Elements below the limits of detection were not included in analyses.

**Table 1.** Summary of marlin samples used in the study by species and sex.

Species	Average short length (cm)	Average weight (kg)	Males	Females	Sex unknown
Black marlin	202.9 ± 16.1	73.6 ± 25.5	15	4	6
Blue marlin	237.5 ± 19.8	148.7 ± 41.7	2	16	7
Striped marlin	225.6 ± 9.5	76.3 ± 10.4	8	7	10

Initial analyses compared elemental profiles obtained from three sampling locations along the fin spine: one condyle's width from the base (C1), three condyles' width from the base (C3), and the spine tip. Our results showed that the C1 location produced the most consistent and reliable data, as evidenced by the stable levels of silica detected in these sample locations, indicating that infinite thickness was being met, and X-rays were not penetrating through the sample (Supplementary Fig. 1). As such, all fin spines were analysed and statistically compared using data from the C1 sampling location. Furthermore, we excluded 22 elements from our analyses (Ag, Au, Bi, Ce, Co, Cr, In, La, Nb, Ne, Pb, Pd, Pt, Sb, Si, Ta, Te, Th, U, V, W, and Y) due to absences in their  $K\alpha 1$  or  $K\beta 2$  peaks during the extended diagnostic analysis (90 s per beam, 270 s total), ensuring only diagnostically and biologically relevant elements were considered in subsequent analyses.

### Statistical analysis

Elemental profiles were compared among species by using a permutational multivariate analysis of variance (PERMANOVA) with Monte Carlo simulations. This was run on the Euclidean distances of the square-root transformed elemental concentration data and counts-per-second data. PERMANOVA tests were used to assess the effects of species, sex, and short-length as a proxy for age, on elemental profiles. When PERMANOVA main tests showed significant differences for a factor, pairwise tests were performed to identify significant differences between factor levels. The average distance among species and the elements driving the separation between species was determined using a similarity percentage (SIMPER) test on the square-root transformed elemental profiles.

Non-metric multidimensional scaling (nMDS) was applied to the Euclidean distances of the square-root transformed elemental data to visualize patterns, groupings, and dispersion within and among species. This technique was chosen for its ability to effectively represent multi-dimensional data in a two-dimensional space while preserving the relative relationships between data points (Couturier et al. 2020). The effectiveness of the nMDS ordination was evaluated using the stress value, with values below 0.2 indicating a reliable representation of the data structure in two-dimensional space (Clarke 1993).

### Classification analysis

Random forest classification was applied to predict marlin species based on elemental composition and to identify key elements contributing to classification. This method was selected for its ability to model complex, non-linear relationships and its resilience to overfitting (Moradi et al. 2024). The data were randomly split into training (70%) and testing (30%) sets and each random forest model was trained using 5000 trees and 10 variables per split. The trained model

was then applied to the independent test set, and classification performance was evaluated using a confusion matrix, which provided key metrics, including accuracy, sensitivity, specificity, and precision. To account for variability in data partitioning and ensure robustness, we repeated the process 25 times, aggregating per-species classification metrics across runs. The final model performance was summarized as the mean and standard deviation of accuracy, sensitivity, specificity, and precision across iterations. This approach allowed for a rigorous evaluation of elemental data in species classification and enabled a direct comparison between elemental concentration data and counts-per-second data in distinguishing marlin species.

Canonical analysis of principal coordinates (CAP) was used to assess the ability of elemental composition data to distinguish between species and to test for classification accuracy. CAP is a constrained ordination technique that maximizes the separation between groups by finding axes that best discriminate predefined categories while preserving the underlying dissimilarity structure of the data (Anderson and Willis 2003). For this analysis, Euclidean distances were used as the dissimilarity measure to ensure that differences in elemental concentrations were accurately represented. The analysis was conducted using the PRIMER software package, which provides a robust framework for multivariate analysis of ecological data. The significance of the group separation was tested using permutation tests (4999 permutations) to evaluate whether the observed classification was better than random chance at a  $\alpha$ -level of 0.01 (Anderson 2001). The resulting CAP plot provided a visual representation of the separation between groups, with samples projected onto the constrained principal coordinate axes. Classification accuracy was further assessed by examining the assignment of samples to their respective groups, helping to determine the robustness of the elemental composition in distinguishing species.

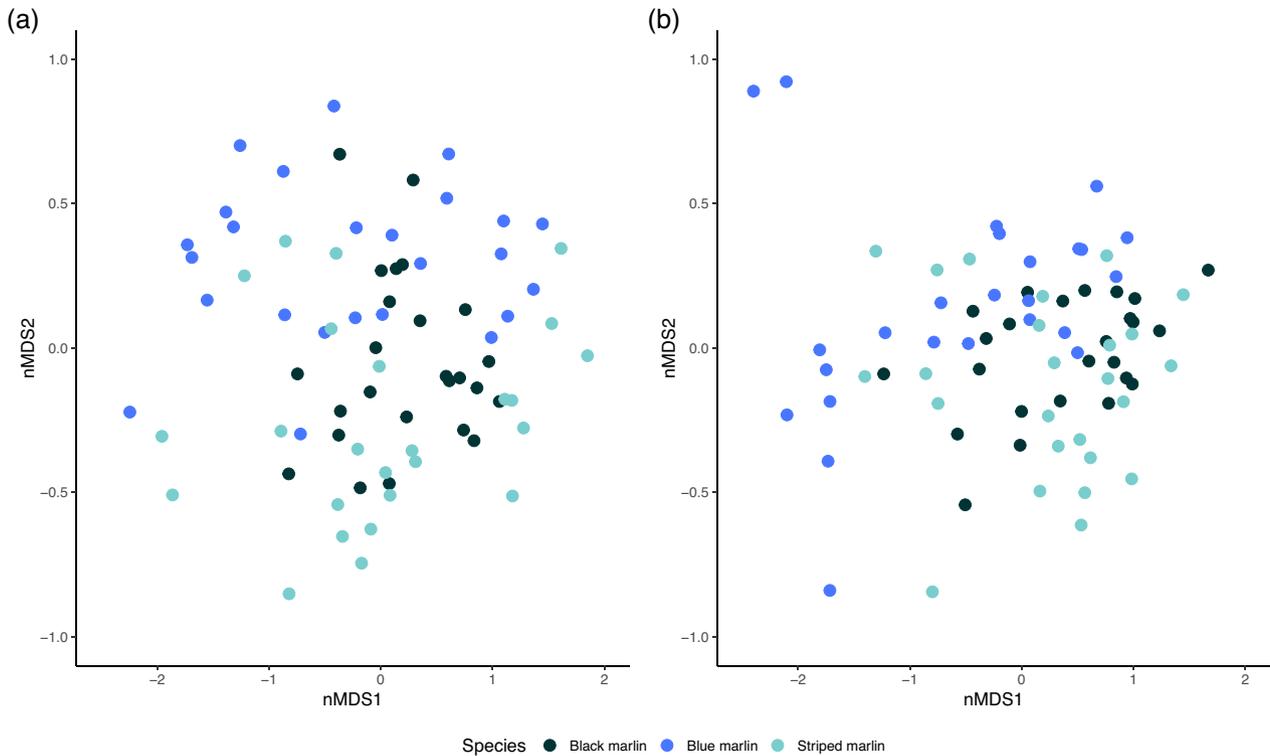
## Results

### Results summary

Our analyses revealed significant differences in elemental profiles among marlin species (PERMANOVA,  $P < 0.05$ ), with blue marlin showing the most distinct elemental signatures. Analysis of Principal Coordinates demonstrated considerable classification potential, achieving 68.0% accuracy for concentration data and 81.3% accuracy for counts-per-second data. Random forest models further supported species differentiation, with overall classification accuracies of 51.5% ± 1.73% for concentration data and 62.6% ± 1.44% for counts-per-second data—both substantial improvements over the naïve classifier's 33% accuracy, with blue marlin consistently achieving the highest classification success. Sensitivity and precision metrics highlighted robust classification for blue marlin and black marlin, though striped marlin showed greater misclassification rates.

**Table 2.** PERMANOVA results showing pairwise comparisons of elemental concentration data and count-per-second data between marlin species, including  $t$ -values,  $P$ -values, and Monte Carlo  $P$ -values.

	Groups	$t$	$P(\text{perm})$	$P(\text{MC})$
Concentration data	Black marlin, blue marlin	3.41	0.01	0.01
	Black marlin, striped marlin	1	0.33	0.32
	Blue marlin, striped marlin	2.76	0.01	0.01
Counts-per-second data	Black marlin, blue marlin	2.26	0.02	0.02
	Black marlin, striped marlin	1.3	0.18	0.17
	Blue marlin, striped marlin	2.03	0.03	0.03



**Figure 2.** nMDS plot illustrating the multivariate distribution of elemental concentration data (a) and counts-per-second data (b), with points representing samples positioned based on their compositional similarity in reduced-dimensional space and specified by species.

### Identifying species-specific elemental profiles

The elemental profiles of the different marlin species were analysed using a PERMANOVA. Elemental profiles were significantly different between species for both concentration data (Pseudo-F = 7.33, perm = 4999,  $p(\text{MC}) = 0.01$ ) and for counts-per-second data (Pseudo-F = 3.72, perm = 4999,  $p(\text{MC}) = 0.02$ ). Pairwise comparisons of both elemental concentration data and counts-per-second data revealed that blue marlin were significantly different from black and striped marlin, while black and striped were not significantly different from each other (Table 2). PERMANOVAs did not reveal any significant differences in elemental profiles for sex or length, regardless of data type.

### Contributions to group differences

To further explore the differences in elemental profiles between species, a similarity percentage (SIMPER) analysis was conducted. In concentration data, P, Ca, and S contributed the most to the observed dissimilarities, accounting for the majority of the overall variance between groups

(Supplementary Table 1). In count-per-second data, Ca\_K\_10, P\_K\_10, and Sn\_L\_10 contributed the most to the observed dissimilarities, accounting for the majority of the overall variance between groups (Supplementary Table 2).

### Multidimensional Scaling

The nMDS plot provided a visual representation of the differences in elemental profiles between species for elemental concentration data and counts-per-second data. Clustering was limited by species, with some general trends suggesting differences in elemental profiles in multi-dimensional space (Fig. 2, stress value = 0.05).

### Species identification using elemental profiles

#### Canonical analysis for species differentiation

A canonical analysis of principal coordinates (CAP) was used to evaluate whether elemental profiles could reliably differentiate between marlin species. For elemental concentration data, a cross-validation using leave-one-out allocation of observations to groups was performed, resulting

**Table 3.** Assignment tables showing the percentage of correct and incorrect classifications for each marlin species based on random forest and canonical analysis of principal coordinates (CAP) models applied to elemental concentration and counts-per-second data.

		Predicted species			Correct classification
		Black marlin	Blue marlin	Striped marlin	
True Species	Random forest assignment table—concentration data				
		Black marlin	Blue marlin	Striped marlin	
	Black marlin	55.0	18.5	26.5	55.0 ± 4.5
	Blue marlin	10.8	72.7	16.5	72.7 ± 3.5
	Striped marlin	47.4	26.0	26.6	26.6 ± 3.3
	Random forest assignment table—counts-per-second data				
		Black marlin	Blue marlin	Striped marlin	
	Black marlin	55.2	15.5	29.4	55.2 ± 3.6
	Blue marlin	7.0	81.7	11.3	81.7 ± 2.9
	Striped marlin	28.7	19.5	51.8	51.8 ± 3.1
	CAP assignment table—concentration data				
		Black marlin	Blue marlin	Striped marlin	
Black marlin	55.0	18.5	26.5	55.0	
Blue marlin	10.8	72.7	16.5	72.7	
Striped marlin	47.4	26.0	26.6	26.6	
CAP assignment table—counts-per-second data					
	Black marlin	Blue marlin	Striped marlin		
Black marlin	55.2	15.5	29.4	55.2	
Blue marlin	7.0	81.7	11.3	81.7	
Striped marlin	28.7	19.5	51.8	51.8	

Values represent the mean percentage of predictions across 25 iterations. For random forest models, the correct classification column includes the mean ± standard error of classification accuracy per species. CAP values represent single-run outcomes without error estimates.

in an overall correct classification of 68.0%, far exceeding the null hypothesis expectation of roughly 33% accuracy for random guessing in a three-class problem (i.e. 100/3 classes = 33.33%; Table 3). The CAP analysis using concentration data demonstrated partial separation among species, with the first two canonical axes explaining 75.7% and 61.8% of the variance, respectively (Fig. 3). Cross-validation results showed that black marlin had a correct classification rate of 68.0%, blue marlin of 68.0%, and striped marlin of 68.0%, indicating good classification performance for all species. For counts-per-second data, leave-one-out cross-validation produced an overall classification accuracy of 81.3%. The CAP analysis using counts-per-second data demonstrated improved separation among species compared to concentration data, with the first two canonical axes explaining 86.9% and 81.7% of the variance, respectively (Fig. 3). Cross-validation results showed that black marlin had a correct classification rate of 76.0%, blue marlin had 88.0%, and striped marlin had 80.0% indicating good classification performance for all species.

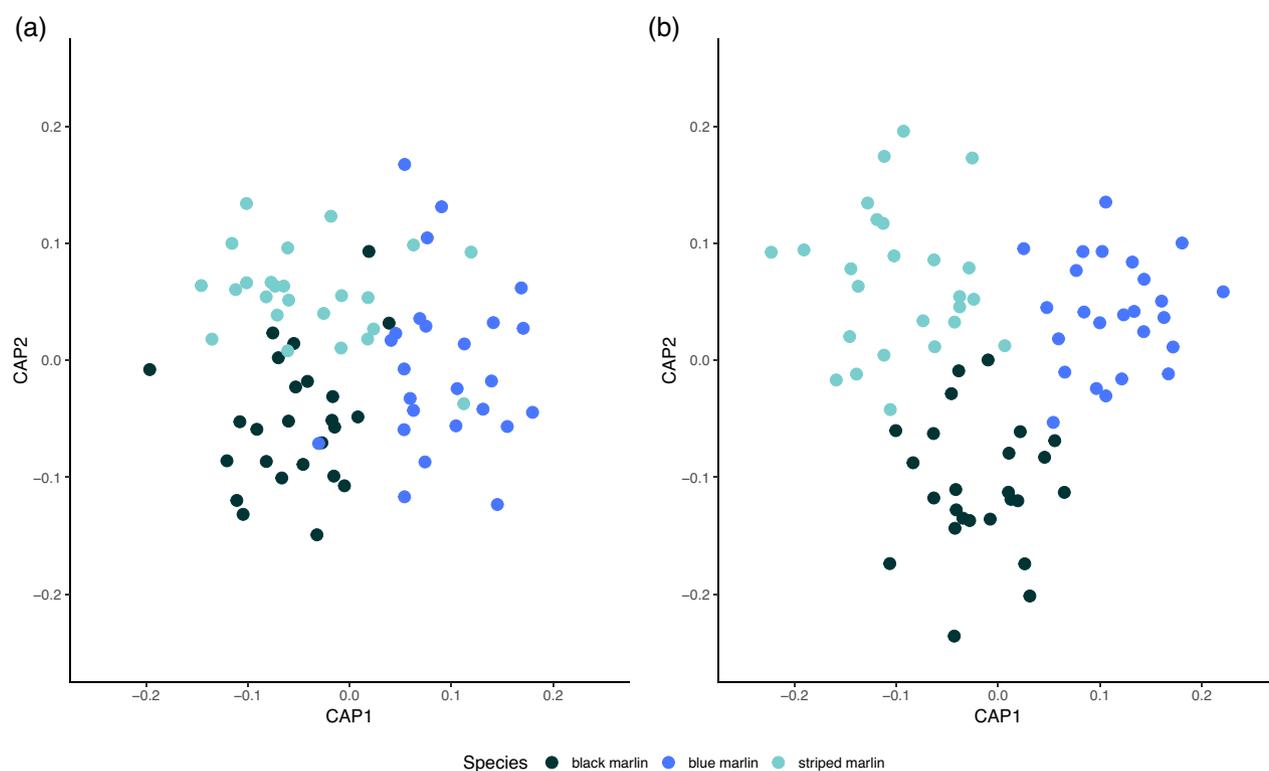
### Machine learning classification

Random forest models were used to classify species based on elemental profiles for concentration and counts-per-second data. For elemental concentration data, the random forest model achieved an overall accuracy of 51.5% ± 1.73% in classifying the species, with blue marlin having the highest classification accuracy and black marlin the lowest, improving on the null hypothesis expectation of roughly 33% accuracy for random guessing in a three-class problem. Sensitivity and specificity metrics varied among species, with blue marlin showing the highest mean (±standard deviation) values (sensitivity = 0.65 ± 0.23, specificity = 0.76 ± 0.11, precision = 0.53 ± 0.18), indicating a significant classification performance. The precision and sensitivity values also highlighted the robustness of the model for blue marlin and black

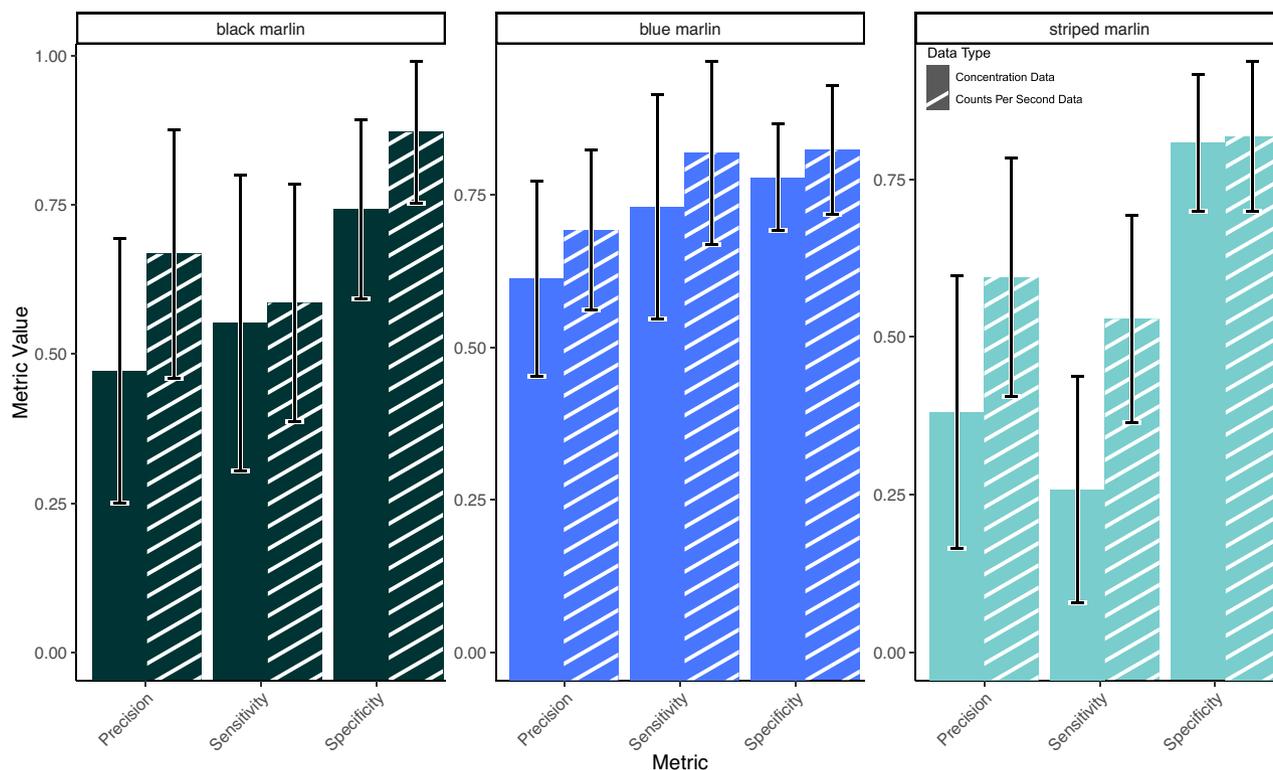
marlin, while striped marlin had lower precision and sensitivity (Fig. 4). For counts-per-second data, the random forest model achieved an overall accuracy of 62.6% ± 1.44% in classifying the species, with blue marlin having the highest classification accuracy and striped marlin the lowest (Fig. 4). Sensitivity and specificity metrics varied among species, with blue marlin showing the highest mean (±standard deviation) values (sensitivity = 0.88 ± 0.12, specificity = 0.85 ± 0.07, precision = 0.75 ± 0.12), indicating a significant classification performance. The precision and recall values also highlighted the robustness of the model for blue marlin and black marlin, while striped marlin had lower precision and recall. The confusion matrix showed that black marlin and striped marlin were most often confused, while blue marlin had fewer misclassifications (Table 3). These results suggest that elemental profiles of both elemental concentration data and counts-per-second data have potential for species identification, with particularly high success for blue marlin.

### Discussion

This study provides evidence for the potential of pXRF as a rapid, accessible tool for species identification in fisheries monitoring. By demonstrating significant differences in the elemental profiles of marlin species, particularly between those of blue marlin and the other two species, this work highlights the potential of elemental profiling to address critical gaps in species monitoring. While the classification success achieved here does not yet match the accuracy of some genetic approaches, such as >90% rates reported for Pacific salmon (Robinson et al. 2024), it represents a substantial improvement over current practices in Australian marlin fisheries, where trained observers have demonstrated only ~33% accuracy based on genetic validation (Williams et al. 2018). These findings contribute to the growing body of research em-



**Figure 3.** Canonical analysis of principal coordinates (CAP) plot visualizing the elemental concentration data (a) and counts-per-second data (b), in multivariate space, constrained by the specified grouping factor to highlight differences among species.



**Figure 4.** Bar plot comparing classification metrics for black marlin, blue marlin, and striped marlin based on elemental data obtained using pXRF. Metrics are displayed separately for concentration data (solid fill) and counts-per-second data (striped fill). Error bars represent standard deviations across 25 model runs. Precision is the proportion of true positive predictions out of all positive predictions made by the model  $[TP/(TP + FP)]$ . Sensitivity (recall): the proportion of actual positives correctly identified by the model  $[TP/(TP + FN)]$ . Specificity: the proportion of actual negatives correctly identified by the model  $[TN/(TN + FP)]$ . TP = true positive, FP = false positive, FN = false negative, TN = true negative.

phasizing the importance of developing cost-effective, field-deployable techniques to complement existing genetic methods, ultimately supporting fisheries management and conservation.

The differences observed in elemental profiles, particularly for blue marlin compared to black and striped marlin, likely reflect distinct ecological and physiological traits among these species (Guillemin et al. 2022). The clearer separation of blue marlin in the ordination analyses aligns with their preference for oceanic habitats and prey types distinct from those of black and striped marlin, at least in certain geographic contexts (Guillemin et al. 2022). Key elements such as phosphorus, calcium, and sulphur—which vary based on dietary sources and environmental availability—appear to drive these observed patterns (Duarte et al. 2022). Specifically, phosphorus and sulphur are typically limited in pelagic ecosystems, suggesting their presence in tissues could indicate adaptations related to foraging strategies or habitat use in nutrient-poor offshore waters (Campana 1999, Walther and Limburg 2012, Bristow et al. 2017).

Although the nMDS analysis showed limited species separation, this outcome is expected given that nMDS is an unsupervised method, designed to visualize general patterns of dissimilarity without directly considering group identities (Anderson and Willis 2003). In contrast, the supervised CAP analysis explicitly optimizes ordination to distinguish predefined groups—here, species—by selecting axes that maximize inter-group differences (Anderson and Willis 2003). This methodological distinction explains why CAP more clearly differentiated blue marlin from the others, underscoring the complementary value of combining supervised and unsupervised approaches to interpreting elemental profile data. To strengthen interpretations further, future research should employ regionally stratified sampling strategies to examine how geographic origin, prey availability, and environmental factors shape elemental signatures at both the species and population levels. Such targeted studies would help validate and extend the applicability of elemental profiling methods across diverse fisheries contexts.

The ability of pXRF to classify species using elemental profiles was further evaluated using canonical analysis of principal coordinates (CAP) and machine learning approaches. CAP analysis achieved a correct classification rate of 68.0% for concentration data and 81.3% for count-per-second data, indicating that raw spectral data may better capture interspecies differences. This is likely because raw data retain the full complexity of elemental signals, including subtle variations in peak intensities and inter-element relationships, that can be lost during conversion to concentration values. Similar approaches have been successfully applied in soil science, where pXRF-derived spectral data combined with machine learning algorithms have yielded high accuracy in predicting soil properties (Silva et al. 2017, Benedet et al. 2021). However, the observed variation in classification accuracy across species—with blue marlin showing the highest rates and black marlin the lowest—may reflect differences in ecological plasticity or variations in sampling conditions that influence elemental incorporation. These findings underscore the potential capacity of pXRF for species discrimination and suggest that optimizing data processing and model development may further enhance its utility in fisheries monitoring.

The random forest model achieved significant overall accuracy of  $51.5\% \pm 1.73\%$  for concentration data, and  $62.6\%$

$\pm 1.44\%$  for counts-per-second data. Considering that a naïve classifier for a three-class problem would yield an accuracy of around 33%, our machine learning results represent a substantial improvement. Moreover, the random forest model provided valuable insights into the importance of specific elements—such as Zn, S, Sr, P, and Fe—that are likely linked to critical physiological and ecological differences among the marlin species (Guillemin et al. 2022). For instance, variations in elemental composition have been linked to differences in species and geographic origin, as demonstrated in studies on farmed fish (Davis et al. 2022). Similarly, changes in elemental deposition in strontium bands in vertebrae of sharks have been attributed to changes in the salinity of the waters that the animals inhabit (Raoult et al. 2016), whereas changes in zinc concentrations in shark vertebrae are more related to physiology and ontogeny (Raoult et al. 2018). While machine learning algorithms show promise, further optimization—such as testing more advanced models or including additional variables—may enhance their predictive power. While further optimization—such as exploring more advanced models or incorporating additional variables—may enhance predictive power even further, these results underscore the strong potential of integrating elemental profiling with computational methods to achieve precise and reliable species identification in fisheries monitoring.

Recent genetic investigations by Williams et al. (2018) have underscored the pervasive challenge of species misidentification among Istiophorid billfishes, with misidentification rates reaching up to 80% in observer-reported data. These findings highlight how traditional morphological assessments, particularly when specimens are processed or partially damaged, can lead to significant errors in species assignment, thereby compromising stock assessments. Our study's application of pXRF-based elemental profiling provides a promising complementary approach by capturing subtle yet diagnostically relevant differences in elemental composition that correlate with species-specific ecological and physiological traits. In doing so, this technique theoretically offers a rapid, non-destructive alternative that could help reconcile observer data with genetic findings, ultimately enhancing the accuracy of catch reports and supporting more robust management decisions. Integrating such innovative methods into routine fisheries monitoring could mitigate the uncertainties arising from misidentification, as reported by Williams et al. (2018), and pave the way for improved conservation outcomes.

From a conservation and management standpoint, accurate species identification is critical—especially for high-value, conservation-sensitive marlin (Collette et al. 2011). Mislabelling in fisheries observer programs, commercial fishery logbooks, and commercial markets underscores the need for innovative and efficient methods to verify catch composition (Walsh et al. 2005, Williams et al. 2018, Blanco-Fernandez et al. 2022). By offering a rapid, field-ready approach, pXRF can complement genetic methods that are often hindered by cost, time, and the requirement for specialized laboratory facilities. More immediate on-site identification can strengthen enforcement of no-take regulations and improve compliance with fisheries policies. This is especially relevant for black and blue marlin, where accurate species recognition can mitigate inadvertent harvest and sale, thus contributing directly to conservation objectives. Moreover, implementing pXRF-based elemental profiling in real-time can bolster efforts to combat illegal, unreported, and unregulated fish-

ing by enhancing traceability and accountability in fisheries landings.

Despite its promise, the use of pXRF for species identification faces several challenges. Overlap in elemental profiles between black and striped marlin complicates their differentiation, possibly due to shared ecological niches or diets (Guillemin et al. 2022), though increasing the number of replicates may help reveal subtle differences. While our models here are accurate, they are trained on cleaned fin spines and therefore cannot be directly converted to fully intact specimens. Expanding pXRF assessments to *in situ* applications and fully intact specimens is crucial for better delineating its technical constraints—such as matrix effects, reduced sensitivity to trace elements, and the challenge of infinite thickness. Notably, we scanned all specimens on a SiO<sub>2</sub> block to avoid background signals and maintain consistency. This approach underscores the necessity for standardized protocols in sample preparation and data acquisition to ensure reproducibility.

Building on these initial findings, future research should focus on validating pXRF's utility across a wider range of species, regions, and environmental contexts. Enlarging the dataset to incorporate additional marlin samples or other pelagic fishes would provide deeper insights into the ecological and physiological factors driving elemental variation and refine model performance (Osisanwo et al. 2017). While this study represents the first application of pXRF for fish species identification, we recognize that the approach remains in its early stages and will require further development and benchmarking—particularly in comparison to well-established genetic methods. Rather than replacing genetics, we envision pXRF as a complementary tool that could add value in scenarios where genetic approaches are impractical due to cost, turnaround time, or infrastructure constraints. Combining pXRF with other techniques could form a robust multi-modal framework, especially in cases where elemental overlap exists between species. Machine learning offers additional opportunities to extract meaningful patterns from high-dimensional pXRF data, and incorporating environmental metadata may further improve predictive accuracy. Field-based validation using portable instruments will be essential to assess feasibility and guide method optimization. With continued refinement, pXRF holds promise as an accessible and scalable technique to enhance fisheries monitoring, particularly for high-value, data-poor, or visually unidentifiable specimens.

## Conclusion

This study underscores the promising potential of pXRF as a complementary tool for species identification in fisheries monitoring, representing an important first step in demonstrating its utility. By providing a rapid, non-destructive method for measuring elemental profiles, pXRF addresses critical challenges in current monitoring practices, including the need for cost-effective, field-deployable solutions. These findings serve as an initial proof-of-concept, however, further research is essential to validate its effectiveness in *in-situ* environments. Future studies should focus on testing pXRF on live or more intact specimens to refine its discriminatory power and explore its applicability across diverse field applications. Ultimately, integrating pXRF into fisheries management could enhance species traceability, reduce misidentification, and support the

sustainable management of marine resources, paving the way for the development of advanced forensic conservation tools.

## Acknowledgements

We thank the New South Wales Game Fishing Association and the many individual game fishing club officials and anglers who provided samples for this work.

## Author contributions

Patrick J. Burke (Conceptualization [equal], Data curation [lead], Formal analysis [lead], Funding acquisition [equal], Investigation [lead], Methodology [lead], Project administration [lead], Software [lead], Validation [lead], Visualization [lead], Writing – original draft [lead], Writing – review & editing [lead]), Kyle Zawada (Conceptualization [equal], Methodology [equal], Supervision [equal], Writing – review & editing [equal]), Tristan Guillemin (Investigation [equal], Project administration [equal], Resources [equal], Writing – review & editing [equal]), Julian Pepperell (Data curation [equal], Resources [equal], Writing – review & editing [equal]), and Jane E. Williamson (Supervision [equal], Writing – review & editing [equal])

## Supplementary material

**Supplementary data** is available at *ICES Journal of Marine Science* online.

*Conflict of interest:* None declared.

## Funding

This work was partly funded by the Macquarie Minds and Intelligence Initiative at Macquarie University.

## Data availability

The data and analysis underlying this article is open and available here: [10.6084/m9.figshare.29044922](https://doi.org/10.6084/m9.figshare.29044922).

## References

- Ahles S, DeWitt CAM, Hellberg RS. A meta-analysis of seafood species mislabeling in the United States. *Food Control* 2025;171:111110. <https://doi.org/10.1016/j.foodcont.2024.111110>
- Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 2001;26:32–46.
- Anderson MJ, Willis TJ. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* 2003;84:511–25. [https://doi.org/10.1890/0012-9658\(2003\)084\[0511:CAOPCA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2)
- Benedet L, Nilsson MS, Silva SHG et al. X-ray fluorescence spectrometry applied to digital mapping of soil fertility attributes in tropical region with elevated spatial variability. *Anais Da Academia Brasileira de Ciências* 2021;93:e20200646. <https://doi.org/10.1590/0001-3765202120200646>
- Blanco-Fernandez C, Erzini K, Rodriguez-Diego S et al. Two fish in a pod. Mislabelling on board threatens sustainability in mixed fisheries. *Front Mar Sci* 2022;9:841667. <https://doi.org/10.3389/fmars.2022.841667>
- Brandis KJ, Francis RJ, Zawada KJA et al. Advancing the application of pXRF for animal samples. *PLoS One* 2024;19:e0297830. <https://doi.org/10.1371/journal.pone.0297830>

- Brandis KJ, Meagher P, Schoppe S *et al.* Determining the provenance of traded wildlife in the Philippines. *Animals* 2023;13:1–14. <https://doi.org/10.3390/ani13132165>
- Bristow LA, Mohr W, Ahmerkamp S *et al.* Nutrients that limit growth in the ocean. *Curr Biol* 2017;27:R474–8. <https://doi.org/10.1016/j.cub.2017.03.030>
- Bromhead D, Pepperell J, Wise B *et al.* *Striped Marlin: Biology and Fisheries*. Bureau of Rural Sciences. Canberra, Australia 2004, 260.
- Buddhachat K, Thitaram C, Brown JL *et al.* Use of handheld X-ray fluorescence as a non-invasive method to distinguish between Asian and African elephant tusks. *Sci Rep* 2016;6:24845. <https://doi.org/10.1038/srep24845>
- Butler I, Patterson H, Bromhead D *et al.* Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra, *Fishery Status Reports*.2023
- Campana S. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar Ecol Prog Ser* 1999;188:263–97. <https://doi.org/10.3354/meps188263>
- Campana SE. Transboundary movements, unmonitored fishing mortality, and ineffective international fisheries management pose risks for pelagic sharks in the Northwest Atlantic. *Can J Fish Aquat Sci* 2016;73:1599–607. <https://doi.org/10.1139/cjfas-2015-0502>
- Clarke KR. Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol* 1993;18:117–43. <https://doi.org/10.1111/j.1442-9993.1993.tb00438.x>
- Collette BB, Carpenter KE, Polidoro BA *et al.* High value and long life—double jeopardy for tunas and billfishes. *Science* 2011;333:291–2. <https://doi.org/10.1126/science.1208730>
- Couturier LIE, Michel LN, Amaro T *et al.* State of art and best practices for fatty acid analysis in aquatic sciences. *ICES J Mar Sci*. 2020;77:2375–95. <https://doi.org/10.1093/icesjms/fsaa121>
- Cundy ME, Santana-Garcon J, McLennan AG *et al.* Seafood label quality and mislabelling rates hamper consumer choices for sustainability in Australia. *Sci Rep* 2023;13:10146. <https://doi.org/10.1038/s41598-023-37066-4>
- Davis R, Boyd C, Ahyani N *et al.* The role of species and geography in the elemental profiles of farm-raised shrimp from Indonesia. *Environ Sci Pollut Res* 2022;30:15830–41. <https://doi.org/10.1007/s11356-022-23318-9>
- NSW Department of Primary Industries. Illegal sale of blue marlin nets fishers \$11 000 in fines. *NSW Department of Primary Industries*. 2021 [https://www.dpi.nsw.gov.au/about-us/media-centre/releases/2021/illegal-sale-of-blue-marlin-nets-fishers-\\$11,000-in-fines](https://www.dpi.nsw.gov.au/about-us/media-centre/releases/2021/illegal-sale-of-blue-marlin-nets-fishers-$11,000-in-fines), Accessed 22/01/25
- Duarte B, Mamede R, Carreiras J *et al.* Harnessing the full power of chemometric-based analysis of total reflection X-ray fluorescence spectral data to boost the identification of seafood provenance and fishing areas. *Foods* 2022;11:2699. <https://doi.org/10.3390/foods11172699>
- Ducharme-Barth N, Pilling G, Hampton J. *Stock Assessment of SW Pacific Striped Marlin in the WCPO*. WCPFCSC15-2019/SA-WP-07. Western and Central Pacific Fisheries Commission .... 2019. [https://www.spc.int/DigitalLibrary/Doc/FAME/Meetings/WCPFC/SC15/SC15\\_SA\\_WP\\_07\\_SWP\\_striped\\_marlin\\_assessment.pdf](https://www.spc.int/DigitalLibrary/Doc/FAME/Meetings/WCPFC/SC15/SC15_SA_WP_07_SWP_striped_marlin_assessment.pdf) (11 March 2025, date last accessed).
- FAO. *The State of World Fisheries and Aquaculture* 2024. FAO. Rome, Italy, 2024. <https://doi.org/10.4060/cd0683en>
- Gil-Delgado A, Ibáñez-Insa J, Sellés A *et al.* Noninvasive elemental XRF characterization of mudstone lagerstätten for provenance identification: advantages and limitations. *Palaeontol Electron* 1 1–20 2025. <https://doi.org/10.26879/1415>
- Guillemin TA, Pepperell JG, Gaston T *et al.* Deciphering the trophic ecology of three marlin species using stable isotope analysis in temperate waters off southeastern Australia. *Front Mar Sci* 2022;9:1–11. <https://doi.org/10.3389/fmars.2022.795436>
- Guillemin TA, Pepperell JG, Schilling HT *et al.* 90 years of catch data reveal changes in catch composition in the Australian east coast recreational marlin fishery. *Rev Fish Biol Fish* 2025;35:371–89. <https://doi.org/10.1007/s11160-024-09906-7>
- Kalish J, Campbell R, Davis T *et al.* Assessment of black marlin and blue Marlin in the Australian fishing zone. *Report of the Black and Blue Marlin Working Group*. Department of Agriculture, Fisheries and Forestry Australia. Canberra, ACT 2002.
- McWhinnie SF. The tragedy of the commons in international fisheries: an empirical examination. *J Environ Econ Manage* 2009;57:321–33. <https://doi.org/10.1016/j.jeem.2008.07.008>
- Moradi M, Zhai Z-M, Nielsen A *et al.* Random forests for detecting weak signals and extracting physical information: a case study of magnetic navigation. *APL Machine Learn* 2024;2:016118. <https://doi.org/10.1063/5.0189564>
- Myun Park J, Powell NN, Gillings MR *et al.* Phylogeny and form in fishes: genetic and morphometric characteristics of dragonets (*Foetorepus* sp.) do not align. *Acta Zool* 2020;101:218–26. <https://doi.org/10.1111/azo.12287>
- Nganvongpanit K, Buddhachat K, Klinhom S *et al.* Determining comparative elemental profile using handheld X-ray fluorescence in humans, elephants, dogs, and dolphins: preliminary study for species identification. *Forensic Sci Int* 2016;263:101–6. <https://doi.org/10.1016/j.forsciint.2016.03.056>
- Osisanwo FY, Akinsola JET, Awodele O *et al.* Supervised machine learning algorithms: classification and comparison. *Int J Comp Trends Technol* 2017;48:128–38. <https://doi.org/10.14445/22312803/IJCTT-V48P126>
- Pardo MÁ, Jiménez E, Pérez-Villarreal B. Misdescription incidents in seafood sector. *Food Control* 2016;62:277–83. <https://doi.org/10.1016/j.foodcont.2015.10.048>
- Raoult V, Howell N, Zahra D *et al.* Localized zinc distribution in shark vertebrae suggests differential deposition during ontogeny and across vertebral structures. *PLoS One* 2018;13:1–14. <https://doi.org/10.1371/journal.pone.0190927>
- Raoult V, Peddemors VM, Zahra D *et al.* Strontium mineralization of shark vertebrae. *Sci Rep* 2016;6:1–10. <https://doi.org/10.1038/srep29698>
- Robinson ZL, Stephenson J, Vertacnik K *et al.* Efficient species identification for Pacific salmon genetic monitoring programs. *Evol Appl* 2024;17:e13680. <https://doi.org/10.1111/eva.13680>
- Silva SHG, Teixeira AFDS, Menezes MDD *et al.* Multiple linear regression and random forest to predict and map soil properties using data from portable X-ray fluorescence spectrometer (pXRF). *Ciência Agrotecnol* 2017;41:648–64. <https://doi.org/10.1590/1413-70542017416010317>
- Storch D, Šimová I, Smyčka J *et al.* Biodiversity dynamics in the Anthropocene: how human activities change equilibria of species richness. *Ecography* 2022;2022:ecog.05778. <https://doi.org/10.1111/ecog.05778>
- Teletchea F. Molecular identification methods of fish species: reassessment and possible applications. *Rev Fish Biol Fish* 2009;19:265–93. <https://doi.org/10.1007/s11160-009-9107-4>
- Walsh WA, Ito RY, Kawamoto KE *et al.* Analysis of logbook accuracy for blue marlin (*Makaira nigricans*) in the Hawaii-based longline fishery with a generalized additive model and commercial sales data. *Fish Res* 2005;75:175–92. <https://doi.org/10.1016/j.fishres.2004.11.007>
- Walther BD, Limburg KE. The use of otolith chemistry to characterize diadromous migrations. *J Fish Biol* 2012;81:796–825. <https://doi.org/10.1111/j.1095-8649.2012.03371.x>
- Williams SM, Pepperell JG, Bennett M *et al.* Misidentification of istiophorid billfishes by fisheries observers raises uncertainty over stock status. *J Fish Biol* 2018;93:415–9. <https://doi.org/10.1111/jfb.13738>