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Biodiversity science of ancient fisheries: Archaeological indicators of eelgrass meadow health and indigenous (Wiyot) aquaculture, Humboldt Bay, CA

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A B S T R A C T

Humboldt Bay, California, mirrors many coastal estuaries impacted by historical development and climate change, leading to biodiversity loss. Disintegrating habitats also affect local Indigenous communities, whose deep-rooted histories include stewardship of these biologically and culturally essential places. Understanding human-fisheries dynamics is crucial for biocultural restoration. We present a snapshot of fisheries use at *Digawututklh* (CA-HUM-23), a Late Holocene ethnographic Wiyot village on the north spit of *Wigi* (Humboldt Bay), an area experiencing the highest rate of level rise in California that it is exacerbated by the high rate of subsidence where the Juan de Fuca Plate dives under the North American Plate. To expand knowledge of species use, we employ complementary faunal and genetic analyses and test the capabilities of ancient DNA (aDNA) barcoding methods. We employ a broad-spectrum sampling approach to identify previously unidentifiable elements and confirm aDNA identification of fish bone is feasible on a wide range of non-vertebral elements and tiny, fragile fragments. Our findings highlight the potential of this methodology and the need for further sequencing of modern fish bones to refine aDNA species identification. This biodiversity-centered approach provides a more comprehensive picture of historical fisheries in this endangered northern California estuary. Contrary to a salmon-focal economy assumed in prominent developmental models, results reveal complex interactions with multiple fish species, employing diverse capture methods and technologies, such as tidal weirs, nets, and spears. Identified fish species, along with shellfish, crab, waterfowl, and mammals recovered at the site, indicate significant record of human connectivity with eelgrass (*Zostera marina*) meadows. The eelgrass biome of Humboldt Bay —a highly productive habitat that supplies nutrients, shelter, and nursery grounds for diverse species—was a key target for early indigenous aquaculture, practiced by the some of the earliest sedentary plank house villagers in coastal northwest California. The study provides insight into Wiyot sustainable practices, informed by traditional ecological knowledge of food webs and seasonal spawning and migratory patterns.

1. Introduction

Marine estuaries are among the most biodiverse and productive ecosystems on the planet. They are also among the most threatened. Estuaries—coastal bays and wetlands where freshwater meets saltwater—are unique and productive ecosystems vital for wildlife and human communities. For thousands of years, Indigenous communities of the northeast Pacific Rim have sustainably interacted with these rich aquatic habitats (Erlandson et al., 2015; Tushingham, 2023). Human impacts, especially post-Euro-American contact, have accelerated negative effects on estuaries. Fish are particularly vulnerable. Numerous species are adapted to complex, often threatened, local microhabitats associated with tidal channels and marshes, including estuarine nurseries, havens for pelagic and freshwater species during their early development (Beck et al., 2001; Moyle and Leidy, 1992; Vasconcelos et al., 2015). These issues also negatively impact Indigenous communities who have deep ancestral ties with these places. Climate change and environmental stressors are projected to amplify negative impacts,

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and while there is a pressing need for effective conservation strategies, efforts are hampered by a lack of baseline knowledge and historical records for many estuary systems (Gillanders et al., 2022). There is also a critical need to integrate Indigenous histories and Traditional Ecological Knowledge into fisheries management plans, sea level rise research and planning (Richmond et al., 2023). *Wigi* is in the heartland of the Wiyot Tribe, whose ongoing stewardship is evident in their leadership of diverse cultural and natural restoration projects (Shaughnessy et al., 2017; Richmond et al., 2023; Wiyot Tribe, 2017).

Archaeology is well-positioned to contribute to our understanding of past fisheries and their use by people over significant time scales. Signatures of fishing include diverse artifacts such as harpoons, net weights, hooks, nets and lines, and other fishing-related tools and technology, as well as facilities for capturing fish such as weirs and traps, and those associated with drying, processing, and storing fish. Ichthyofaunal remains (bones, scales) can be recovered under ideal preservation conditions, for instance at specialized fish camps and village middens. Because of the diverse life histories and characteristics of different fish, species identification of fish bone can be particularly illuminating for understanding past fishing strategies and human impacts on fisheries. For example, people may have targeted certain fish species due to their seasonal availability, nutritional content (like fat), storability, and co-occurrence with other species. Fish are also useful indicators of environmental health, and archaeological data can help build long-term records of aquatic biodiversity, fish population dynamics, and climate, first because diverse fish species can be indicators of different environmental conditions, and second because they have a major impact on the range and distribution of other organisms and hence may reflect the health and character of estuarine food webs (Moyle and Leidy, 1992; Whitfield and Elliott, 2002). Coupled with zooarchaeological records, archaeo-genomics and ancient DNA (aDNA) methods are excellent tools for documenting plant and wildlife baselines and evaluating climatic and anthropogenic impacts on biodiversity over time (Hofman et al., 2015). Advances in the genomic analysis of fish have added to our understanding of past fisheries and their use, especially where they have been wiped out or suffered major declines due to overfishing and other factors (Oosting et al., 2019; Rodrigues et al., 2018). These studies provide valuable long-term ecological data on ancient fisheries, far exceeding historical catch records. The growing fields of conservation paleobiology and applied historical ecology emphasize the potential for applying such data to inform biological conservation and management by leveraging archaeological, geological, and historical data to better understand the interplay between humans and the environment through the Anthropocene (Dietl et al., 2015; Swetnam et al., 1999). The approach can provide a powerful means for land managers, policy makers, scientists, and Tribal and local communities to develop and monitor conservation and climate change plans.

Conservation and food web restoration efforts can be informed by high-resolution records of sustainable Indigenous fisheries and past management practices. To realize the potential of archaeological data for biodiversity science, several sampling and methodological challenges must be addressed. Zooarchaeological (or faunal) analysis involving comparative morphometric analysis of fish bone has broadened our understanding of fisheries use. However, faunal identification to the species-level is often challenging because many fish families (e.g., salmon, shark, herring, and smelt) are difficult or impossible to identify to species by physical traits alone (e.g., Moss et al., 2014, 2022). Taphonomic processes often leave fish remains partially preserved, further complicating faunal assessments, which are also influenced by varying levels of analytical expertise (Hawkins et al., 2022). The ability to identify ancient fish bones via "DNA barcoding," has significantly advanced over the past two decades, broadening our capacity to identify various fishes globally. Improved methodologies have led to a notable uptick in archaeological applications—particularly in the northeast Pacific Rim. Collagen fingerprinting ("ZooMS") is also increasingly used for species identification and documenting historical fisheries baselines (Guiry et al., 2020; Harvey et al., 2022; Rick et al., 2019). However, aDNA methods currently offer a broader sequence database, making them more suitable for characterizing biodiversity and evaluating the limits of species identification. Nonetheless, several research areas remain unexplored in aDNA species identification due to sampling biases and untested methodological frontiers. Conventional sampling strategies are typically driven by initial faunal analyses and research priorities focusing on a narrow group of fish families/species. For instance, regional studies of salmonids (*Oncorhynchus* spp.), rockfish (*Sebastes* spp.), and smelts (e.g., Grier et al., 2013; Lanman et al., 2021; Moss et al., 2022; Palmer et al., 2018; Speller et al., 2012; Yang et al., 2004) has led to better aDNA identification capabilities for these fish, while others, like flatfish, remain under-researched. This creates a circular bias: as research prioritizes certain fish, aDNA barcoding analytical capabilities become more refined for these species/families, which may lead to more successful/frequent identifications of focal species. These issues are compounded by conventional aDNA sample selection, which often occurs after faunal analyses are complete and focuses on easily identifiable fish bone (e.g., vertebrae), rather than more challenging elements (spines, cranial elements, pectoral and pelvic girdles), and non-diagnostic fragments. Unidentified "miscellaneous" fish bones often dominate fish bone assemblages, representing a rich but largely untapped source of biodiversity data. A handful of studies have attempted to identify numerous fish species and elements. Hlinka et al. (2002) conducted a pilot study on aDNA identification of (mostly vertebral) fish bones, but achieved only a 2 % success rate, concluding that aDNA analysis was not yet viable. Grealy et al. (2016) used bulk sampling of fish bones to determine species presence/absence in different stratum. Natural and cultural taphonomic factors can also make some fish species archaeologically invisible if only vertebral elements are studied, for instance when butchery and discard patterns result in only non-vertebral fish parts being discarded at a site.

We target Humboldt Bay, California's second largest estuary (after San Francisco Bay), which spanned 27,000 acres in the 1850s. Development through diking, logging, draining, damming, and siltation has since reduced this habitat by two-thirds, including 90 % of its original 9000 acres of salt marshes (Barnhart et al., 1992). Habitat loss and degradation profoundly impacted food webs and local fisheries (Moyle et al., 2011). Despite these declines, Humboldt Bay remains a vital ecosystem, with the largest eelgrass habitat in California (Gilkerson and Merkel, 2017). It continues to support large and diverse wildlife populations, serves as a critical nursery for numerous fish and invertebrate species, and a key unit of the Pacific Flyway for migratory waterfowl (Barnhart et al., 1992). Modern fish surveys document the presence of numerous species in Humboldt Bay's remnant tidal marshes (Shaughnessy et al., 2017). Yet, many questions remain about their past distribution and use by people, and conservation efforts can benefit from enhanced historical documentation.

Our research addresses past fish biodiversity and Indigenous capture and consumption patterns at *Digawututklh*[1] (CA-HUM-23), an important Wiyot ethnographic village on the west shore of *Wigi* at Samoa on the north spit of the Bay and 0.67 miles east of the Pacific Ocean strand. Loud (1918) observed that the site was the largest shellmound in the region and posited that it was one of its most ancient villages based on the size of the midden and relatively stable position of the shellmound. Several ethnographies indicate that CA-HUM-23 was the location of a named dance for the *Wiki* (Humboldt Bay) Wiyot (Loud, 1918:269; See also Nomland and Kroeber, 1936:42; Supplemental Text 1). Limited excavations in 2015, radiocarbon dated to 1175 cal BP, retained depth and integrity of deposits and had excellent faunal preservation (Roscoe, 2016). In collaboration with the local Wiyot Tribal Historic Preservation Officers (THPOs) of the Wiyot Tribe, Bear River Band of Rohnerville

[1] *Digawututklh* is the Wiyot placename for CA-HUM-23 at Samoa per approval of the Wiyot Tribe's Culture Committee and Tribal Council.

Rancheria, and Blue Lake Rancheria, we developed a research plan to analyze the fish bone to expand understanding of human-fisheries interactions in Humboldt Bay. Detailed analysis of fish bones has only been conducted at one other site in Humboldt Bay, CA-HUM-321 (Tushingham et al., 2016), located two miles north on the North Spit at Manila, which focused on identifying smelt species using fine mesh screening and aDNA techniques (Palmer et al., 2018). However, similar analyses have not been extended to other fish species, despite the Wiyot having historical access to a diverse range of fish from the Bay, the Pacific Ocean, and Mad and Eel rivers, among other streams, in Wiyot ancestral territory.

This study aims to deepen our understanding of ancient fishing practices through the evaluation of DNA barcoding, refinement of faunal identifications, and the mitigation of biases in faunal analysis. Key questions include: Which species were central to Wiyot fishing approximately 1200 years ago? What insights do these findings provide about habitat use, fishing technology, and dietary traditions? How do they reflect the historic health and biodiversity of Humboldt Bay? We took a biodiversity-centered approach to reconstruct a more comprehensive picture of historical fisheries by employing a broad-spectrum sampling strategy and complimentary analytical tools, including morphological faunal and genetic analyses, to identify fish bones recovered at the site. To address the limitations of conventional faunal analysis and enhance the scope of aDNA research, we explored the efficacy of broad-spectrum sampling and aDNA techniques in identifying species represented by small, non-vertebral fish elements and small, non-diagnostic fragments and detritus. This work built on studies developed in partnership with Tribal governments in Humboldt and Del Norte Counties, CA, showing that genetic species level identification was possible on very small fish bone (even weighing less than 1–5 mg) (Palmer et al., 2018; Tushingham et al., 2019). While these studies analyzed smelt vertebrae, the present study seeks to refine analytical resolution across a broader range of species and skeletal elements.

We applied polymerase chain reaction (PCR) with "universal" primers targeting a section of the mitochondrial DNA 12S gene (Jordan et al., 2010) across different taxonomic groups and on a broad spectrum of skeletal elements and previously unidentified fish bone detritus (72 % of the 226 fish bones that were classified as "miscellaneous fish", and thus essentially archaeologically "invisible."). While notable that these primers can amplify DNA originating from both bony and cartilaginous fishes (Palmer et al., 2018), but even "universal" primers have limitations and their effectiveness across taxonomic groups is not guaranteed. For example, in recent investigations, Kemp (unpublished results) has found that the forward of this primer pair biases against amplification of this section of the mitochondrial genome in groupers (subfamily Epinephelinae), as its 3' base mismatches to the targets. With this consideration, we tested the potential of aDNA to improve diagnostic resolution by positively identifying diverse fish species and bone elements, including spines, pectoral and pelvic girdles, cranial parts, and other traditionally "non-diagnostic" fragments.

Our biodiversity-focused, broad-spectrum sampling approach is unique in several ways. First, rather than focusing a single class or family of fish, we sought to identify diverse species and elements by analyzing individual—often very small—fish bones, enabling species-specific questions and quantification of identified fish. Second, approximately half of our sample comprises morphologically unidentified "miscellaneous fish" bones (i.e., spines, cranial parts, and non-diagnostic fragments), differing from other studies that refine or confirm faunal fish bone identifications (e.g., Grier al 2013; Lanman et al., 2021). It is notable too that aDNA analysis can identify systematic misidentification by morphometric means alone (e.g., Moss et al., 2022), providing novel observation of prehistoric fishing practices. Through these efforts, we aim to advance our understanding of past estuarine ecosystems and contribute valuable data for biodiversity conservation and restoration initiatives in *Wigi* and beyond. Ultimately, the findings will enrich historical ecological records, inform conservation strategies, and support the integration of Indigenous knowledge into contemporary fisheries management.

2. Background

The Wiyot are Algic-speaking peoples whose ancestral lands encompass *Wigi* (Humboldt Bay), the Mad River, and lower Eel River. At the time of Euro-American contact, the Wiyot practiced "aquatic foraging," a unique hunting, gathering, and fishing way of life common in mid to high latitudes that involves a focus on aquatic resources, watercraft as a main mode of transport, and "collector-type" strategies, e.g., logistical procurement of seasonally available foods, sedentism, storage at a home base village (Ames, 2002; Binford, 1990; Tushingham, 2023). Wiyot villages consisted of clusters of substantial redwood plank houses, similar in form to houses to the north in the Pacific Northwest Coast, but smaller, and organized into clusters of politically independent households.

The Wiyot were "preeminently a fisher folk" (Loud, 1918:238). They captured a wide variety of fish using varied and specialized procurement strategies and technologies (Curtis, 1924; Hewes, 1947; Kroeber and Barrett, 1960; Loud, 1918) (Supplemental Text 2). Fish, along with other plant and animal resources, were processed, carefully preserved, and stored within houses. Like other Native communities in northwest California, river-caught fish were important, especially salmonids, as well as other anadromous fish including sturgeon, lamprey and eulachon. The Wiyot homelands, however, were unique and provided exceptionally biodiverse resource base: "Humboldt Bay, in possession of the Wiyot, was the only sheltered body of salt water in the region, and more kinds of sea fish were taken there, probably, than in all the remainder of the coast" (Kroeber and Barrett, 1960:5). While there is much to learn about past use of the Bay's biodiverse habitats, Dwight Simon's ground-breaking catchment analysis study of CA-HUM-351/H on the north margin of Wigi at Arcata (Simons, 1993) provides a foundational predictive framework for understanding the spatial and ecological dimensions of regional site use human-environmental dynamics.

Sedentary villages were in place throughout the region by 1300 cal BP, and the mass harvest of fish (especially smelt) and intensive shellfish procurement was practiced by that time (Palmer et al., 2018; Tushingham and Bencze, 2013; Tushingham and Christiansen, 2015; Tushingham et al., 2013, 2016, 2019; Tushingham, 2020). Salmonids often take center-stage in regional developmental models because of their economic potential and widely documented ethnographic and ongoing cultural significance (e.g., Hewes, 1947; Kroeber and Barrett, 1960). Archaeologists have increasingly grappled with "salmonopia" or "the inability to see all the food resources because of salmon" (Monks, 1987: 119). Archaeological fish bone are an important source of data, but such studies are rare in north coastal California, and systematic recovery methods, including bulk soil collection, flotation, and fine mesh screening is a relatively recent phenomenon (Tushingham and Christiansen, 2015). Fishing persists as an important part of native culture and sustenance despite major declines and extirpations.

CA-HUM-23 is associated with the Wiyot village of *Digawututklh*, the Wiyot placename adopted by the Wiyot Tribe, also reported by Loud (1918) to be named *witki* (Supplemental Text 1) , located on the narrow (10-mile long by one-mile wide) Samoa Peninsula (or North Spit) of Humboldt Bay (Fig. 1; Supplemental Fig. 1, 2). The site, known to have the highest rate of sea level rise in California at 18.6 inches per century, was situated near the tidal flats of the estuary to the east and the Pacific Ocean to the west (Russell and Griggs, 2012). The village benefited from its proximity to productive eelgrass beds and mudflats of Humboldt Bay, as well as salmonid-bearing freshwater streams and smelt spawning beds to the north. Loud (1918) reported that it was the largest shellmound in the region and hypothesized it was also one of the region's oldest (Loud, 1918). The village, inhabited until the early 1800s, was reportedly referred to as *witki* after a specific dance that was held there (Loud, 1918); Nomland and Kroeber, 1936:42; Supplemental Text 1).

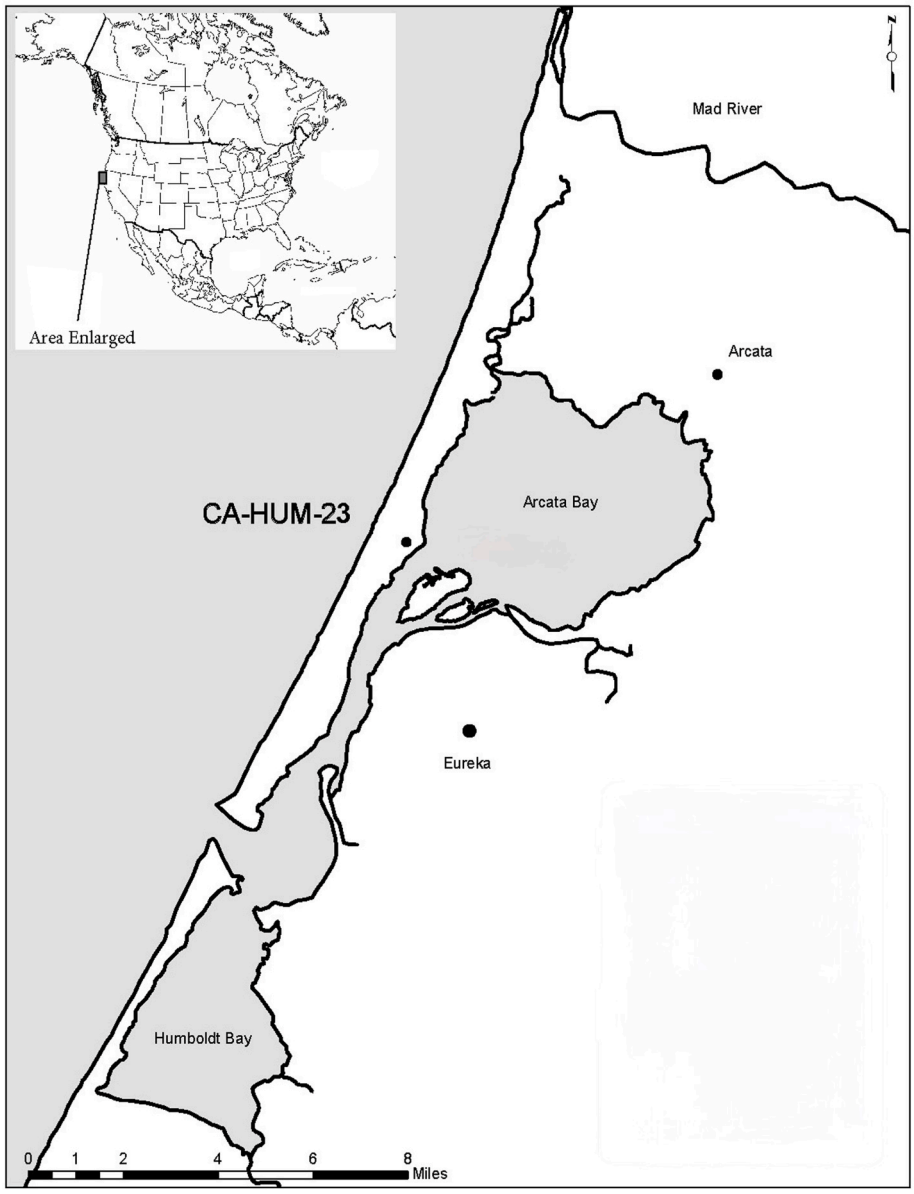

Fig. 1. Location of Site CA-HUM-23 on the north spit (Samoa Peninsula) of *Wigi* (Humboldt Bay), California.

Originally held at *Digawututklh*, the dance was later also held at CA-HUM-17, another village on the north spit of Humboldt Bay according to Jerry James, a Wiyot survivor of the *Tuluwat* (CA-HUM-67) massacre, (Roscoe, 2016:17; Nomland and Kroeber, 1936:42).

Fish bone analyzed in this study were recovered during 2015 excavations at the site as part of a cultural resource assessment for a Pacific Gas and Electric (PG&E) pole replacement project (Roscoe, 2016). In consideration of project impacts to this National Register of Historic Places (NRHP) eligible site, a mitigation plan was developed in consultation with THPOs representing the Wiyot Tribe, Bear River Band of Rohnerville Rancheria, and Blue Lake Rancheria (Roscoe, 2016:7, 19, 35; Supplemental Text 1). The plan implemented three key components: (1) initial assessment of subsurface deposits at four utility pole locations, (2) excavation of a 1 × 1 m unit in an area containing substantial intact midden deposits, and (3) systematic analysis and radiocarbon dating of recovered midden constituents, including materials recovered through flotation of bulk soil samples (Roscoe, 2016; Supplemental Table 1) (Roscoe 2016; (Supplemental Table 1).

For this study, the research team and partnering Tribes aimed to document the biodiversity and Wiyot usage of fisheries, contributing data relevant to traditional indigenous "First Foods" revitalization, fisheries conservation, and ecological restoration. Excavations at CA-HUM-23 resulted in the recovery of cultural materials dominated by lithic artifacts, shellfish, and faunal remains. Radiocarbon dated *Saxidomus* shell from the base of the unit (60–70 cmbs) yielded a conventional radiocarbon age of 1830 ± 30 RCYBP with a calibrated result (95 % probability) of 1260-1055 Cal BP and intercept of 1175 Cal BP (BETA-418909). This date, supported with source-controlled obsidian hydration analysis performed on six obsidian flakes sourced from the northern Medicine Lake Highlands (specifically the Lost Iron Wells and Grasshopper Flat locations) (Origer, 2015), indicates a late Holocene, early *Tuluwat* Pattern, for initial site occupation (Roscoe, 2016; Supplemental Text 1).

3. Materials and methods

Fish bone was separated from other field collected faunal remains that were processed and analyzed at Washington State University (WSU). The initial aDNA study was conducted at WSU (in 2015), and a second round of analyses, utilizing an additional PCR amplification

method, at the University of Oklahoma (in 2018).

3.1. Faunal assemblage and analysis

The fish bone assemblage (n = 226) was initially analyzed via a traditional skeletal analysis, identifying bones to the highest taxonomic level possible based on morphological traits. Paige Hawthorne conducted a preliminary faunal sorting and analysis of shellfish, bird, and mammal remains. The faunal fish bone analysis, reported here, was conducted by Justin Hopt, utilizing the comparative faunal collection at WSU, representative photos of bones from the Portland State University faunal collection, the virtual zooarchaeology of the arctic project website (https://vzap.iri.isu.edu/), and available written manuals (Cannon, 1987).

3.2. aDNA fish bone sample

Sample selection of 63 fish bones for the aDNA pilot study took place at Tushingham's lab, then at WSU. Prior to analysis, all specimens were photographed, weighed, and described (Fish Bone Photos in Supplemental Fig. 3). Following a biodiversity-focused, "broad-spectrum approach," and to test whether aDNA could identify small and varied fish detritus, most of the selected samples (n = 43) were previously unidentified "miscellaneous fish" elements. We analyzed 31 vertebrae and 32 other elements, including six teeth, 11 cranial bones, three pectoral bones, six spines, and six unknown fish bones, aiming to capture a more comprehensive snapshot of the fish assemblage. We included appropriately-sized specimens to test whether the absence of morphologically identified salmonids in the faunal analysis was due to the absence of easily identifiable parts. Additionally, 20 vertebrae previously identified by faunal analysis (Table 1), were sampled, including unknown species of flatfish (n = 4), greenling (n = 3), houndshark (n = 5), rockfish (n = 1), smelt (n = 3), and surfperch (n = 4). Due to time and cost constraints not all faunal-identified fish bone could be included in the aDNA study, including two cabezon, two Plainfin midshipman, and two lingcod bones. Other fish families were partially sampled (four of 14 flatfish, three of 13 greenling, one of five rockfish, four of 13 surfperch). All faunal identified houndshark and smelt were sampled (5/5 and 3/3 bones, respectively), due to tribal and research interest in these species. Once selected, the subsample was transferred to the Kemp DNA laboratory team blind to them of any faunal identification data.

3.3. Genetic Analysis—Washington State University

In 2015, initial pre-polymerase chain reaction (PCR) activities were conducted at Kemp's aDNA laboratory, then at WSU. Highly degraded DNA samples were separately housed from where PCR and post-PCR activities are conducted, and strict contamination protocols were practiced (Kemp and Smith, 2010). Approximately ≤50 mg was subsampled from each of the 63 specimens using a one-time-use razor blade. Subsamples were decontaminated by submersion in 6 % (w/v) sodium hypochlorite for 4 min (Barta et al., 2013), rinsed twice in DNA-free water, and transferred to 1.5 mL tubes.

Next, the bone samples were transferred to 1.5 mL tubes, aliquots of 500 mL of 0.5M ethylenediaminetetraacetic acid (*EDTA; pH 8.0)* were added, and tubes gently rocked at room temperature for >48 h. An extraction negative control, without bone material, was included in each extraction batch, typically at a ratio of 1:7. DNA extraction followed Kemp et al. (2014) except for using 2.5 % "resin" (2.5 % Celite in 6M guanidine HCl) instead of 2.0 % resin.

DNA eluates were evaluated for inhibitors and repeat silica extracted as per Kemp et al. (2014) until inhibition was sufficiently subdued to permit PCR amplification of the positive aDNA control. At WSU, the positive control was pooled DNA from various archaeological Aleutian Cackling Goose (*Branta hutchinsii leucopareia*) bones. Conditions for the inhibition PCRs are described by Kemp et al. (2014).

"Standard" and rescue PCR at 25 % increase (Johnson and Kemp, 2017) were conducted on inhibition-"free" eluates to amplify a 189 base pair (bp) section of the 12S rRNA gene [relative to comparative sample *Oncorhynchus mykiss* (NCBI Accession DQ288271)], yet with possible length variants, it is more conservative to describe the amplicon to be 189 ± bp in length], following Kemp et al. (2020). Primers for PCR amplification are those described by Jordan et al. (2010), with the corrected reverse orientation: "OST12S-F" (5′-GCTTAAAACCCAAAGGACTTG-3′) and "OST12S-R" (5′-CTACACCTCGACCTGACGTT-3′)]. With primers removed, this amounts to a 148 bp DNA barcode [relative to comparative sample *Oncorhynchus mykiss* (NCBI Accession DQ288271)], yet with possible length variants, it is more conservative to describe the barcode to be 148± bp in length].

Two μl of PCR products were separated and visualized on 2 % agarose gels to confirm amplification. Gels were run at 180 V for 15–20 min, stained with GelRed, and visualized under UV light excitation. All amplicons were Sanger sequenced in both directions by commercial vendors. Sequences were aligned in Sequencher v5.4.6., primer sequences removed, and the resulting electropherograms (i.e., the DNA barcodes) visually inspected for quality. Basic Local Alignment Search Tool (BLAST) search of NCBI with the blastn algorithm was used to identify the closest matches in the database.

We reported species with top percentage identities (100 % or less) and 100 % query coverage to the blasted sequence. We used on-line databases (i.e., Wikipedia and FishBase) to gather information about geographic distribution of the fishes pertinent to this study to narrow our identifications. Geographic origin aids identification, as seen with 12S sequence of Pacific herring (*C. pallasii*), which is identical to Atlantic herring (*C. harengus*). However, only Pacific herring remains are expected in pre-European contact California (Palmer et al., 2018). While ideally, each specimen would be identified to the species level (using morphology and/or genetic identification), any taxonomic refinement provides useful information about the types of fish harvested by ancestral Wiyot at CA-HUM-23. For example, differentiating between a salmon (*Oncorhynchus* spp.) and a herring (*Clupea* spp.) is valuable even if the exact species is unknown. As another example, of the 99 rockfish species with available comparative 12S sequence data (Hyde and Vetter, 2007), 13 can be identified to species with this section of the genome, while the rest would produce "rockfish-like" sequences, unidentifiable to the species level (Moss et al., 2022). All such logic was applied as we compare varied lines of evidence and reconstruct the CA-HUM-23 fish bone assemblage.

Table 1
Faunal analysis results, site CA-HUM-23.

Family/Species	Common Name	NISP[a]
Batrachoididae	Toadfish	–
Porichthys notatus	Plainfin Midshipman	2
Cottidae	Sculpin	3
Scorpaenichthys marmoratus	Cabezon	2
Embiotocidae	Surfperch	13
Hexagrammidae	Greenling	13
Ophiodon elongatus	Lingcod	2
Osmeridae	Smelt	3
Pleuronectidae	Flatfish	14
Scorpaenidae	Rockfish	5
Triakidae	Houndshark	5
Unknown	Unknown	164
TOTAL ASSEMBLAGE:		**226**

[a] NISP=Number of Identified Specimens.

3.4. Genetic analysis (application of additional PCR Applications)—University of Oklahoma

In 2018, the second round of aDNA analysis, all pre-PCR activities were conducted at the aDNA laboratory in the Laboratories of Molecular

Anthropology and Microbiome Research (LMAMR) at the University of Oklahoma. This facility specializes in processing aged, degraded, and/or low copy number aDNA samples, with strict precautions to minimize and monitor contamination.

DNA was re-extracted from subsamples of the same 63 fish remains as described under WSU methods. An inhibition test was conducted using ancient turkey (*Melleagris gallopavo*) aDNA control following Kemp et al. (2020). PCR amplification followed WSU methods except that 1:10 dilutions of the resulting aDNA eluates (pre-inhibitor removal) were included. Additionally, to standard PCR and rescue PCR, PCR enhancer cocktail-P (PEC-P; DNA Polymerase Technology) was employed as described by Kemp et al. (2020).

4. Results

4.1. Fish bone identification: morphological faunal results

Morphological faunal analysis of the CA-HUM-23 fish bone assemblage (n = 226 initially classified as "miscellaneous fish") resulted in the identification of six species: two Plainfin Midshipman (*Porichthys notatus*), two Cabezon (*Scorpaenichthys marmoratus*) and two Lingcod (*Ophiodon elongatus*) (Table 1; Supplemental Table 2). Family or genus level identifications include three sculpin (Cottidae), 13 surfperch (Embiotocidae), 13 greenling (Hexagrammidae), three smelt (Osmeridae), 14 flatfish (Pleuronectidae), five houndshark (Triakidae), and 5 rockfish (*Sebastes* spp.). The remaining 164 specimens (72.6 % of the sample) were unidentified "miscellaneous fish" (n = 164).

4.2. Fish bone identification: aDNA results

DNA was recovered from 23/63 samples (36.5 %). DNA sequences recovered in this study are present in Fast-All (FASTA) format in Supplemental Text 3). Nine of 23 positive results were replicated between WSU and OU laboratory studies. The remaining 14 positive results were observed once in one of the two laboratories, but not both (Table 2; Supplemental Table 3). Nevertheless, of the nine haplotypes observed across the 23 samples, six were shared by two or more specimens. These observations are consistent with results largely not compromised by post-mortem damage to the bases. Typically, post-mortem damage of these forms will result in an artificially derived haplotype (or longer than expected branch length) within a species, not a species misidentification (see for example the cases for rockfish sequences likely compromised by such damage; Moss et al., 2022) The three unique haplotypes are discussed in further detail below as means to evaluate this hypothesis.

Five samples were identified as likely surfperches, exhibiting four haplotypes (samples 16 and 25 share a haplotype; samples 50 and 52 share a haplotype). Sample 8 matches 100 % to white seaperch (*Phanerodon furcatus*).

Four specimens (1, 2, 13, and 14) are likely houndshark (family Triakidae), all exhibit a single haplotype that shares 99 % identity with various sharks. Species matches for smoothhound sharks (*Mustelus* spp.) mostly reside in the Atlantic Ocean. This haplotype also matches 99 % to the leopard shark *(Triaskis semifasciata*), a species of hound shark found in the Eastern Pacific Ocean, from Oregon to the Gulf of California.

Nine specimens are likely flatfish (family Pleuronectidae). The haplotype from sample 10 reveals 97 % identity to 19 species of flatfishes (i.e., various soles and flounders, one dab, one plaice, and one halibut). Samples 21, 24, 26, 30, 38, 53, 54, and 55 share a haplotype that has 100 % identity to various soles, flounders, and plaices. While it also matches to black gemfish (*Nesiarchus nasutus*), this non-flatfish resides in the tropics far from the study area.

Samples 23, 31, and 35 share a haplotype matching 100 % to jack silverside (*Atherinopsis californensis;* family Atherinopsidae). The morphological faunal identification for sample 31 was an unknown flatfish, and the other two were unknown "miscellaneous fish."

Sample 44 is likely a Pacific salmonid (genus *Oncorhynchus, family* Salmonidae**)**. While study primers used are generally useful for discriminating Pacific salmonids (Jordan et al., 2010), the sequence showed 99 % identity to various salmons and trouts. In our experience with ancient salmonid DNA, the sequence is likely chum salmon (*O. keta*) with an additional C > T transition at position 712 [relative to the full mitochondrial genome of a rainbow trout (*O. mykiss*), Genbank accession DQ288271 (Brown et al., 2006)]. However, because the observation could not be repeated, the additional mutation could be either a product of post-mortem genetic damage or it truly represents a previously unknown chum salmon mitochondrial lineage.

Finally, sample 36 is likely a Pacific herring (*Clupea pallasii*). Though also a 100 % match to Atlantic herring and European sprat (*Sprattus sprattus*), we exclude these species as they reside in the Atlantic Ocean.

4.3. Faunal and genetic fish bone analysis summary

Comparing the 63 fish bone analyzed by both methods, morphological faunal analysis identified 20/63 fish bones (31.7 %), twelve of these twenty samples were identified without corresponding genetic identification (Table 3; Supplemental Table 4). Ancient DNA analysis identified 23/63 (36.5 %), fifteen of these twenty-three samples were identified without corresponding morphological identification. In total, eight samples were identified by both methods of identification, five of which (samples 1, 2, 13, 14, and 30) matched in identification. Three samples were assessed with resulting mismatches in identification: 1) sample 8 was identified as smelt morphologically and as white seaperch with aDNA, 2) sample 31 as a flatfish via faunal analysis and a jack silverside genetically, 3) sample 44 as a greenling morphologically and as a pacific salmonid with aDNA. The origin of these mismatched identifications is unclear and would require additional analyses. However, it is notable that all three samples are of vertebrae, a typically more "diagnostic" element among fish remains.

The absence of genetic identification of Plainfin midshipman, Cottidae (Sculpin and Cabezon), Hexagrammidae (Greenling and Lingcod), Smelt, and Rockfish is of interest. However, we note that the "universal" primer set used in our study has been used successfully to identify remains of Cottidae (Irish Lord and Sculpin Cabezon), Hexagrammidae (Greenling), Smelt, and various Rockfish (Grier et al., 2013; Moss et al., 2022; Palmer et al., 2018). This suggests that inadequate DNA preservation prevented identification, not a particular bias against these taxonomic groups in the genetic laboratories.

4.4. Fish identification results by skeletal element

Our study confirms that aDNA can identify a variety of fish elements including small and fragmentary bones. Genetic analysis provided identifications for 15 previously unidentified bones: six cranial elements (including one vomer, one basioccipital, one dentary, and one quadrate element), three pectoral bones (including one pectoral girdles), three

Table 2
aDNA Analysis Results, Site CA-HUM-23.

Family/Species	Common Name	Total
Atherinopsidae	Neotropical silverside	–
Atherinopsis californensis	Jack silverside	3
Clupeidae	Herring	–
Clupea pallasii	Pacific herring	1
Embiotocidae	Surfperch	4
Phanerodon furcatus	White seaperch	1
Pleuronectidae	Flatfish	9
Salmonidae	Salmonid	–
Oncorhynchus spp.	Pacific salmonid	1
Triakidae	Houndshark	4
Unknown	Unknown	40
TOTAL SAMPLE:		**63**

Table 3
Comparative faunal and genetic species results of CA-HUM-23 fish bone sample (n = 63).

aDNA ID#	LEVEL	ELEMENT	FAUNAL SPECIES ID	GENETIC SPECIES ID	COMPARATIVE	COMBINED ID	QTY	WEIGHT
1	40–50 cm	Vertebrae	Houndshark	Houndshark	MATCH (FAUNAL = aDNA results)	Houndshark	1	0.03
2	40–50 cm	Vertebrae	Houndshark	Houndshark	MATCH (FAUNAL = aDNA results)	Houndshark	1	0.35
3	40–50 cm	Tooth	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.05
4	40–50 cm	Tooth	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.08
5	40–50 cm	Vertebrae (Atlas)	Rockfish	No DNA	FAUNAL ID only	Rockfish	1	0.19
6	40–50 cm	Spine	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.04
7	40–50 cm	Spine	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.05
8	40–50 cm	Vertebrae	Smelt	White seaperch	MISMATCH (FAUNAL ≠ aDNA results)	White seaperch	1	<0.01
9	40–50 cm	Vertebrae	Smelt	No DNA	FAUNAL ID only	Smelt	1	0.01
10	40–50 cm	Cranial (Vomer)	Unknown/Misc. Fish	Flatfish	aDNA ID only	Flatfish	1	0.02
11	40–50 cm	Vertebrae	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.18
12	40–50 cm	Vertebrae	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.28
13	50–60 cm	Vertebrae	Houndshark	Houndshark	MATCH (FAUNAL = aDNA results)	Houndshark	1	0.05
14	50–60 cm	Vertebrae	Houndshark	Houndshark	MATCH (FAUNAL = aDNA results)	Houndshark	1	0.01
15	60–70 cm	Vertebrae	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.17
16	60–70 cm	Vertebrae	Unknown/Misc. Fish	Surfperch	aDNA ID only	Surfperch	1	0.08
17	60–70 cm	Vertebrae	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.06
18	60–70 cm	Vertebrae	Houndshark	No DNA	FAUNAL ID only	Houndshark	1	0.01
19	60–70 cm	Vertebrae	Smelt	No DNA	FAUNAL ID only	Smelt	1	<0.01
20	60–70 cm	Cranial	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.33
21	60–70 cm	Cranial	Unknown/Misc. Fish	Flatfish	aDNA ID only	Flatfish	1	0.21
22	60–70 cm	Cranial	Unknown/Misc. Fish	No DNA	aDNA ID only	White seaperch	1	0.09
23	60–70 cm	Pectoral (Pectoral Girdle)	Unknown/Misc. Fish	Jack Silverside	aDNA ID only	Jack Silverside	1	0.04
24	60–70 cm	Spine	Unknown/Misc. Fish	Flatfish	aDNA ID only	Flatfish	1	0.15
25	60–70 cm	Spine	Unknown/Misc. Fish	Surfperch	aDNA ID only	Surfperch	1	0.08
26	60–70 cm	Spine	Unknown/Misc. Fish	Flatfish	aDNA ID only	Flatfish	1	0.09
27	60–70 cm	Spine	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.06
28	60–70 cm	Vertebrae	Flatfish	No DNA	FAUNAL ID only	Flatfish	1	0.1
29	60–70 cm	Vertebrae	Flatfish	No DNA	FAUNAL ID only	Flatfish	1	0.08
30	60–70 cm	Vertebrae	Flatfish	Flatfish	MATCH (FAUNAL = aDNA results)	Flatfish	1	0.07
31	60–70 cm	Vertebrae (Precaudal)	Flatfish	Jack Silverside	MISMATCH (FAUNAL ≠ aDNA results)	Jack Silverside	1	0.06
32	60–70 cm	Tooth	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.07
33	50–60 cm	Tooth	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.02
34	50–60 cm	Tooth	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.02
35	50–60 cm	Cranial (Basiooccipital)	Unknown/Misc. Fish	Jack Silverside	aDNA ID only	Jack Silverside	1	0.17
36	50–60 cm	Unknown	Unknown/Misc. Fish	Herring	aDNA ID only	Herring	1	<0.01
37	40–50 cm	Cranial	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.19
38	40–50 cm	Cranial	Unknown/Misc. Fish	Flatfish	aDNA ID only	Flatfish	1	0.35
39	40–50 cm	Cranial	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.14
40	40–50 cm	Cranial	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.14
41	40–50 cm	Unknown	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.21
42	40–50 cm	Tooth	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.1

(*continued on next page*)

Table 3 (*continued*)

aDNA ID#	LEVEL	ELEMENT	FAUNAL SPECIES ID	GENETIC SPECIES ID	COMPARATIVE	COMBINED ID	QTY	WEIGHT
43	60–70 cm	Vertebrae	Greenling	No DNA	FAUNAL ID only	Greenling	1	0.03
44	60–70 cm	Vertebrae	Greenling	Pacific salmonid	MISMATCH (FAUNAL ≠ aDNA results)	Pacific salmonid	1	0.08
45	60–70 cm	Vertebrae	Greenling	No DNA	FAUNAL ID only	Greenling	1	0.07
46	60–70 cm	Vertebrae	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.02
47	60–70 cm	Vertebrae	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.17
48	60–70 cm	Vertebrae	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	<0.01
49	60–70 cm	Unknown	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.06
50	60–70 cm	Unknown	Unknown/Misc. Fish	Surfperch	aDNA ID only	Surfperch	1	0.05
51	60–70 cm	Pectoral (Pelvic girdle)	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.01
52	50–60 cm	Cranial (Dentary)	Unknown/Misc. Fish	Surfperch	aDNA ID only	Surfperch	1	0.04
53	50–60 cm	Unknown	Unknown/Misc. Fish	Flatfish	aDNA ID only	Flatfish	1	0.16
54	50–60 cm	Cranial (Quadrate)	Unknown/Misc. Fish	Flatfish	aDNA ID only	Flatfish	1	0.21
55	50–60 cm	Unknown	Unknown/Misc. Fish	Flatfish	aDNA ID only	Flatfish	1	0.12
56	40–50 cm	Vertebrae	Surfperch	No DNA	FAUNAL ID only	Surfperch	1	<0.01
57	40–50 cm	Pectoral (Scapula)	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.04
58	40–50 cm	Vertebrae (Precaudal)	Surfperch	No DNA	FAUNAL ID only	Surfperch	1	<0.01
59	40–50 cm	Vertebrae (Precaudal)	Surfperch	No DNA	FAUNAL ID only	Surfperch	1	0.06
60	40–50 cm	Vertebrae (Precaudal)	Surfperch	No DNA	FAUNAL ID only	Surfperch	1	0.05
61	40–50 cm	Vertebrae	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.03
62	40–50 cm	Vertebrae (Atlas)	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	<0.01
63	40–50 cm	Vertebrae (Atlas)	Unknown/Misc. Fish	No DNA	ZERO IDs	Unknown	1	0.03

spines, one vertebra, and four unknown elements) (Tables 3 and 4). It also confirmed five previously identified vertebrae and corrected faunal identifications for greenling (aDNA = Pacific salmonid), flatfish (aDNA = jack silverside), and smelt (aDNA = white seaperch).

4.5. Comparison of faunal and genetic analyses (total fish bone assemblage; n = 226)

A more comprehensive understanding of the overall fish bone assemblage is provided by collating aDNA and faunal analysis results (Table 5; Fig. 2). Of the 226 bones in the faunal analysis, 164 were unidentifiable. The aDNA analysis of 63 specimens reduced the unidentifiable count to 149 (Table 3; Supplemental Table 4). It confirmed the presence of numerous fish taxa, adjusted several counts, identified several taxonomic groups for the first time (herring, jack silverside, white seaperch and Pacific salmonid) (Fig. 2).

4.6. Complementary faunal results: shellfish, bird, and mammals

In addition to fish bone, the CA-HUM-23 faunal assemblage included diverse shellfish, avian, terrestrial and marine mammal remains that provide additional clues about subsistence and local environmental interactions (Supplemental Text 1). In brief, the avian bone assemblage (n = 289) largely consists of highly fragmentary and unidentifiable bones (88 % of total bird remains). However, identifiable bird families are represented by ducks, (Anatidae; n = 12), gulls (Laridae; n = 7), hens (Rallidae; n = 1), loons (Gaviidae; n = 1), and passerines (Icteridae; n = 1), as well as size-classed remains, “Small-Medium Avian” (n = 11), and “Medium-Large Avian” (n = 1). Mammal remains (n = 151 elements) are also mostly fragmentary and unidentifiable. Identified/size classed specimens include: mule deer (*Odocoileus hemionus*; n = 6, rib fragments and long bones as well as a single antler tine, possibly a tool), harbor seal (*Phoca vitulina* spp.; n = 1, small molar), dog (*Canus familiaris*; n = 1 premolar), rodent (Rodentia; n = , likely non-cultural/invasive borrowers), small terrestrial mammal (n = 4), medium terrestrial mammal (n = 1), and micromammal (n = 5).

Table 4
Comparative results: By skeletal element, site CA-HUM-23.

Comparative Result	*Skeletal Element*						
	Tooth	Cranial	Pectoral	Spine	Vertebrae	Unknown	Grand Total
aDNA ID only	–	7	1	3	1	4	**16**
FAUNAL ID only	–	–	–	–	12	–	**12**
MATCH (FAUNAL = aDNA results)	–	–	–	–	5	–	**5**
MISMATCH (FAUNAL ≠ aDNA results)	–	–	–	–	3	–	**3**
ZERO IDs	6	4	2	3	10	2	**27**
Grand Total	**6**	**11**	**3**	**6**	**31**	**6**	**226**

Table 5
Final fish bone assemblage (combined faunal and aDNA study results).

Family/Species	Common Name	NISP
Atherinopsidae	Neotropical silverside	–
Atherinopsis californensis	Jack Silverside	3
Batrachoididae	Toadfish	–
Porichthys notatus	Plainfin Midshipman	2
Cottidae	Sculpin	3
Scorpaenichthys marmoratus	Cabezon	2
Clupeidae	Herring	1
Embiotocidae	Surfperch	16
Phanerodon furcatus	White seaperch	1
Hexagrammidae	Greenling	12
Ophiodon elongatus	Lingcod	2
Osmeridae	Smelt	2
Pleuronectidae	Flatfish	21
Salmonidae	Salmonids	–
Oncorhynchus spp.	Pacific Salmon	1
Scorpaenidae	Rockfish	5
Triakidae	Houndshark	5
TOTAL:		**76**

Unfortunately, the highly fragmentary nature of the bird and mammal assemblage in particular limits detailed taxonomic identification using traditional zooarchaeological methods. Future aDNA analysis of these materials may help improve taxonomic resolution and provide additional insights into species composition and diversity.

5. Discussion

The archaeological and genetic evidence from CA-HUM-23 reveals a complex picture of Indigenous precontact fishing practices and settlement patterns in *Wigi*. This multi-proxy investigation provides new insights into the relationship between Wiyot people and their marine environment, particularly regarding fishing strategies, resource utilization, and the role of estuarine ecosystems. These findings challenge simplistic models of coastal resource exploitation and highlight the sophisticated ecological knowledge and diverse subsistence practices of Indigenous communities.

5.1. Indigenous fishing strategies at CA-HUM-23

The assemblage from CA-HUM-23 demonstrates that the Wiyot people employed a diverse array of fishing strategies tailored to specific species and environmental conditions. This adaptability and deep ecological knowledge are evident in both the archaeological record and ethnographic accounts, which document various specialized techniques for different target species.

Contrary to a focus on salmonids or any specific fishery, study results point to complex Wiyot interactions with varied fish species and habitats, inferring diverse capture methods and technologies. Ethnographic data provide details about catchment strategies for most identified fish (see Supplemental Text 2). ***Mass harvested fish*** include anadromous **salmon**, which provided an important staple for the Wiyot and other northwestern groups, and their presence is well-documented in *Wigi* (Barnhart et al.,1992; Hewes, 1947; Kroeber and Barrett, 1960; Loud, 1918), though salmon is sparsely represented in the small study sample. Diverse and specialized catchment strategies and technologies are associated with salmon mass harvesting practices. This included such technologies as weirs in rivers and tidal sloughs (with and without basketry traps) and several types of large nets (including drag nets, plunge nets, gill nets, seine nets) (Hewes, 1947; Kroeber and Barrett, 1960). Channels in Humboldt Bay also "could be used in trawling for salmon at the time of the semiannual runs" (Loud, 1918,304).

Smelt are small forage fish that were netted in great numbers. Beach spawning smelts were captured with V-shaped nets, including **Surf Smelt** (*Hypomesus pretiosus*) and **Night Smelt** (*Spirinchus starksi*), the latter of which is smaller and spawns in the evening (Kroeber and Barrett, 1960). Surf and Night Smelt bones are abundantly represented in the shell midden site at Manila (CA-HUM-321), located on the North Spit

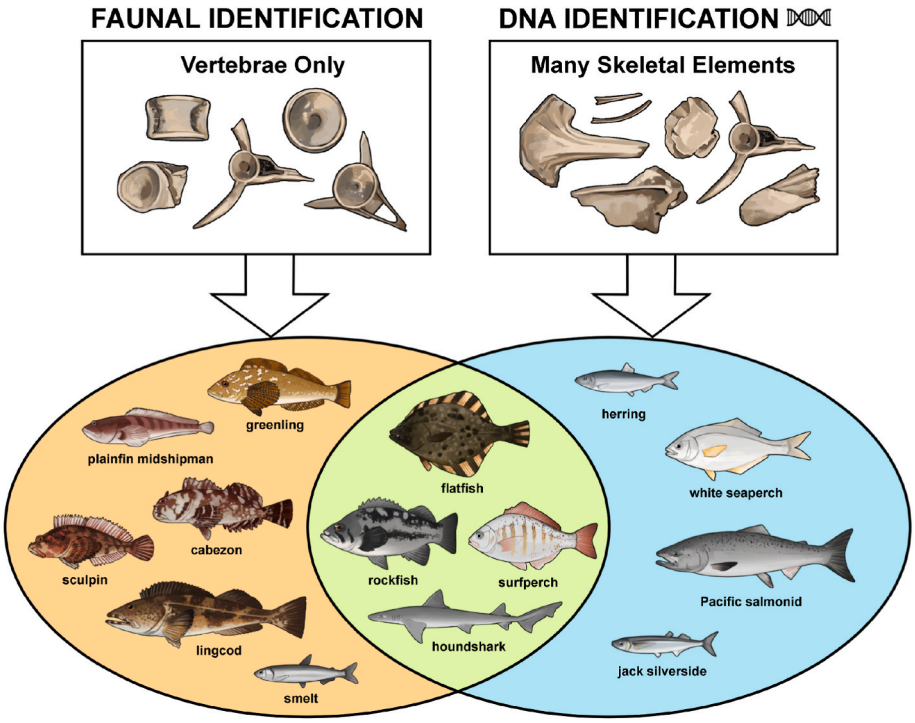

Fig. 2. Summary of biodiversity-focused study materials, methods, and results: Fourteen fish species/families were identified at CA-HUM-23. Of these, six were identified via faunal analysis of vertebrae (orange region in Venn diagram), four via aDNA of varied elements and non-diagnostic fragments (blue), and four identified with both methods (green). (Artwork courtesy of Joseph Wu.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

0.67-mile north of the *Digawututklh* at Samoa and about mid-way between *Wigi* and the Ocean, and it is C14 dated about the same time as CA-HUM-23 (Palmer et al., 2018; Tushingham et al., 2016). Smelt also include anadromous **Eulachon** (*Thaleichthys pacificus*), a very oily fish that could be targeted in both the saltwater bay or freshwater streams with plunge nets (Kroeber and Barrett, 1960). **Pacific Herring** are small schooling fish likely caught *en masse* when spawning on eelgrass (Barnhart et al., 1992; Gleason et al., 2017). The Wiyot captured herring by impounding with portable weirs in shallow waters and sloughs and scooping up in dip nets as tidewaters receded (Kroeber and Barrett, 1960).

Flatfish include species like Starry Flounder and English Sole, which were caught with hook and line and speared with both a toggle harpoon and a sharpened pole/lance (Kroeber and Barrett, 1960). **Surfperch and flatfish** were taken with small dip nets after being impounded within movable intertidal weirs in shallow waters and mud flats (Hewes, 1947; Kroeber and Barrett, 1960). **Houndshark** were historically targeted for their livers, and so abundant "during the early years of the white settlement these fish were so numerous that twenty to thirty boats, two men per boat, found it a profitable business to spear them for their oil" (Loud, 1918:281). Humboldt Bay is a known nursery for a critical population of genetically distinct Leopard Shark, *Triakis semifasciata* (Ebert and Ebert, 2005; Lewallen et al., 2007). Loud (1918) suggested that archaeological harpoon heads were fishing equipment used by the Wiyot for shark fishing.

Plainfin midshipmen are bioluminescent fish with a fascinating life cycle. Although they spend much of their lives in deep water, they move to shallow waters to breed. After females lay eggs, males guard the nests in rocky intertidal zones. While it is possible that midshipmen were procured during their deep-water intervals, it is more likely that people obtained them in a much less costly way by taking advantage of their spawning behavior. During breeding season, Plainfin midshipmen bury themselves in the sand and mud of intertidal zones, making them vulnerable to harvest, particularly with the use of tidal traps and weirs. People may have taken advantage of their nocturnal feeding habits, using torches or firelight to attract the fish, a practice used for nighttime salmon fishing. Additionally, the males guarding their nests in shallow waters could have been susceptible to being speared or hand-harvested during this time. **Lingcod** and **Cabezon** spend much of their lives in deep water environments and may have been procured in pelagic settings. However, it is much more likely that they were procured locally. Cabezon are common in deep and shallow channel tunnels of the Bay, and eelgrass meadows are lingcod and cabezon nursery habitat (Barnhart et al., 1992; Garwood and Mulligan, 2013).

5.2. Eelgrass meadows, biodiversity, and human settlement of Humboldt Bay

Notably, all identified fish inhabit *Wigi's* productive eelgrass (*Zostera marina*) meadows at some point in their life histories, for example using them as nurseries, shelter, or feeding grounds (Barnhart et al., 1992; Ebert and Ebert, 2005; Garwood and Mulligan, 2013; Gleason et al., 2017). Estuaries, often called "the nurseries of the sea," offer critical shelter and nursery habitats for numerous fish and wildlife, supporting their breeding and life cycles. Eelgrass (*Zostera* sp.), a flowering seagrass found in temperate-zone estuaries and shallow bays, plays a fundamental role in maintaining the health and biodiversity of these ecosystems. As an "ecosystem engineer," eelgrass enhances water quality, increases light availability, and stabilizes sediments (Barnhart et al., 1992; Gilkerson and Merkel, 2017). Humboldt Bay hosts the most extensive eelgrass meadows in California, which are regarded as the most significant driver of primary productivity in the Bay (Gilkerson and Merkel, 2017). Beyond benefiting plant and animal communities through shelter and nutrients, eelgrass meadows hold deep cultural and ecological importance for the Indigenous communities of Humboldt Bay.

While impossible to know exactly where and how fish were procured, much fishing was likely tied to the productive eelgrass meadows of *Wigi*, where people employed portable fish weirs, nets, and similar technologies for individual and mass capture strategies (Driver, 1939; Hewes, 1947; Kroeber and Barrett, 1960). Such strategies were much more efficient and less dangerous than pelagic fishing. The Wiyot occasionally fished for pelagic species with heavy lines and sinkers, venturing "out two or three miles beyond the line of breakers" (Kroeber and Barrett, 1960,89) However, mussels and sea lions, not fish, were typically the target of these forays to offshore rocks (Kroeber and Barrett, 1960). Wiyot settlement was near the coast but centered on *Wigi*: "Every bay settlement was on tidewater. The Wiyot thus were as 'coastal' in residence as a people could be … they used the ocean very little for either subsistence or travel" (Nomland and Kroeber, 1936:45).

In addition to fish bone data, the CA-HUM-23 and CA-HUM-321 shellfish and faunal assemblage provides further clues to estuarine habitat use and eelgrass meadow connectivity (Supplemental Text 1; Tushingham et al., 2016). The shellfish assemblage reflects an emphasis on easily accessible taxa found in mud and sand that could be harvested in intertidal flats in *Wigi* exposed at low tides near the sites, including Pacific Littleneck clam (*Protothaca staminea*), Gaper (*Tresus nuttallii*), Butter Clam (*Saxidomus giganteus*), and Basket Cockle (*Clinocardium nutallii*). Bent Nose clam (*Macoma nasuta*) is found in mudflats underlying eelgrass beds (Schlosser and Eicher, 2012). Crab (*Cancer* spp.) was also recovered from CA-HUM-23, and eelgrass beds are nursery ground for both Dungeness crab (*C. Magister*) and Rock crabs (*C. antennarius* and *C. productus*) (Barnhart et al., 1992; Schlosser and Eicher, 2012). The identified avian assemblage is consistent with species that inhabit wetland or marshy habitats, including include duck (Anatidae), Gulls (Laridae), Hens (Rallidae), Loons (Gaviidae), and Passerine (Supplemental Text 1). *Wigi* is on the Pacific flyway and is an important wintering area for these and other migrating water birds (Barnhart et al., 1992). Eelgrass beds are vital to the Pacific Flyway because they provide food for many species of birds that migrate along the Pacific Coast. Various birds eat eelgrass blades or seeds, feed on invertebrates and fish in eelgrass beds, and in some cases eat herring eggs deposited on eelgrass blades (Barnhart et al., 1992; Schlosser and Eicher, 2012). Waterfowl were commonly netted in Bay wetlands. Harbor seal was also identified, a species that was likely procured when entering the Bay through its narrow and treacherous inlet to feed on fish and invertebrates.

5.3. Aquaculture, sedentism, and Wiyot Stewardship of Wigi

Wiyot people have long utilized low-impact methods to harvest fish and other foods in ways that sustain eelgrass biome health, demonstrating a deep understanding of ecological systems and the critical balance between human needs and environmental protection. Their traditional fishing techniques, including portable fish weirs and nets, minimize ecosystem disruption through selective species capture while enabling natural habitat recovery.

These sustainable practices, particularly hand harvesting and small-scale selective fishing, help maintain healthy eelgrass meadows by avoiding damage to the plants' roots and rhizomes. This careful approach preserved the habitat's essential function as a nursery and shelter for diverse aquatic species, ensuring the ecosystem can continue supporting both wildlife and human communities. Time-tested methods support species regeneration and biodiversity, offer valuable insights for modern eelgrass restoration efforts, and demonstrate the enduring value of Traditional Ecological Knowledge.

The Wiyot Tribe continues to steward their homelands, leading diverse cultural and natural restoration projects, including critical eelgrass restoration and monitoring (Shaughnessy et al., 2017; Richmond et al., 2023). A landmark achievement was the 2004 recovery of Tuluwat (Duluwat) Island, a sacred site that underwent a massive, multi-year restoration and cleanup to remove contaminants and debris (Wiyot Tribe, 2017). Eelgrass restoration includes clearing invasive

cordgrass and debris from eelgrass beds and mudflats to facilitate native eelgrass recovery. The Tribe also partners with academic institutions and agencies to study and monitor natural resources, integrating Tribal values and knowledge to develop equitable and resilient adaptation strategies for environmental challenges like sea-level rise (Richmond et al., 2023).

More than 100 years ago, Llewellyn L. Loud proposed that CA-HUM-23 was one of the region's oldest shellmounds, based on its considerable size and location (Loud, 1918; Supplemental Text 2). To-date, archaeological data infer that CA-HUM-23 and CA-HUM-321 are the earliest known locations sedentary village life in coastal northwest California, although no house structures have yet been excavated (Tushingham et al., 2016). Both sites are strategically located on the Northern Spit of *Wigi*, with direct access to a biodiverse estuarine landscape. Their proximity to productive eelgrass meadows and expansive mud flats at low tide (Supplemental Fig. 2). provided favorable conditions for communities to thrive. Indigenous practices also sustained environmental health, with aquaculture developing in a way that nurtured and enhanced this thriving ecosystem, which in turn provided abundant resources for the community. Eelgrass meadows offered a stable, predictable food source while protecting shorelines by stabilizing sediment and reducing erosion, making these areas ideal for permanent settlement.

In addition, transport via canoe or foot provided access to extended social networks and seasonal resources, such as salmon from the Mad and Eel rivers, and smelt spawning beaches along the Pacific Coast. Maintained trails linked CA-HUM-23 to fire-managed meadows, which were inland "oases" for hunted and gathered resources, and other places in the Wiyot homelands (Loud, 1918; Kroeber and Barrett, 1960; Nomland and Kroeber, 1936). The village also held significant ceremonial importance for the Wiki (Humboldt Bay) Wiyot. It served as a starting point for visitors traveling to the island village of *Tuluwat* (CA-HUM-67), the origin place of the Wiyot people where the annual World Renewal Ceremony took place—an event central to Wiyot ceremonial life. Notably, *Digawututklh* is in clear view of Tuluwat, which is situated 0.87-mile southeast of the site.

5.4. Limitations

Several factors may limit our understanding of the full scope of Indigenous fishing practices at CA-HUM-23. First, small fish species, such as herring, surfperches, and smelt, are likely underrepresented in the assemblage due to the use of 1/8″ mesh or larger for screening during excavation (Roscoe, 2016). Second, the small sample size of this pilot study constrains broader conclusions. Additionally, natural and cultural taphonomic processes hinder the recovery of certain fish species. For instance, sturgeon and lamprey, being cartilaginous, are less likely to preserve, while salmonid bones are prone to fragmentation and decomposition. Fish butchery at special-use sites before transportation to CA-HUM-23, and off-site discard of bones after butchery at the site, may result in underrepresentation of certain species.

While aDNA analysis successfully identified some species, taxonomic resolution was limited for others. The limitations of our genetic approach means some identifications could only be made at the family level, such as flatfish, which could include various flounder or sole species. Surprisingly, both smelt and salmon species were also unidentifiable, which may be a sample size issue. However, development of primers that target addition mitochondrial DNA barcoding regions to better differentiate specific fish groups is possible (e.g., Moss et al., 2022). Once achieved, the residual DNA extracts may be reanalyzed for species-level identification of the samples.

6. Conclusion

Marine estuaries such as Humboldt Bay support biodiverse plant and animal populations, making them essential ecological and cultural landscapes. This study highlights the Wiyot's sophisticated and sustainable fishing practices, deeply rooted in their connection to marine habitats, particularly the rich eelgrass biome of Humboldt Bay. Refined species identification revealed previously undocumented fish species at CA-HUM-23 and helped to discriminate interactions with local ecosystems. Fishing focused on nearby mudflats and channels, with tidal weirs, nets, and other technologies, as well as at local beaches and rocky intertidal zones. The findings reflect nuanced understanding of fish migration and spawning patterns and illustrate the enduring human connection to California's largest remaining eelgrass meadows, a sanctuary and nursery habitat for numerous fauna.

Prior to Euro-American settlement, Wiyot aquaculture was deeply entwined with the abundant and predictable resources of the Humboldt Bay estuary. The CA-HUM-23 fish bone assemblage provides substantial evidence of human interaction with fish and other species linked to eelgrass meadows, underscoring the importance of these habitats for both sustenance and cultural practices. Our biodiversity-centered approach has revealed a 1200-year record of sustainable aquaculture, informed by the Wiyot's traditional ecological knowledge of seasonal spawning and migratory patterns. These practices were violently disrupted during the mid-1800s Gold Rush period, when the Wiyot endured brutal massacres and forced displacement from their homelands, severing access to their ancestral fishing, hunting, and gathering grounds.

Historic degradation and transformation of *Wigi* was also substantial, and there continue to be numerous anthropogenic and environmental stressors that impact eelgrass meadows and the descendant communities connected to them. Eelgrass meadows today face threats including sea level rise, ocean acidification, eelgrass wasting disease, and thermal stress (Gilkerson and Merkel, 2017). While diminished, Humboldt Bay still contains California's largest eelgrass habitat (Gilkerson and Merkel, 2017). The growing recognition of eelgrass meadows as crucial global "blue carbon" storage zones and biodiversity hotspots underscores the need for conservation efforts. Understanding the historical ecology of these estuarine ecosystems, along with the sustainable practices of the Wiyot, provides critical insights for ongoing biodiversity conservation and restoration. A truly comprehensive restoration honors both ecological health and cultural heritage (Richmond et al., 2023). As the Wiyot Tribe reconnects with their ancestral lands and waters, their ongoing leadership in projects is essential not only to restoring the *Wigi* ecosystem but also preserve and revitalize Wiyot cultural practices and knowledge (Shaughnessy et al., 2017; Richmond et al., 2023; Wiyot Tribe, 2017). The intertwined heritage of the Wiyot people and this significant estuary calls for a holistic approach to conservation, enabling both the ecosystem and the cultural traditions it sustains to flourish once more.

CRediT authorship contribution statement

Shannon Tushingham: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Justin Hopt:** Writing – original draft, Formal analysis. **Colin Christiansen:** Formal analysis. **Paige Hawthorne:** Formal analysis. **Brittany Bingham:** Formal analysis. **Erica Palmer:** Formal analysis. **Umar Sandhu:** Formal analysis. **James Roscoe:** Writing – original draft, Resources, Investigation, Funding acquisition. **Janet P. Eidsness:** Writing – review & editing. **Brian M. Kemp:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None.

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Appendix A. Supplementary data

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