SEX-SPECIFIC GROWTH AND LONGEVITY OF ‘EHU’, *Etelis carbunculus* (FAMILY LUTJANIDAE), WITHIN THE HAWAIIAN ARCHIPELAGO

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAÍI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

MARINE BIOLOGY

April 2019

By

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Keywords: Commercial Bottomfish, Age, Growth, Sexual Dimorphism, Radiocarbon dating, Longevity

ACKNOWLEDGEMENTS

I would like to thank my committee chair Dr. Erik Franklin for his support, insight and guidance throughout this process. I owe a great amount of gratitude to my committee members Dr. Edward DeMartini, Dr. Allen Andrews and Dr. Jeffrey Drazen for their mentoring, advice and paitence through the course of my research. I want to thank you all for your time, effort and the continued support.

I would like to ackowledge NOAA Advanced Studies Progam (ASP) for providing the funding for my research and NOAA PIFSC FRMD leadership, especially Dr. Christopher Boggs and Beth Lumsden for providing the matching assistanship. I thank the NOAA PIFSC LHP staff for assisting with processing the many otoliths in this study; especially, Dr. Joseph O'Malley, Dr. Brett Taylor, Robert Humphreys and Erin Reed. I am grateful for all members of the Erik Franklin Lab for assistance during my reseach.

Thanks to Dr. Corey Wakefield and Dr. Ashely Williams for teaching me the standardized techniques used for Etelinae species in Indo-Pacific. Thanks to Clay Tam who coordinated much of the fish sample delivery to my door. Thanks to the Hawaiian Archipelago commercial bottom fishing fleet, who were always willing to collect and donate samples. I am especially thankful to Captain's Layne Nakamura, Eddie Ebisui, and Uncle Roy Morioka for always looking out for potential samples!

Lastly, I thank my family for you continued support and forgiveness when field sampling, lab work and writing took me away from important family time. I am extremely grateful to have the patience, understanding and support of my wife, Tanya Nichols, who has held the family together while I focussed on school.

ABSTRACT

Across their Indo-Pacific distribution, deep-water snapper (Lutjanidae) species have supported valuable commercial, cultural and subsistence fisheries for over a century; however, species-specific information related to demographic variation in life history has been less understood in the deep-water snapper biology and a continuing shortfall. Developing an understanding of the life history of exploited species provides critical information relevant to fisheries conservation and management. In the Hawaiian Islands, the pygmy ruby snapper or ehu (*Etelis carbunculus*) is a moderately slow growing, long-lived, gonochoristic teleost, that is exploited as part of a deep-water, multi-species complex within the bottomfish fishery. Using collections over a 40-year period (1978-2018) from the main (MHI) and northwestern (NWHI) Hawaiian Islands, this study expands the data on *E. carbunculus* by examining two important components of the species life history. First, I examined length and age-specific patterns in the demography of *E. carbunculus* across the subregions of the Hawaiian Archipelago by using an analysis of otolith growth zones and age-validation from bomb-radiocarbon dating. The results indicated females grow larger and live longer than males in the NWHI. In the MHI, maximum sizes were greater in females than males, yet age structure was not different. A conspicuous age truncation, of both sexes, in the MHI compared to the NWHI suggested potential effects from fishing (from greater effort in the MHI), variability in biological traits, or differences in the marine environment between subregions. Second, I investigated sexual dimorphism of this species, using external sex identification of the urogenital region and urogenital distances. The method accurately determined the sex of 96% of 157 specimens ranging between 18.7 – 45.7 cm fork length

(FL) for females and 16.7 – 49.7 cm FL for males. Males and females significantly differed in total antero-ventral length of the urogenital region (urogenital distance, UD) and in length of the papilla septum (papilla septum distance, PSD). Although both distances were larger for females, the latter metric (PSD) was the more accruate predictor of sex, based on a logistic regression. The results herein illustrate the general importance that life history traits should not be considered similar between sexes of the deep-water snapper complex, age structure should be considered for exploited species, and the use of external sex determination can facilitate better understanding of sex-specific life history and fisheries data for species of Lutjanid snappers.

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Historical significance

Throughout the tropical and subtropical Indian and Pacific Oceans, deep-slope fisheries are utilized by small-scale artisanal, subsistence and commercial fishing endeavors supporting both domestic and export markets (Gomez et al 2015). Given the wide distribution of deep-slope fisheries and the historical exploitation of most target species within the deep-slope fisheries, it is possible that multiple nations are exploiting the same stocks (Gaither et al. 2011). With an ever-increasing demand on these resources, coupled with the potential for species-specific variations in life history information, across the species distribution, there is an increasing urgency to understand the implications of their exploitation. In general, the life history characteristics of deep-water fishery species include extended longevity, moderatly-slow growth, delayed maturity, and low resilience to fishing pressure. All of these traits should be central to fisheries management plans that include the respective life histories of target species in their stock assessments and management strategies (Norse et al. 2012; Brodziak et al. 2014). Life history information is a critical aspect to the sustainable management and conservation of these fisheries, which the disregard may be of extreme consequence to an unmanaged fishery (Morato et al. 2006; Drazen and Haedrich 2012; Newman et al. 2015).

Historically, the high values of these deep-slope fishery resources have been influenced by early Hawaiian, Polynesian and Asian cultures throughout the Indo-Pacific distributions (Dalzell 1992; Ikehara 2003). Strong local economic and cultural values have placed these fisheries in a precarious position of commerce for centuries. Many species comprise the deep-slope fishery, with primary landings being snappers (Lutjanidae), followed by groupers

(Epinephelidae) and lastly emperors (Lethrinidae) (Williams et al. 2012). The family Lutjanidae has a circumtropical and subtropical distribution and is divisible into four discrete geographical faunas: eastern Pacific, Indo-West Pacific, eastern Atlantic, and western Atlantic. The family comprises 17 genera and approximately 192 species - species richness declines with distance from the Indo-Pacific faunal center (Rivas 1970; Allen 1985; Randall 2007). A review of the snappers found in the western and central Pacific, divided the family into four subfamilies (Carpenter and Niem 2001). One of the most commercially valuable Lutjanid subfamilies is the eteline snappers, which consists of 5 genera, representing 21 snapper species found across the tropical to subtropical waters of Indo-Pacific and Atlantic (Randall 2007).

Within the Hawaiian Archipelago, deep-slope fisheries (specifically, members of Lutjanidae) have been exploited for several centuries, but detailed records only date to the early 1900s (Cobb 1902; Ralston and Polovina 1982; Watson and Morato 2013). In contrast, fishing in the wider western Central Pacific only recently (the last several decades) began focusing on seamounts, continental slopes, or other deep bathymetric features that provide habitat for these deep-water fish, with the intent to reduce exploitation of inshore reef fish populations (Dalzell and Preston 1992; Misa et al. 2013; Newman et al. 2015). Early exploitation of deep-slope resources in Hawaiʻi can be attributed to an affinity for these highly revered species by the ruling classes of the historical Kingdom of Hawai’i (Mitsuyasu 2003). Prior to European colonization, the indigenous people of Hawaiʻi had developed traditional fishing practices to harvest deep-slope fisheries, known locally as bottomfish, and many of the same techniques are still used today by commercial fishers (Moffitt 2003). Originally, it was the native Hawaiian fishermen (po’o lawai’a) who primarily fished specific fishing areas (koa) for bottomfish (Cobb 1902; Schug 2001). The Hawaiian cultural practices of the time were subsistence, and the catch was reserved for the Ali'i or royal ruling classes of Hawaiian society, until the arrival of Captain Cook and the influence of Western society.

Hawaiian deep-slope fishery

It was not until European colonization (early nineteenth century) and the development of a cash economy and commodity-based market in Hawaiʻi that a commercial exploitation of deep-slope fisheries started (Mitsuyasu 2003; Haight et al. 1993a). Records indicate that fish commodities were sold near Honolulu waterfront in 1832, with markets established on surrounding islands as well. Deep-slope fish were reported as being taken commercially during an investigation by U.S. fish and Wildlife in 1900; however, the initial commercial exploitation of the fishery began when a flux of immigrants in the late nineteenth century began targeting this fishery (Cobb 1902). Many of these immigrants were Japanese and with them came the use of traditional Japanese fishing vessels called "sampans" and their traditional methods of fishing, which increased the fishing pressure. The initial efforts for the bottomfish fishery were concentrated around the Main Hawaiian Islands (MHI), but with the advent of motor-powered vessels and the end of World War II, the range and extent of the fishing fleet increased to cover the entire Hawaiian Archipelago (Mitsuyasu 2003).

After WWII the fishery had expanded geographically, driven by a reduction in catch within the MHI, and the increased range in fishing allowed by technological improvements (Moffitt 2003). During this time, the fishers moved towards the more remote Northwestern Hawaiian Islands (NWHI), a region that is dramatically different, both geographically and geologically, from the MHI. The NWHI extend for more than 1000 miles across the tropical Pacific Ocean and consists of low islands that are unpopulated, atolls with no surface lands, and submerged banks (Fig. 1.1). The NWHI islands and bathymetric features of this subregion are geologically different from those in the MHI, due largely to an ancient progression in volcanic origin, and the NWHI is very limited in terrestrial habitat with an impoverished land fauna (Shomura 1980; Kelly et al. 2006). Conversely, the geography of the MHI consist of high elevations and considerably larger land masses. The MHI bottomfish habitat is considerably narrower, less extensive, and the fishing areas around specific features are impacted by greater fishing pressure, primarily due to the increased number of fishers and accessibility, compared to the NWHI (Parke 2007).

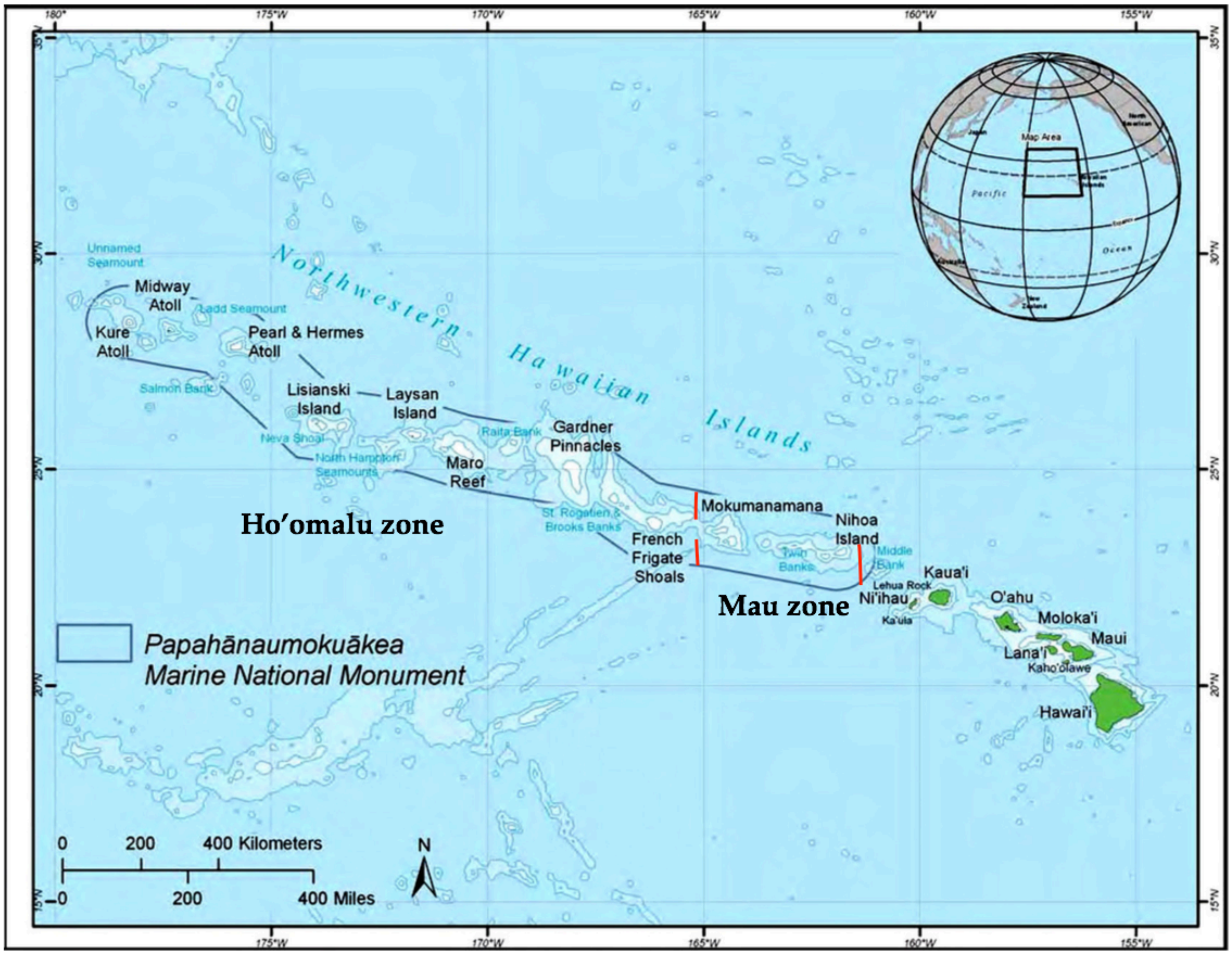

Figure 1.1. Map of Hawaiian Archipelago. Islands associated with the MHI sub-region are indicated as green colored islands. The Papahānaumokuākea Marine National Monument (PMNM) include the islands of the NWHI subregion and are encircled by a black line. The red line indicates the separation of the Mau and Ho’omalu fishing zones of the NWHI. Map courtesy of NOAA PIFSC.

With increased effort across the Hawaiian Archipelago, the cumulative total landings of deep-water snappers in the MHI and NWHI initially peaked in the 1950s at ~500t (Fig. 1.2; Haight et al. 1993a; Langseth et al. 2018). A majority of the cumulative landings during the 1950's peak consisted of eteline snappers (subfamily Etelinae). Since the 1950s, the Eteline snappers continue to be the highest proportion of catch and have a high commercial value through to present day (Brodziak et al 2011, 2014). The Eteline snappers are known to inhabit deeper shelf waters, and are typically concentrated on seamounts or continental and insular slopes (Leis and Lee 1994; Misa et al. 2013; Newman et al. 2016). Only one species of eteline snapper ("uku" grey snapper, *Aprion virescens*) commonly occurs in open-waters from near-shore to at least 100 m in Hawai'i (Randall 2007).

Management of the deep-slope fishery in Hawaii

During the late 1980s, a second peak occurred in cumulative catch and concerns over localized depletion led both State and Federal governments to consider formal management strategies (Fig. 1.2**;** WPRFMC 1986). The Fishery Management Plan (FMP) for Bottomfish and Seamount Groundfish Fisheries in the Western Pacific Region became effective on August 27, 1986 (51 FR 274132), this FMP initially focused on measures to prohibit certain destructive fishing techniques, established a moratorium on the commercial harvest of seamount groundfish stocks at the Hancock Seamounts, and implemented a permit system for bottomfish fishing in the waters of the Exclusive Economic Zone (EEZ) around the Northwestern Hawaiian Islands (NWHI). The plan also established a management framework that provided for regulatory adjustments to be made, such as catch and size limits, area or seasonal closures, fishing effort limitations and gear restrictions, access limitations, permit and/or catch reporting requirements, and a rules-related notice system (WPRFMC 1986).

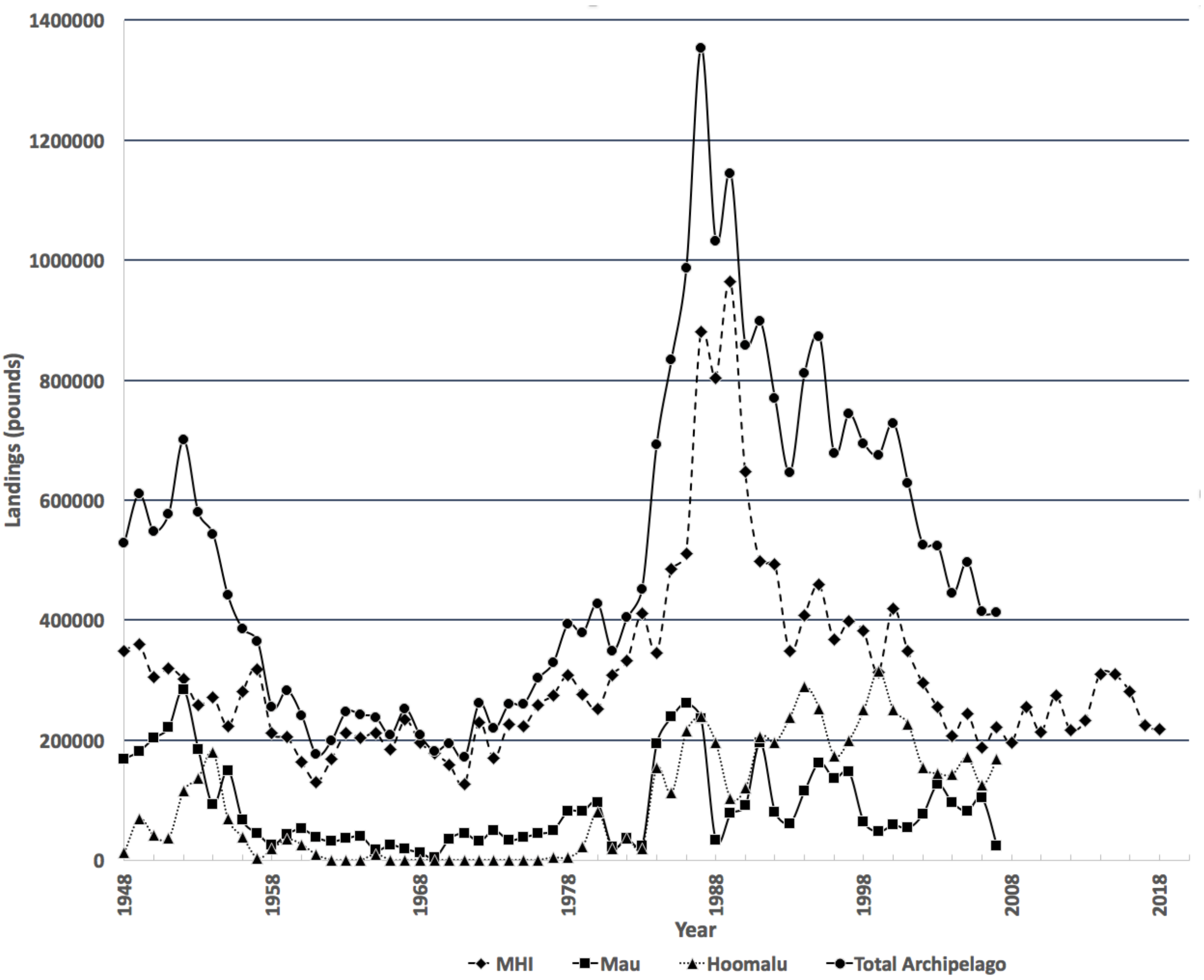

Figure 1.2. Cumulative total landings (in pounds) for BMUS by management zones from 1948-2018. The federal fisheries management regime includes three fishing zones: the main Hawaiian Islands (MHI) Zone, and two zones in the Northwestern Hawaiian Islands (NWHI), the Mau Zone and the Hoomalu Zone. These three fishing zones are reported from 1948-2008, post-2008 bottomfish fishing is only reported from the MHI zone due to the closure of the Northwestern Hawaiian Islands under Presidential Proclamation 8031. (figure adapted from Brodziak and Moffitt 2009 and Langseth et al. 2018).

Prior to 1993 the state issued Commercial Marine Licenses (CML) on a fiscal year base, expiring on June 30th, regardless of initial permit issue date. In 1993, the state established bottomfish fisheries regulations including vessel registration identification and non-commercial bag limits. Additionally, a bottomfish fishing year was federally established from September through August of the following year and permanent CML fishing licenses were issued that could be renewed. Since the 1993 implementation of the FMP, effort and landing decreased in the MHI (Fig. 1.2). The substantial declines in fishery production and total landings from the historical peak and indications that the spawning potential ratio of eteline snappers was < 20% (Moffitt 2003), led the state to initiated a series of management approaches. These approaches included the creation of 19 fishery spatial closure areas (i.e., fishery marine reserves) around the MHI (called "bottomfish restricted fishing areas" or BFRAs), establishment of a bottomfish boat registry, and introduction of a noncommercial bag limit for the Deep 7 Bottomfish species complex, a group of 6 snappers and 1 grouper species (Table 1.1; WPRFMC 2005; Hospital and Beavers 2011). Following the implementation of these approaches, fishing production stabilized; however, the following assessment on the status of Hawaiian bottomfish indicated that while the archipelagic management unit was not overfished it was experiencing overfishing (Moffitt 2003). Fishing mortality for the archipelagic bottomfish management unit was then estimated to be 24% above the overfishing threshold. As a result, management measures were crafted to reduce fishing mortality on MHI bottomfish where overfishing was occurring; these included fishery closures and total allowable commercial catch limits (TACs) (Moffitt 2003).

Management of the fishery changed again in 2007, when BRFAs in the MHI were reduced in overall numbers (from 19 to 12) but expanded, in total, the areas covered (from 838.0 Km^2 to 690.3 Km^2; Parke 2007). This determination was made following an analysis of the State's original BRFA network that found only 9.2 % of "suitable habitat" for the deep-slope bottomfish was inside the BRFAs; while the newly enacted 12 BRFAs encompass 11.2 % of bottomfish suitable habitat (Parke, 2007). One of the most critical changes in the management of the fishery, that impacted the MHI occurred in 2010, when the areas within the NWHI were closed to fishing with the creation of the Papahānaumokuākea Marine National Monument (PMNM) under Presidental Proclamation 8031. This closure of the NWHI region of the fishery meant that the exploitation of this resource would now become restricted to the most heavily populated region, the Main Hawaiian Islands. Within Hawaiian federal waters outside of three nautical miles from shore, the Western Pacific Regional Fishery Management Council continued to manage the Deep-7 bottomfish species under the Hawaiʻi Fishery Ecosystem Plan (Table 1.1; WPRFMC 2016). In Hawaii state waters from shoreline to (within three nautical miles), the State of Hawaiʻi, Department of Land and Natural Resources, Division of Aquatic Resources manages the deep-water bottomfish fishery in the Main Hawaiian Islands (MHI) under a joint management arrangement with the National Marine Fisheries Service (NMFS), Pacific Islands Regional Office (PIRO) and the Western Pacific Regional Fishery Management Council (WPRFMC).

The Deep-7 bottomfish complex has been evaluated under the Magnuson-Stevens Fisheries Conservation and Management Act (MSA) as a single archipelagic-wide multi-species stock (WPRFMC 1986, 2008). Within Hawaiʻi's MHI commercial bottomfish fishery, the major management unit, which contains the Deep-7 complex, is called a Bottomfish Management Unit Species (BMUS). In addition to the Deep-7, the BMUS consists of a complex of 14 species that include nine snappers, four jacks, and a single species of grouper. The distribution occupied by these 14 species primarily differ based on their respective shallow-water and deep-water habitats (Parke 2007; Misa et al. 2013). Eteline snappers managed as part of the Deep-7 complex species have consistently represented most of the annual reported and estimated annual unreported catches of Hawaiian bottomfish species present in records of the State of Hawai‘i Department of Aquatic Resources (HDAR; Brodziak et al. 2014; DeMartini 2016). Most of the stock assessments completed on deep-water snapper species in the Pacific Ocean (e.g., Hawai‘i, Tonga and Vanuatu) have been limited to simple production models for the entire multispecies stock, because of lack of comprehensive life history information for the species, especially size and age of catch (Martell et al. 2011; Newman et al. 2015). The most recent stock assessment on the Hawaiian Deep-7 complex, which utilized a Bayesian surplus production model fit to bottomfish catch and effort data spanning nearly 70 years, determined that the stock is not overfished and overfishing is not occurring as of 2018 (Langseth et al. 2018). Based on projections from the 2015 assessment and inclusion of recommendations from an external review, the recent assessment determined the over fishing limit (OFL) equal to 604,000 lbs. The previous 2015 annual catch limit (ACL) for deep-7 was set at 360000 lb, based on the maximum sustainable yield (MSY) estimated at 404,000 lbs and an overfishing limit at 352,000 lbs (Langseth et al. 2018). Continued decreases in effort and participation in the commercial fishery has meant that there were 496 current active CML fishers as of 2018, but many recreational catches have been historically unreported and may bias estimates of catch and effort. The current ACL and stock assessment use estimates of unreported catches (recreational and commercial) that equal the amount of reported catches (Zeller et al. 2008; Brodziak et al. 2014).

Table 1.1. The Bottomfish Management Unit Species (BMUS) complex as currently managed in the Hawaiian Archipelago. “Deep-7” indicate the major management unit of snapper species and “other Bottomfish”. The latter comprise species commonly caught in the fishery but are not targeted for commercial sale.

	Bottomfish Management Unit Species (BMUS)	
	DEEP-7 Bottomfish Species	
Scientific name	**Common name**	**Hawaiian name**
Eelis carbunculus	Pygmy ruby snapper	Ehu
Etelis coruscans	Longtail snapper	Onaga
Pristipomoides filamentosus	Pink snapper	O’Pakapaka
Pristipomoides seiboldii	Lavendar snapper	Kalekale
Pristipomoides zonatus	Banded snapper	Gindai
Aphareus rutilans	Silver jaw jobfish	Lehi
Hyporthodus quernus	Sea bass	Hapu’upu
	Other Bottomfish Species	
Pristipomoides auricilla	Yellow tail snapper	Yellowtail Kalekale
Aprion virescnes	Grey jobfish	Uku
Lutjanis kasmira	Blue stripe snapper	Ta’ape
Caranx ignoblis	Giant Trevally	White ulua
Caranx lugubris	Black Trevally	Black ulua
Pseudocaranx cheilio	Thick lipped trevally	Butuguchi
Seriola dumerili	Greater amberjack	Kahala

Although the most recent assessment indicated that fishing mortality on the Deep 7 species complex is at a sustainable level, and stocks are not overfished, the direct fishing impacts on and stock status of the individual bottomfish species are unknown. To attempt to address information on individual bottomfish species, a previous stock assessment included external reviewer’s recommendations that included length frequency information from biological sampling and inclusion of life history parameters and published length-age relationships that better approximate natural mortality and related traits for individual species (Neilson 2015). The current stock assessment included a single species stock assessment using parameters for the most numerically abundant species in the complex and highest proportion of catch, the opakapaka (*Pristipomoides filamentosus*) (Langseth et al. 2018). However, this species varies greatly in the published life history parameters from those estimated for other species within the managed complex, so may not adequately serve as an indicator for other species in the bottomfish complex (Andrews et al. 2012; Newman et al. 2015). It is further important to note that previous assessments concluded that overfishing was occurring for the entire Deep 7 species complex. The previous 2004 assessment indicated that the two species of greatest concern were "onaga" or flame snapper (*Etelis coruscans*) and pygmy ruby snapper (*Etelis carbunculus*) (Moffit et al. 2006), which constitute the second and third highest reported catches in the complex, respectively (Langseth et al. 2018).

Life history of *E. carbunculus*

One of the key Eteline snappers managed in the BMUS is *Etelis carbunculus* (family Lutjanidae, subfamily Etelinae). This species is more commonly referred to as ehu or pygmy ruby snapper due to its highly revered red color that symbolizes luck in Asian cultures and its popular use in ceremonial events (Ikehara 2003). *E. carbunculus* has been described using specimens limited to the Northwestern Hawaiian Islands (NWHI), Fiji, and Western Australia, although it has a wide Indo-Pacific distribution (Fig. 1.3; Smith and Kostlan 1991; Williams et al. 2015; Newman et al. 2016). Confounding the identification of these fish across the Indo-Pacific distributions has been the misuse of synonymous species nomenclature for *E. carbunculus*. *Etelis carbunculus* was previously considered synonymous with and often referred to as *E. marshi* (Anderson 1981; Andrews et al. 2014) but the holotypes used for the original morphological comparison to distinguish between the two species do not differ in meristic or morphometric charcteristics between them (Randall 1993). Characteristics of the specimen collected from the Central Pacific near Honolulu (USNM 50714, ca 318 mm SL, Jenkins 1903), agree closely with those of a specimen collected from Mahe, Seychelles Archipelago (MNHN 6603, 248 mm, Cuvier 1828) used during the original classification and interchangeably through the 1990's (Smith and Kostlan 1991; B. Mundy, Pacific Islands Fisheries Science Center, Honolulu, HI, personal communication, 2017). The correct nomenclature has resumed the specific designation of *E. carbunculus,* with *E. marshi* becoming a junior synonym. However, recent advances in genetic analyses has provided even more concern over potential misidentification of a cryptic species related to *E. carbunculus* within the genus.

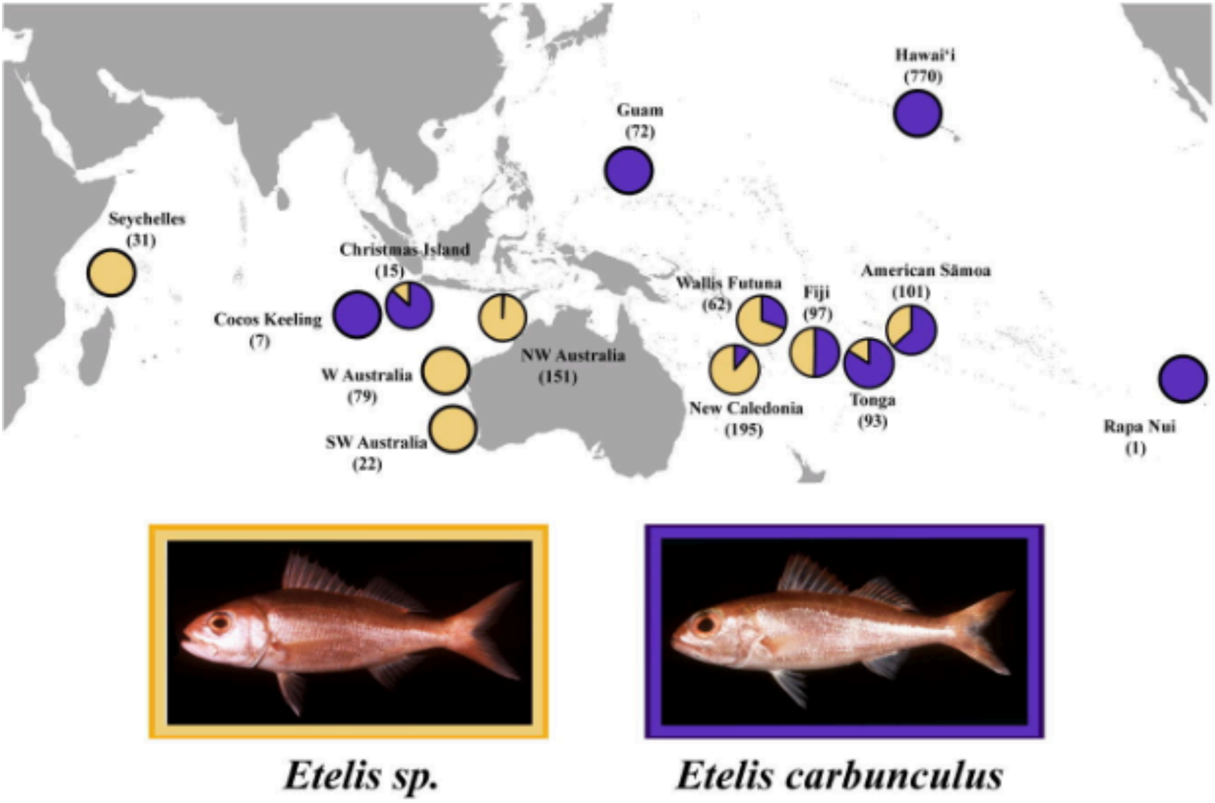

Figure 1.3. Distribution of *Etelis carbunculus* cryptic pair. Yellow and purple circles indicate confirmed collections of each of the species across the Indian and Pacific Oceans. (modified from K. Andrews et al. 2016).

Initial consideration for *E. carbunculus* comprising more than a single species was supported by the presence of significant differences in the maximum sizes achieved by *E. carbunculus* between regions of the Pacific Ocean. This large range in maximum sizes indicated either geographically structured intra-specific variation in growth patterns or the presence of different species (Smith and Kostlan 1991; Smith 1992; Williams et al. 2012). Bimodal distributions of length of the species between regions were also considered evidence for different species or inter-annual recruitment variability (Lokani et al. 1990). However, advances in genetic research, focusing on populations genetics of *E. carbunculus* collected in New Caledonia, found two distinct mitochondrial DNA (mtDNA) control region lineages with deep divergence (Loeun et al. 2013). This work sparked subsequent taxonomic and phylogenetic investigation by K. Andrews et al. (2016b), who examined the DNA sequences from mtDNA gene cytochrome b (ctyb) across the species‘ distribution range and realted this to a further comparison of morphology. The latter comprehensive study indicated that *E. carbunculus* consists of two species which, although generally similar in morphology, differ in coloration of the upper-caudal fin tip and the shape of the opercular spine. The study has provided conclusive evidence that the nominal *E. carbunculus* is composed of two species—a yet to be named species *Etelis* sp. and *E. carbunculus* (K. Andrews et al. 2016b; Newman et al. 2017). Williams et al. (2013) and Wakefield et al. (2014) found that, despite phenotypic similarity, the *E.* sp. (reported as *E. carbunculus* in previous studies) attains a much larger maximum size than *E. carbunculus* (reported as *E. marshi* in previous studies). Archived otoliths have been used to further distinguish between the two species; using canonical discriminant analysis (CDA) and simple otolith morphometry (length, width, thickness and weight), accuracy in identifying the cryptic speceis has been increased to 99.6-100% accurate (Wakefield et al. 2014).

Implications of non-standard methodologies for describing *E carbunculus*

Etelis carbunculus is a mesocarnivorous fish, that inhabits island slope waters of mesophotic depths and deeper (100-300m), and is strongly correlated with the presence of benthic structures (Misa et al. 2013). Trophic studies indicate that *E. carbunculus* occupies a higher trophic position than most other deep-water snapper species and its diet primarily consists of benthic fishes (Haight et al. 1993a; Sacket et al. 2014, 2017). Studies of *E. carbunculus* in the Hawaiian Archipelago have focused primarily on its reproductive biology, documenting that this fish is a gonochore with an active spawning season ranging from July through October (Everson 1984; DeMartini and Lau 1999; DeMartini 2016). Additional evidence, from studies elsewhere in the Pacific Ocean, indicate this protracted season corresponds to the respective latitudinal late spring and early fall (Williams et al. 2017). Spawning season timing is yet to be verified in the farthest western reaches of its Indo-Pacific distribution so it isn't known if the protracted timing is similar across the entire species range. All studies though have indicated regional differences in the maximum size observed for femles compared to males and a preponderance of females in sampled populations. Sex ratios have been female biased in all locations (~2:1 or greater; Everson 1984; Williams et al. 2017). Until recently, information on the growth and age structure of *E. carbunculus* has been lacking or highly variable, especially within the Hawaiian Archipelago.

Reported estimates of age-at-length parameters for *E. carbunculus* have varied considerably across the Indo-Pacific (Ralston and Williams 1988; Smith et al. 1991; Williams et al. 2017). And recently, age-demographic studies have increased, across the species distribution, with the confirmation of the cryptic species pair (*E. carbunculus* and *E.* sp*., K.* Andrews et al. 2016b). The variation in life history parameters submerged within with these cryptic species has been a recent focus, since studies prior to 2014 may have unknowingly incorporated samples that contained individuals from both *Etelis* species. Failure to recognize the cryptic pair may have unknowingly influenced the parameters estimates published for this species, as the maximum sizes vary considerably, presumably the life history parameters are different, and direct comparisons were confounded by confusing nomenclature used.

Additional variation in the age structure and/or growth rates reported by these studies may have been influenced by a variety of other factors besides inclusion of the cryptic species. Many studies were influenced by gaps in size classes sampled, either being unable to account for either pelagic stages, juveniles (<19cm FL), or the largest adults (>50cm FL) (Ralston et al. 1988; Newman et al. 2015). Limited sample sizes and the lack of younger age classes, may have influenced the estimation of initial growth, lowered confidence in the overall growth estimated by sex, and possibly truncated estimates of the L_∞ parameter. All the aforementioned studies used different techniques to assess ages and qualified the findings because of ineffective sectioning techniques, lack of standardized methodology, inability to validate the periodicity of annual marks, or some combination of each factor. Another confounding issue has been the potentially sex-specific growth of *Etelis*. Only two of the studies described sex-specific life history and age structure, whereas all of the studies commented on the significant differences of sex specific length frequency distributions in their respective study samples.

The lack of standardized methods to estimate life history parameters and to validate ageing criteria of eteline species has historically perpetuated the limited quantitative information available for use by fisheries management (Brodziak et al. 2014). First, the variation found in age derivations resulting from a multitude of ageing methods have been questioned primarily because of large differences in both the reported growth coefficient and maximum body length.

Both of these calculations are impacted depending on the method used to age each sample. Secondly, the standardized protocols for assessing size-at-age and validation of ageing criteria were not established for Indo-Pacific studies prior to 2015 and therefore one could not make direct comparisons across Indo Pacific regions (Williams et al. 2012; Wakefield et al. 2016). To respond to these limitations in quantitative information, there have been recent advances in the estimation of longevity (Andrews et al. 2011, 2012), the accuracy of age interpretation of bottomfish species (Wakefield et al. 2010), and in standardized techniques (Newman et al. 2015, 2016) to overcome the limitations of obtaining important life history information. However, addressing these limitations with the methodology described above only focuses on the species-population and not sex-specific variation. Advancing the data available for sex-specific life history comparisons and understanding the potential variability between the sexes would be of great benefit to fisheries managers and in data-poor regions.

Identifying the demographic differences of a species has been of interest to scientists since Darwin's work in 1858 on natural selection and increasingly of interest in marine fishes and their management (Newman et al. 2016). Oftentimes the cost (e.g., processing time, effort and financial cost per specimen) associated with determining sex from histological examination and in completing a comprehensive maturation study is prohibitive. In many data-poor regions of the Indo-Pacific, the facilities and infrastructure to conduct these studies are not presently available (Longenecker et al. 2017; Williams et al. 2017). Simplified methodologies for recognizing sexual dimorphism are rarely employed or lacking for many marine fish, including the deep-water snapper species (Smith et al. 2014). Non-lethal, macroscopic sexual identification methods using genital papillae; however, have been incorporated into some studies for marine teleosts (Vecsei et al. 2003; Smith et al. 2011). The sex of deep-water teleosts has been successfully identified with great accuracy (~95%) using these external methodologies (Luers et al. 2018). By successfully incorporating the use of genital papilla into the macroscopic identification of sex methodology, it is possible to increase the accuracy of sex determination without the added burden associated with histological preparation.

These advances increase the accuracy and precision in age and longevity estimates of bottomfish species and the sex-specific data collections increase the data available for these deep-slope species. Additionally, a broader comparison can be made with Indo-Pacific populations of *E. carbunculus*. This broader comparison will be advantageous for deep-slope fisheries in many countries and territories throughout the Indo-Pacific, which are either data-poor and/or resource-poor. Resource-poor areas will benefit from the nascent research on species-specific life history information, which may facilitate the development of more robust datasets from data-poor regions.

The primary goals of my research and this thesis therefore are as follows: 1) incorporate standardized otolith sectioning methodology and ageing protocols to evaluate bias and precision of age data for *E. carbunculus*; 2) describe the sex-specific growth and longevity characteristics of this species within the Hawaiian Archipelago; 3) explore potential subregional Hawaiian Archipelagic (MHI vs. NWHI) variation in life history traits; 4) standardize the data within the Hawaiian Archipelago to allow for direct comparison of the life history characteristics across the species range; and 5) evaluate the feasibility of an external sex identification method for individual *E. carbunculus* using genital papillae.

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CHAPTER 2: Females of the pygmy ruby snapper, ‘ehu’ (Etelis carbunculus) are more abundant, grow faster, and live longer than males in the Hawaiian Archipelago

ABSTRACT

Developing an understanding of the life history of exploited species provides critical information relevant to fisheries conservation and management. In the Hawaiian Islands, the pygmy ruby snapper or ehu (*Etelis carbunculus*) is one of seven deepwater fish species managed as a species complex within the bottomfish fishery. Using collections from the main (MHI) and northwestern (NWHI) Hawaiian Islands, this study compared the growth and longevity between sexes and geographic subregions of the pygmy ruby snapper over a 40-year period (1978-2018). Using an analysis of otolith growth zones and age-validation from bomb-radiocarbon dating, significant differences in intraspecific growth were present between sexes and geographic subregions (MHI, NWHI). The results indicated females grow larger and live longer than males in the NWHI. In the MHI, maximum sizes were greater in females than males, yet age structure was not different. A conspicuous age truncation, of both sexes, in the MHI compared to the NWHI suggested potential effects from fishing (from greater effort in the MHI), variability in biological traits, or differences in the marine environment between subregions.

INTRODUCTION

Understanding the life history attributes of exploited marine fishes is imperative to effective fisheries conservation and management. Life history traits are intrinsically vulnerable to variability in the marine environment, biology of the species, and fishery exploitation levels (Roff 1991; Rochet et al. 2000; Williams et al. 2017); however, determining the magnitude of specific effects on fish populations from these sources of variability can be difficult (Hutchinson 2002; Rouyer et al. 2014). Historically targeted on smaller scales, the deepwater snappers have increasingly been caught by commercial fisheries across the Indo-Pacific (Moffitt 2003; Williams et al. 2012; Williams et al. 2015; Newman et al. 2016). Concern over variability in biological traits and increasing exploitation levels of some deepwater snappers (i.e., Family Lutjanidae: subfamily Etelinae) has warranted re-evaluation of their life history attributes (Williams et al. 2015; Newman et al. 2016). Updated life history information for deepwater fishes can provide fisheries managers with the best scientific information available to effectively manage Indo-Pacific populations (King and McFarlane 2003; Newman et al. 2016).

Within the Hawaiian Archipelago, a complex of deepwater bottomfishes including six species of eteline snappers and one grouper has been commercially fished for over a century (Cobb 1904; Dalzell and Adams 1992; Langseth et al. 2018). One of these deepwater snappers, the pygmy ruby snapper, *Etelis carbunculus* Cuvier 1828 has been long revered for its red color, and held in great cultural and economic esteem for centuries throughout the Hawaiian Archipelago (Moffitt 2003; Williams et al. 2012). *E. carbunculus* is a medium to large mesocarnivorous fish that inhabits deeper (100-400m) seamounts, and continental and insular slopes with affinity to demersal macrohabitat features like rugose substrates and natural cavities (Misa et al. 2013; Newman et al. 2016; Oyafuso et al. 2017). It is considered a slow to modest growing, long-lived species across its Indo-Pacific distribution (Brodziak et al. 2014; Williams et al. 2017). In contrast, prior growth studies of *E. carbunculus* in the Hawaiian Islands demonstrated marked variations in maximum sizes, age structures, rates of growth, and longevities most likely due to gaps in sampled size classes and different ageing methods (Smith and Kostlan 1991; Ralston and Williams 1988; Williams et al. 2011; Williams et al. 2015; Newman et al. 2017). For the Hawaiian Islands, prior studies of *E. carbunculus* growth and age structure did not adequately address intraspecific variability nor provide reliable parameter estimates as inputs to stock assessments and fishery management decisions.

This study provides the first comprehensive and robust estimates of age, growth, and longevity for *E. carbunculus* for the Hawaiian Islands from samples collected over a 40-year period (1978-2018). We report the first bomb radiocarbon validation of periodicity in increment formation for this species in the Indo-Pacific. We derive aggregated and sex-specific life history parameters for the exploited Hawaiian stock of *E. carbunculus* and compare estimates across the two subregions of the Hawaiian Archipelago, with reference to other regions of the Indo-Pacific. The intent is that the updated life history information will be used for fisheries assessment and management of *E. carbunculus* in the Hawaiian Islands and applicable to the Indo-Pacific.

METHODS

Study area and fish sample collection

A total of 1,334 *E. carbunculus* were identified for life history analysis from archived NOAA Fisheries collections (n = 591) or opportunistically collected (n = 743) from seamounts, deep island-slope reefs, and associated hardbottom features throughout the Hawaiian Archipelago (HA), a collection of islands, atolls, and seamounts between 18 to 28º N and -155 to -176º W

(Fig 2.1). The HA has two geographic subregions: (1) the main Hawaiian Islands (MHI) from the Big Island of Hawaii to Niihau where human populations of the HA are concentrated and (2) the northwestern Hawaiian Islands (NWHI) from Nihoa to Kure Atoll. Specimens were collected from January 1977 to October 2009 in the NWHI and from January 1998 to January 2017 in the MHI using both fisheries-independent scientific surveys and fishery-dependent sources. Fish were collected using verticle droplines with multiple hooks from depths ranging between ~ 100–300 m, and most fishing occurred during daylight (0600-1930 hours). Specimens of non-exploitable sizes (< 20 cm FL) were collected within the MHI from January 2012 to December 2017, using similar droplines with smaller hooks. Fork length (FL, to nearest 0.1 cm) and round body weight (RW, to nearest 0.01 kg) of each fish was measured. The sexual identity of all fish was initially assigned based on macroscopic appearance of gonads after dissection; the sex of fish (n = 525) was confirmed by microscopic examination provided by DeMartini (2016). For collected specimens, paired sagittal otoliths (henceforth "otoliths") were removed, cleaned and stored dry in scintillation vials or paper envelopes.

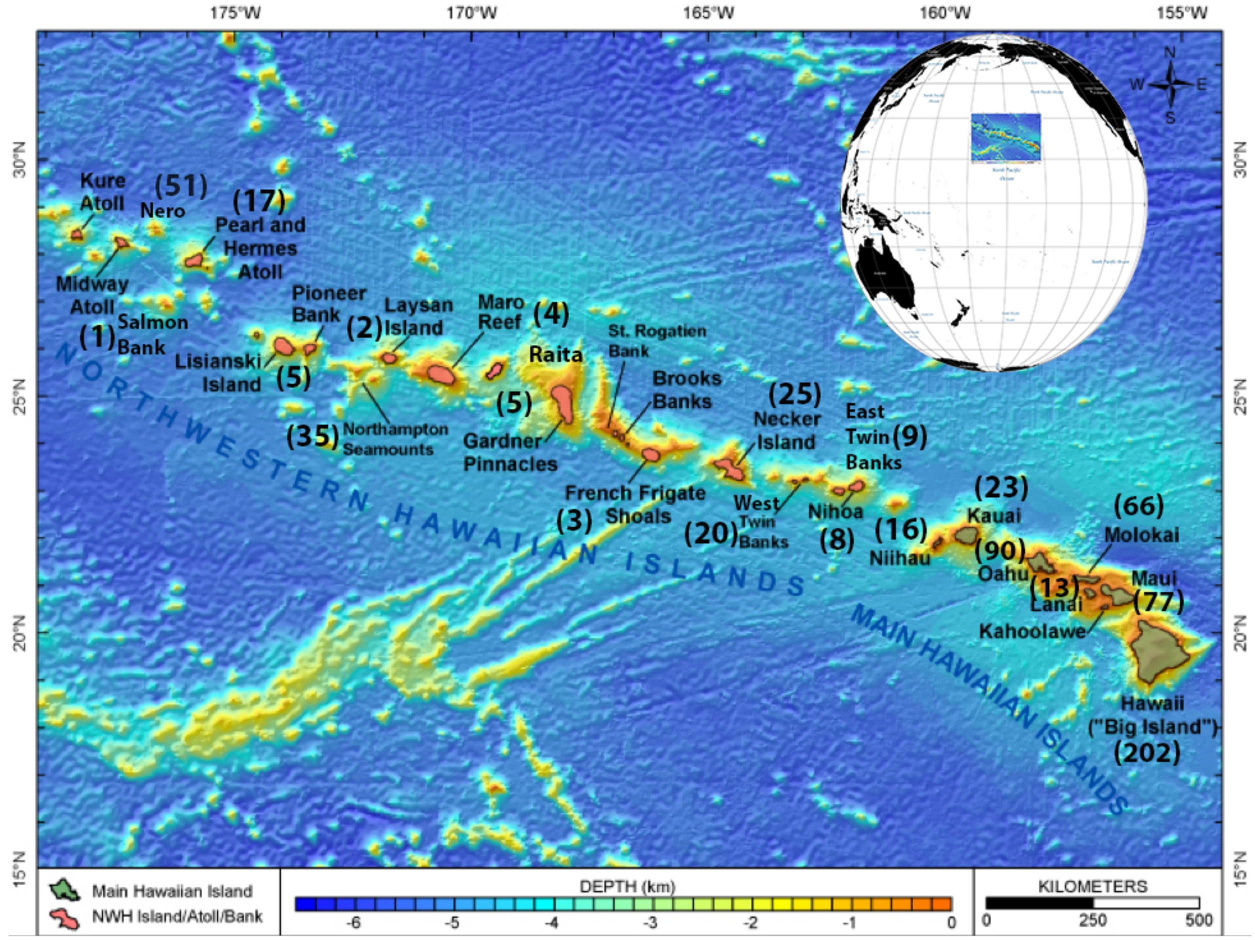

Figure 2.1. Hawaiian Archipelago and sample locations, showing the Main Hawaiian and Northwestern Hawaiian Islands and atolls. Sample locations are shown with respective sample sizes in parentheses (School of Ocean and Earth Sciences and Technology (SOEST), University of Hawaii.

Otolith morphology

A total of 702 *E. carbunculus* otolith samples were selected for analyses of otolith morphology and estimation of age from three time periods of archived and recent survey collections. The time periods and rationale for each were as follows: (1) 1977-1980 NWHI samples (*n* = 55) were used to evaluate longevity and bomb radiocarbon validation of opaque zone deposition; (2) 1998-1999, NWHI (*n* = 138) and MHI (*n* = 160) samples were used for evaluation of intra-specific growth between subregions; and (3) 2010-2017, MHI samples (*n* = 349) samples were used to determine sex specific growth. Samples were selected at random and when possible, the specimens were selected to incorporate 10 individuals per 1 cm length bin, with the focus on selecting five individuals per sex. If bins contained less than five individuals per sex (males: < 20 cm FL and > 38 cm FL; females: < 20 cm FL and > 40 cm FL), then all individuals available in the 1 cm bin were selected.

Each selected otolith was evaluated for condition, any otoliths that were chipped or broken were not used. Several morphometric measurements were obtained from unbroken otoliths with digital calipers (nearest 0.01 mm). The terminology and methods of measurements used to describe the otolith structure follow Wakefield et al. (2014). These dimensions consisted of (1) a length of the otolith from rostrum to post-rostrum, (2) the otolith height from dorsal edge to ventral edge at the widest point, and (3) the thickness from proximal to distal edge. All measurements were taken directly across the primordium (p) **(**Fig. 2.2). The dry weight (mass) of the whole right and left otolith was measured with a glass shielded calibrated balance (AND model 7120, to nearest 0.001g). Right and left otoliths from males and females were each evaluated for possible asymmetry using a paired t-test, and mean values of either right or left otoliths were then compared between sexes by simple t-test after testing for equal variances.

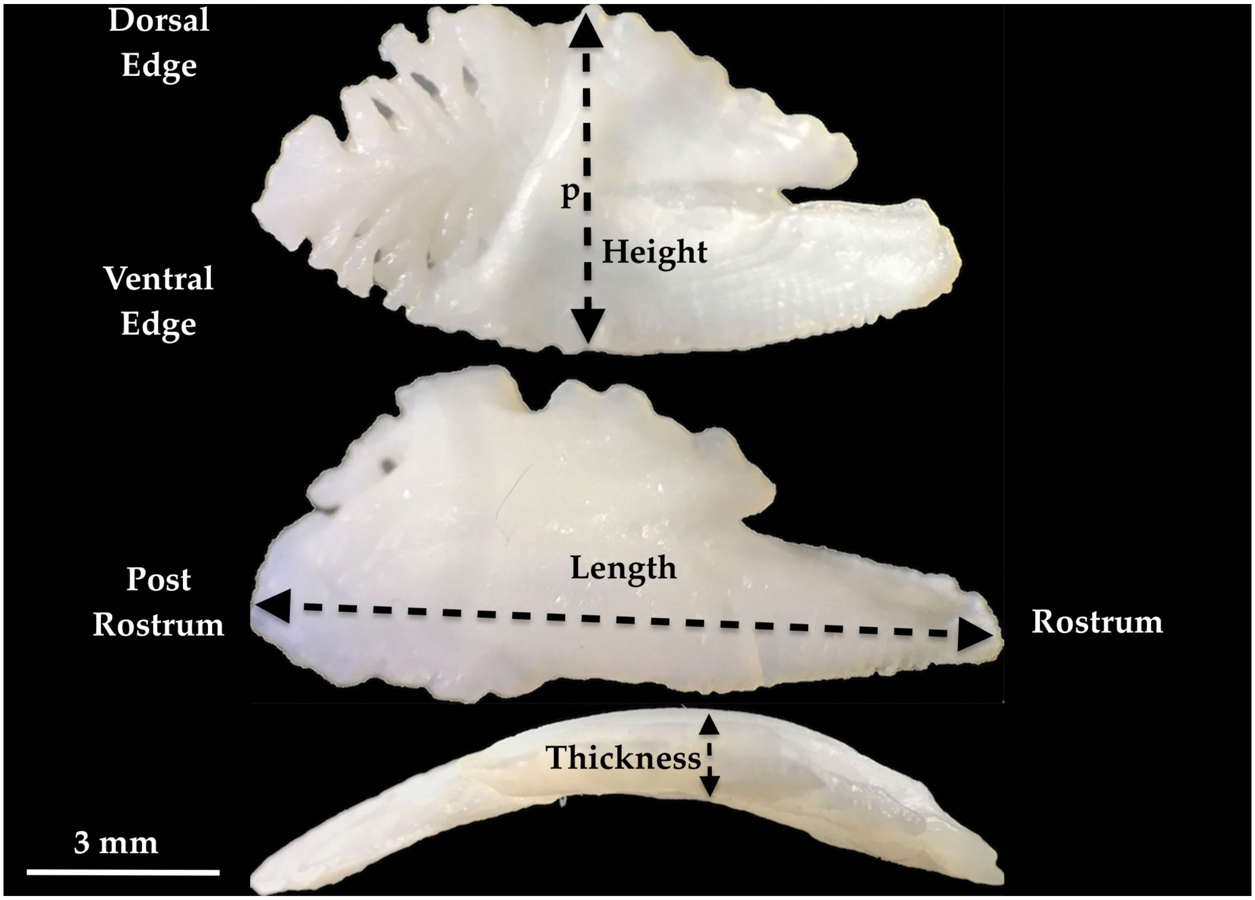

Figure 2.2. Distal (top), Medial (middle), and side profile (bottom) views of the sagittal otoliths of *E. carbunculus* (Fish length 50.7 cm FL). Black dashed lines indicate where measurements were taken for height, length, and thickness. These measurements were taken directly through the primordium (p).

Otolith sectioning

"Annuli", defined as opaque bands or zones alternating with hyaline bands and representing ages in years (Peres and Haimovici 2004), were enumerated using techniques reported previously for deep-water snapper species and, most recently, in a published ageing bias and precision study for etelines (Wakefield et al. 2013a; Wakefield et al. 2013b; Newman et al. 2017). To summarize the standardized protocols, one otolith (either right or left, randomly chosen) was examined under a dissecting scope at 50x for condition; if suitable, the sagitta was marked to identify the primordium on the medial surface along the sulcus acusticus. The primordium was used as a reference point to ensure that the thin transverse section was aligned perpendicular through the core. Next, each individual otolith was mounted in resin and transversely sectioned, perpendicular to the sulcus acusticus using a Buehler precision Isomet saw, or ground using a GEMMASTA GFL8 lapping wheel, to standard thickness (0.15-0.22 mm). Then, to improve the readability of opaque bands, the thin section was swirled in a diluted 2% HCL solution for approximately 10 seconds and placed in a distilled water bath. The HCL etching agent was used to enhance the otolith microstructure by breaking down the chemical composition of the opaque and translucent growth zones—usually the concentrated organic material (translucent zones) more so than the calcified opaque zones (Pannella 1980; Gauldie and Nelson 1990; Gauldie et al. 1990). After a section was removed from the distilled water bath and dried, it was mounted onto a glass slide with crystal bond or resin and covered with a cover slip.

Age estimation and otolith margin analysis

Each opaque band was viewed using a Leica S8 APO stereomicroscope, against a black background and under two obliquely reflecting light sources (one on each side of the microscope stage, at low angles), at 40-80x magnification. The opaque bands were counted across a consistent axis along the ventral portion of the section, from the primordium to the otolith margin. Daily growth increments (DGI) for a selection of individuals (< 18 cm FL) were examined to identify the location of the first annulus. DGIs often require higher magnification for viewing; to do this we used an Olympus BX-51 compound microscope with transmitted light source at 60 -100x magnification. DGIs were counted along a growth axis tracking the annual opaque bands. However, if section quality precluded this primary axis from being used in any reading, then other axes (i.e., the ventral side along the sulcus) were examined for growth zones.

All thin sections were read (both for DGI and annuli) by a primary reader (RSN) without reference to fish length or collection date. Three separate blind counts of each otolith were made over the course of three weeks, in order to evaluate within-reader bias. A final age was accepted when two or more annuli counts agreed; however, in the case where any three counts were in succession (i.e., 5, 6 and 7 for three respective counts), then the mean count was used. In the case of DGI, if there was no agreement among any of the three daily ring readings, then the mean age was chosen as the final age, if within 10% of the median (Taylor and Choat 2014). If the mean age was not within 10% of the median, then the specimen was excluded from analysis. During the interpretation and enumeration of opaque bands, the outer edge-type analysis of the otolith margin was examined to evaluate the periodicity of opaque formation. The margin was noted as either "0" for opaque or "1" for hyaline for all readable samples (Manickchand-Heileman and Phillips 2000).

A random selection of 160 thin transverse otolith sections was read by a second, experienced reader (BMT). Multiple methods were used to calculate between-reader precision as a guide for both readers. Specimens included otolith sections that varied in section quality and across the age distribution. Whenever possible, these included specimens of known age (by bomb-radiocarbon dating, see below) and those whose ages agreed between both readers. The following precision statistics were used to evaluate potential ageing discrepancies: Index of Average Percent Error (IAPE, Beamish and Fournier 1981), and Coefficient of Variation (CV, Campana 2001) .

An acceptable value for the IAPE is 5.5%, as established for temperate species (Campana 2001); higher IAPE values have recently been accepted for long-lived, deep-water snapper species (Newman et al. 2015). A value of 7.6% is considered acceptable for the CV (Campana 2001). Bowker's test of symmetry and Bland-Altman bias plots was used for evaluating between-reader precision using methods described in Bland and Altman (1986) and McBride (2015). The Bowker's unpooled statistic was applied to evaluate bias becasue it considers cross tabulations of ages between readers. The test is designed to detect for departures from symmetry in age distributions and is most sensitive to just a few differences (even a single pair of reader counts) once differences in counts are large enough (McBride 2015).

Bomb radiocarbon validation protocol

To evaluate longevity and provide a validation to annuli counts, methods similar to those previously described in Hawaii were used (Andrews et al. 2012; Andrews et al. 2016a; O'Malley et al. 2016). A total of 29 *E. carbunculus* otoliths were analyzed for ^{14}C in the extracted core (birth year) samples and used to provide validation of the age reading criteria and age estimates. Otoliths were randomly selected from the archived specimens to include both sexes (11 Female, 12 Male, and 6 Unknown sex), and incorporated several age-at-length scenarios. All but one sample was collected between 1977-1980, making the assumption that age estimates were accurate and that these specimens would have been born during the most diagnostic portion of the reference curve, the bomb ^{14}C rise period (~1958-1969; Kalish 1993). The one additional MHI sample was selected from the largest individual collected in 2017 (71 cm FL). The length of specimens used ranged from 26.0 cm to 71.0 cm. The extracted otolith core material was consistent and ranged from 0.9 to 1.2 mg (mean =1.05 mg, SD = 0.11).

To apply the bomb ^{14}C dating methods, whole otoliths were prepared using the following series of steps. Whole otoliths were cleaned with deionized water and a detergent, oven dried overnight, and then weighed (± 0.1 mg) prior to the mounting preparation. The delineation of the otolith target core area for the first year's growth was isolated using information on the dimensions and masses of juvenile otoliths, along with observations of visible opaque regions within the core of each adult otolith specimen. Guidance on locating the target core area of the otolith was from the otolith dimensions of a 11.3 cm FL juvenile specimen: 4.9 mm length, 2.8 mm wide, 0.6 mm thick and weighed 1.01 mg. Using a fine tipped, high speed drill (New Wave Research micromilling machine, ESI-NWR Division, Freemont, CA, and 300 µm bur) the core area was extracted in two successive and overlapping scans at a depth of 130 µm for each pass. Extracted core mass was 1–2 mg.

The extracted $CaCO_3$ core material was submitted to National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at Woods Hole Oceanographic Institute, where routine radiocarbon analyses using accelerated mass spectrometry was conducted on the calcium carbonate. The resulting ^{14}C data were reported as the fractional modern, the measured deviation of the $^{14}C/^{12}C$ ratio from the "modern" sample (e.g., Andrews et al. 2016a). Results were normalized to −25‰ for fractionation correction using a reported $\delta^{13}C$ value and is herein reported as $F^{14}C$ (Reimer et al. 2004).

Existing reference $F^{14}C$ records from the Hawaiian Archipelago (see Andrews et al. 2016a, Kure Atoll and Kailua-Kona, Hawaii Island coral ^{14}C reference record) were selected for bomb ^{14}C dating in this study because these records are from the geographic extreme of the Hawaiian Archipelago and spans the entire bomb ^{14}C chronology (1940s – 2000s). The Kure Atoll coral reference record was accessed through the ^{14}Chrono at Centre for Climate, the

Environment, and Chronology (Queen's University Belfast, United Kingdom) using their CALIBomb interface and analysis routine (http://calib.org/CALIBomb/). The CALIBomb interface provided the opportunity to statistically analyze otolith core $F^{14}C$ results in a moving-average probability fit to the coral reference $F^{14}C$ records, similar to the approach used by Reimer et al. (2004). This study took advantage of the well-defined peak (~1970 to 1980) evident in the $F^{14}C$ Kure coral reference records, to not only utilize the rise period (~1955 to 1970), but also the decline period (after ~ 1980), to accurately apply bomb ^{14}C dating to fish specimens. The Kailua-Kona coral reference record was used to identify and assess variations in the ^{14}C peak and decline periods. The range of dates determined by the CALIBomb analysis were established by projecting the measured $F^{14}C$ values, with a corresponding uncertainty (1–2 standard deviations, SD), through time for an alignment with the $F^{14}C$ reference (Reimer et al. 2004). In addition, a smoothing function, used to compensate for the growth period of the otolith core extraction (~ 0.5 years), was integrated into the CALIBomb analysis routine and tends to average out high-resolution variations in the $F^{14}C$ reference record. The years of formation generated by this analysis routine are based on the probability fits and a subsection of these years focusing on 95% of the primary date range determined from 2 SD (i.e. improbably tailing effects were removed).

Once each target core was removed from its respective otolith, the remaining portion of the whole otolith was sectioned using the grinding technique of Taylor and McIlwain (2010) to prepare for annuli counting. Bomb ^{14}C ages from CALIBomb alignment dates were compared to annuli counts from each respective otolith and any systematic bias was evaluated using age bias plots — the two ages (bomb ^{14}C age and annuli count) were evaluated with the Bowkers test of symmetry (Evans and Hoenig 1998; Kimura et al. 2007).

Size, age, and sex metrics

The length and weight of *E. carbunculus* collected were examined for variations in the fit, slope, and intercept of the length-weight relationship. A the two-parameter power function model was used; $W = aL^b$, where W is whole body weight in kilograms and L is the fork length measured in centimeters. A linearized double-log transformed length-weight model $log(W_i) = log(a) + blog(L_i) + \epsilon_i$, was also examined where *W is* whole body weight, *a* is the coefficient of the power function (intercept), and *b* is the exponent (slope). Comparisons of the linearized length-weight relationship parameters between subregions and between sexes used analysis of covariance (ANCOVA) to test differences in slopes and intercepts. Growth (isometric or allometric) was evaluated using the transformed length-weight model and t-tests were used to evaluate whether slopes differed between subregions and between sexes. The linear model was used to test whether the slope (exponent) parameter was equal to a specific value other than zero (i.e., 3 for isometric growth, Ogle 2017). Kolmogorov-Smirnov (K-S) tests were used to check normality of the length-frequency distributions. Potential outliers were identified using R-studentized residuals and Bonferroni test and were removed, if perturbation of model fit was indicated in post-hoc analysis. The length and age frequency distributions were compared between NWHI and MHI subregions and between the sexes in each subregion using analysis of variance (ANOVA). Sex ratios were evaluated using chi-square goodness of fit to test if the ratio varied from an expected 1:1 ratio.

Von Bertalanffy growth function

A combination of otolith features and fish metrics were used to derive the decimal ages of specimens older than a year. The decimal age incorporated the timing of the annual mark, the birth date of the specimen (measured as the peak of the spawning period), the total enumeration of annual marks, and the capture date. The following formula was used to calculate the decimal age (Taylor et al. 2016):

$$Decimal\ age = if \begin{bmatrix} M_{capture} < M_{deposition}, OZ + \dfrac{M_{capture} + M_{12-birth}}{12} + \dfrac{D_{capture}}{365.25}, \\ M_{capture} \geq M_{deposition}, OZ + \dfrac{M_{capture} + M_{birth}}{12} + \dfrac{D_{capture}}{365.25} \end{bmatrix}$$

where

$M_{capture}$ = month of capture;

$M_{deposition}$ = month of annulus deposition;

OZ = total number of opaque zones interpreted as age;

M_{birth} = month of birth; and

$D_{capture}$ = day of capture within the calendar month.

For simplicity, growth was modeled using either a standard (three parameter) von Bertalanffy growth function (VBGF; von Bertalanffy 1938; Haddon 2011) or a two-parameter version of the VBGF, with t_0 constrained to zero:

$$L_t = L_\infty(1 - e^{-k(t-t_0)}); \qquad (1)$$

$$L_t = L_\infty(1 - e^{-K(t)})\ (2)$$

where

$\boldsymbol{L_t}$= mean fork length (FL, in cm) at age *t;*

$\boldsymbol{L_\infty}$= asymptotic length;

$\boldsymbol{t}$ = a specific age in year;

$\boldsymbol{t_0}$ = hypothetical age at length zero; and

$\boldsymbol{k}$ = growth coefficient.

The von Bertalanffy equation is assumed to adequately describe growth when $t_0 = 0$, and assumes that the pattern of growth remains the same throughout all early life stages. However, in practice, the constant t_0 is extrapolated and must be regarded as largely a modeling artifact and not a biological parameter (Beverton and Holt 1957; Schnute and Fournier 2011).

These parameters were estimated using non-linear least square regression, and an analysis of residual sum of squares (ARSS) was used to determine if the fitted curves differed between regions and between sexes (Haddon 2011). The F –statistic was calculated using Francis (1990):

$$F = \frac{(RSS_p - RSS_s)/(3 * \ (k-1))}{RSS_s/(N - 3 * k)}$$

where

RSS_p = the residual sums of square (RSS) of the VBGF fitted by pooled data (samples from both regions pooled);

RSS_s = the sum of the RSS of each VBGF fitted to each population;

k = the number of curves in the comparison; and

N = the total sample size.

The calculated F value was then compared with the critical F, with $3 * (k-1)$ and $N - 3 * k$, degrees of freedom for the numerator and denominator, respectively (Haddon 2011).

Statistical Analyses

All of the above statistical analyses were performed in R version 3.1.1 (R Core Team 2016) and R studio version 1.3.05 (Rstudio, Inc 2015). The packages and functions used in this software environment were as follows: nlstools, stats, FSA, fishmethods, car, psych, and graphics were created using ggplot2.

RESULTS

Sample fish and length composition

Females were most prominent in collections from both subregions (Fig. 2.3), with populations being significantly females biased in the MHI (1:2.54; $x^2 = 109.16$; $p < 0.001$) and in the NWHI (1:2.6; $x^2 = 39.92$; $p < 0.001$, Appendix A1). The size distributions of *E. carbunculus* for the MHI specimens ranged from 11.3 – 71.0 cm FL ($n = 793$), whereas those from the NWHI ranged from 20.8 – 65.0 cm FL ($n = 541$). Specifically, length frequency distributions differed between sexes for the regions pooled (K-S test; D = 0.158, $p < 0.001$), between subregions for the sexes pooled (K-S test; D = 0.488, $p < 0.001$), between sexes by subregion (MHI Female-Male: K-S test; D = 0.111, $p < 0.001$; NWHI Female-Male: K-S test; D = 0.237, $p < 0.001$) and between subregions by sex (Female MHI-NWHI: Female- K-S test; D = 0.501, $p < 0.001$; Male MHI-NWHI: K-S test; D = 0.266, $p < 0.001$). Mean length also varied between sexes and subregions, with females larger than males in both subregions and fish in the NWHI larger than those in the MHI (Fig. 2.4, Table 2.1). Females attained a larger maximum size compared to males in both regions (71.0 vs. 50.7 cm FL MHI, 65.8 vs. 52.1 cm FL NWHI); no males larger than 53 cm FL were collected from either region.

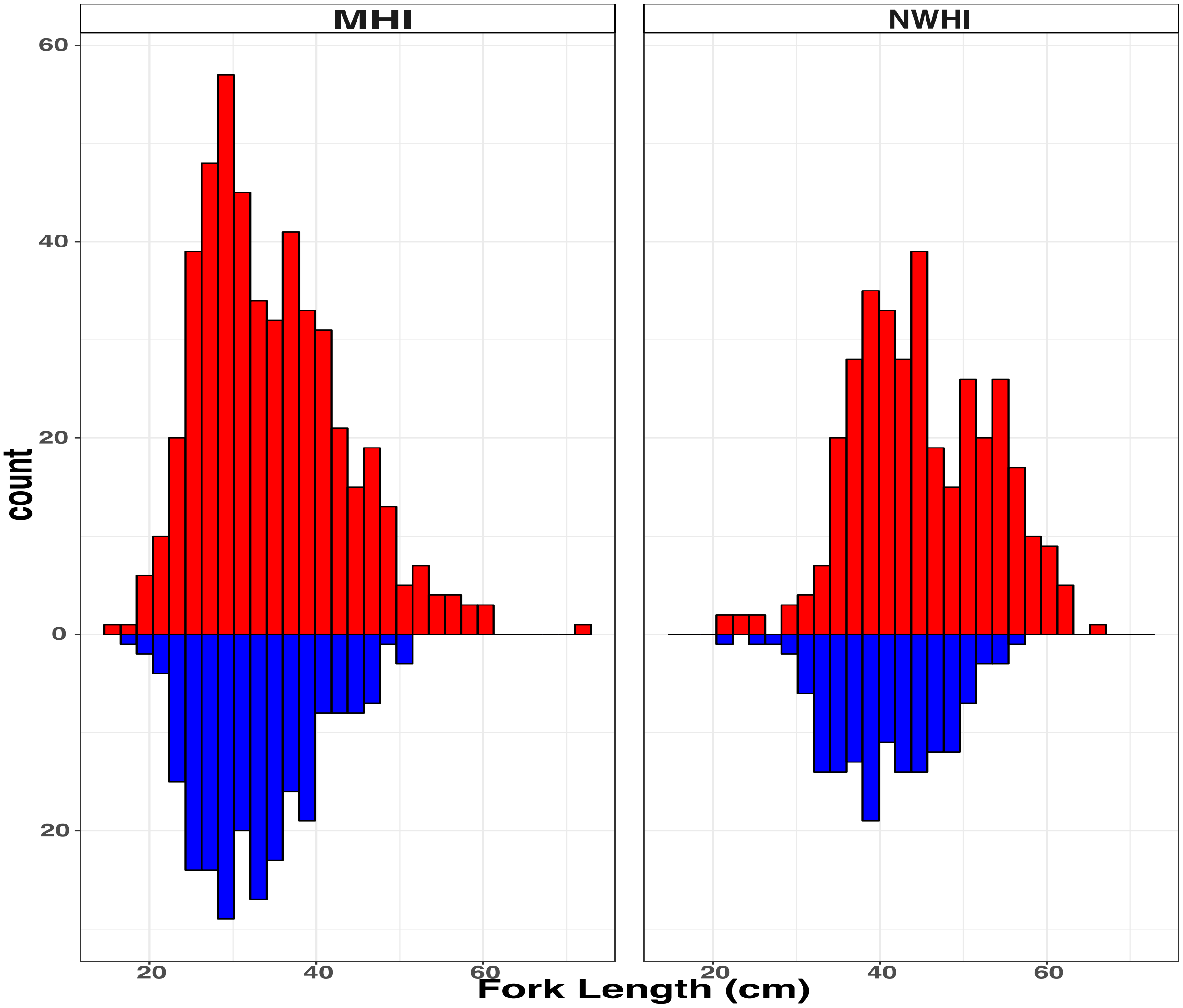

Figure 2.3. Sex-specific length frequency distribution of *E. carbunculus* collected from the Hawaii Archipelago (MHI on right, NWHI on left). The distribution of females is plotted above the line and indicated in red, males are below and indicated in blue.

Table 2.1. Summary statistics for samples and specimens of pygmy ruby snapper *E. carbunculus* collected from the Northwestern Hawaiian Islands (NWHI) and the Main Hawaiian Islands (MHI) during specified time periods. Fork length cm (FL) and Round body weight g (Wt) are noted for archived specimens by subregion (MHI, NWHI) and sex. No. Aged indicates the number of individuals randomly selected from respective groups for sectioning and age determination in this study. Pooled samples did not include fish with unknown sex data (i.e., Unkn sex).

Region Time-series	Variable	Mean	SE	Median	Range	SD	No. of Samples	No. Aged
All samples Pooled subregions	FL (cm)	37.97	0.23	38.10	9.10-71.0	9.44	1694	687
	Wt (Kg)	1.13	0.03	1.00	0.02-5.50	0.64	1523	
MHI Pooled	FL	34.10	0.27	33.30	9.10-71.0	8.54	1028	495
	Wt	1.13	0.02	0.60	0.02-3.76	10.61	863	
Female 2008-2017	FL	35.11	0.35	34.50	9.10–71.0	8.76	629	186
	Wt	0.87	0.03	0.66	0.02–3.76	0.68	523	
Female 1993-1998	FL	33.08	0.62	33.02	18.42-58.60	7.61	153	79
	Wt	0.72	0.04	0.63	0.10-3.71	0.50	153	
Male 2008-2017	FL	33.83	0.41	33.45	13.8–50.7	7.21	314	147
	Wt	0.73	003	0.59	0.10–2.30	0.46	262	
Male 1993-1998	FL	31.08	0.49	30.16	22.23-46.36	5.13	108	53
	Wt	0.55	0.003	0.48	xx-xx	0.28	108	
Unkn sex 2008-2017	FL	27.59	0.92	25.20	11.3 – 50.5	8.49	85	16
	Wt	0.48	0.06	0.28	0.04 – 2.08	0.45	68	
Unkn sex 1993-1998	FL	29.21	2.58	29.85	14.61-39.37	7.31	8	1
	Wt	0.50	0.11	0.44	0.09-1.00	0.30	8	
NWHI Pooled	FL	43.94	0.29	44.00	20.8 – 65.8	7.43	666	192
	Wt	1.56	0.03	1.43	0.16 – 5.50	0.81	660	
Female 1993-1998	FL	45.17	0.35	45.50	20.8 – 65.8	7.42	443	81

	Wt	1.69	0.04	1.46	0.16 – 5.50	0.86	440	
Female 1977-1980	FL	46.34	2.19	46.60	31.40-60.30	9.80	20	25
	Wt	1.95	0.25	1.90	0.53-4.02	1.11	20	
Male 1993-1998	FL	41.58	0.48	42.30	21.7 – 55.6	6.44	177	57
	Wt	1.30	0.04	1.29	0.16 – 3.44	0.56	174	
Male 1977-1980	FL	38.24	1.34	37.80	28.0-53.70	6.31	22	26
	Wt	1.06	0.12	0.95	0.40-2.60	0.57	22	
Unkn sex 1993-1998	FL	41.22	1.23	40.85	26.9 – 62.9	8.33	46	0
	Wt	1.38	0.13	1.23	0.28 – 4.31	0.90	46	
Unkn sex 1977-1980	FL	44.94	5.64	44.20	26.0-62.90	14.92	7	3
	Wt	2.11	0.63	1.55	0.28-4.31	1.66	7	

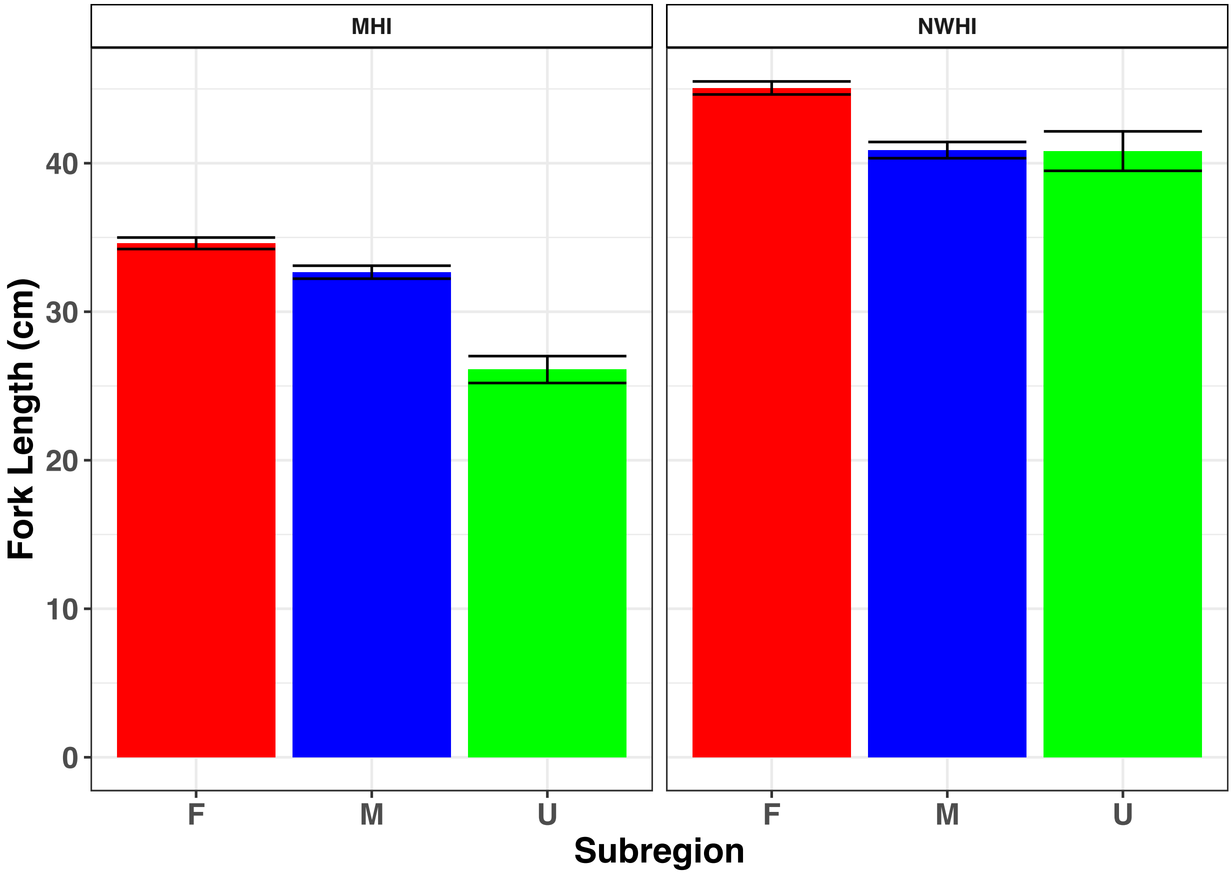

Figure 2.4. Mean fork lengths of *E. carbunculus* collected by subregion and sex. Female (F) are depicted in red, male (M) in blue, and fish of unidentifiable sex (U) are shown in green.

Otolith metrics

No statistically significant difference was detected in otolith metrics (weight, length, height and thickness), between right and left otoliths of individual fish by paired t-test (all $p > 0.38$). Otolith size varied between males and females but not between subregions after controlling for body size effects by using FL as a covariate and truncating uniquely small individuals of both sexes from the MHI (e.g, mass; ANCOVA $p = 0.003$, sex effect; ANCOVA $p = 0.25$, subregion effect). Any differences between sexes and subregions in otolith metrics can be ascribed to sex and subregional difference in fish size (see Age Composition, and Growth results section below). A strong linear relationship was evident between otolith morphometrics and age (r^2 range= 0.79-0.92), with otolith weight being the best to predict age (Fig. 2.5).

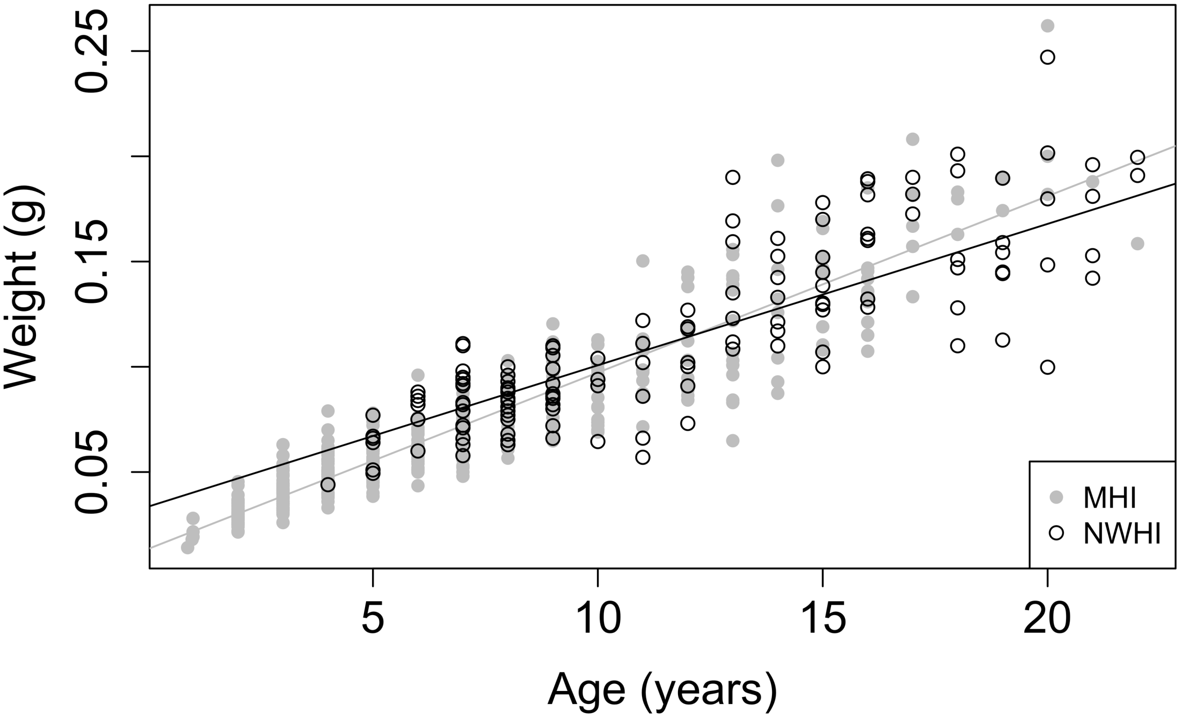

Figure 2.5. Linear relationship of otolith weight (grams) to estimated age (years) of *E. carbunculus* individuals (sexes pooled). Plot shows the subregion MHI in grey, while NWHI is shown in black. The fitted linear regression lines for each subregion are shown.

Otoliths had concentric zones of alternating opaque and translucent bands under reflected light (Fig. 2.6). Although the otoliths of a predefined number of individuals were examined, larger and "older" individuals were added for certain months (May, June, August) to improve comparisons. A single month, July, had only seven individuals, so all were used for margin analysis. Opaque margins were unimodally distributed, began deposition in September and continued through April, with peak formation in February-March (Fig. 2.7). Translucent margins occurred in fish collected from May through July (85%, 34 of 40 fish). A single opaque zone occur annually and therefore can be considered countable annuli that represent age. Seven fish (11.3-18.2 cm FL) whose DGI ranged from 246 – 508 days (mean 368 days) had corresponding annular ages ranging from 0.71 – 1.39 years (mean 1.09 years), indicating that the first annulus is fully formed at an age of one year (see “Growth” section below).

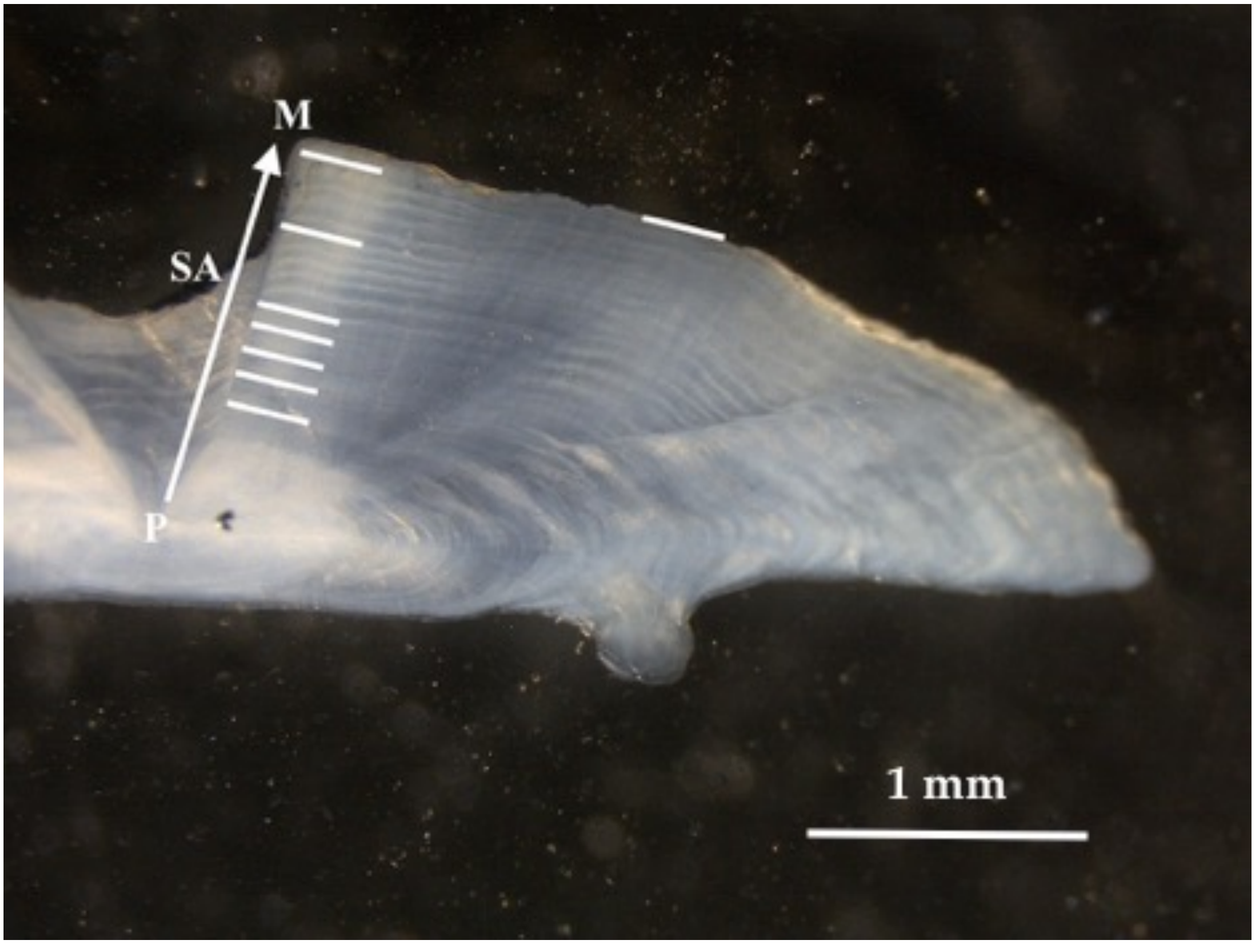

Figure 2.6. Thin transverse section (~170-200 µm) of a sagittal otolith from *E. carbunculus*. (Layne 22-8: female, 42.9cm FL, 16 years). The first five annual increments are denoted by white boxes, followed by the 10th, 15th and a newly formed opaque edge at year 16. The arrow indicates the typical counting path along the sulcus acusticus (SA) axis, from the primordium (P) to the outer margin (M). Scale bar is 1 mm.

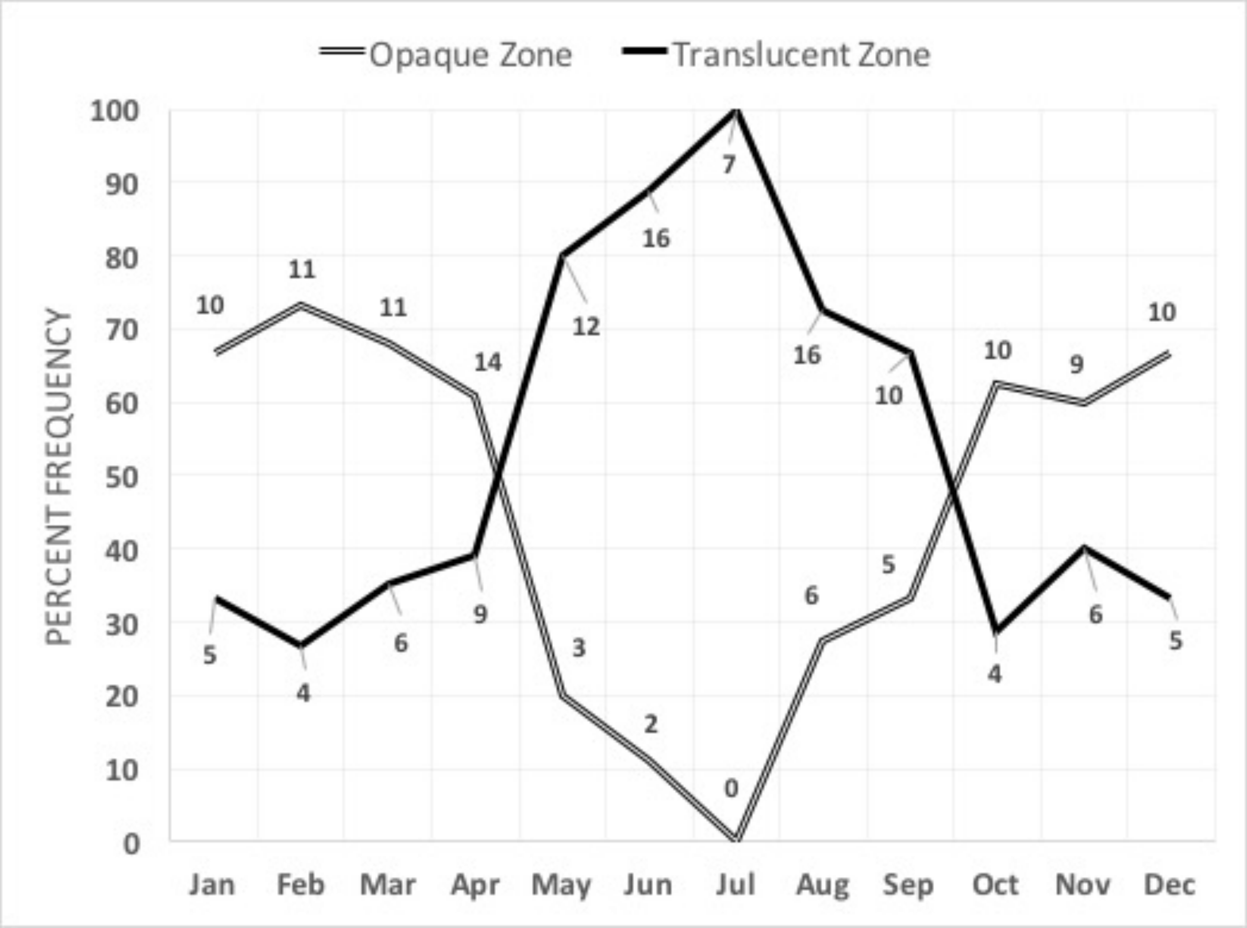

Figure 2.7. Marginal increment (translucent and opaque zone) frequencies by month. The number of individuals with either an opaque or translucent zone, is listed above their respective line and month. All examined otoliths came from MHI subregion

Reader precision and bias

Within reader precision was acceptable (APE = 3.576%, CV= 5.06%). The initial annual counts matched final counts for 478 of the 702 estimated ages, differed by one for 205 estimates, and differed by more than two for the remaining 19 estimaed ages. Otoliths were re-read for the 19 specimens whose initial counts differed by more than two; for these, the temporary final count was replaced with the mean count of the three reads (e.g., estimated mean age 6, when three counts were 5, 6, 7). Final counts were thereby re-assigned for 11 of the 19 fish, and these eight fish were subsequently not used for evaluating size-at-age. The percentage agreement (PA) was 68 % and 98% within 1-2 years, respectively.

The agreement between readers was moderately high, with an IAPE of 3.73% and a CV = 5.27%. However, both the observed IAPE and CV were within the accepted levels of precision (IAPE 5%, CV $\leq$ 7.6%; Campana 2001; Wakefiled et al. 2017a). The otoliths age estimates of *E. carbunculus* were therefore without significant bias and with acceptable agreement between readers (Bowker-Hoenig, df=27, X^2= 36.17, p=0.111). Direct agreement (PA) between readers was 53% and 92% within 1 year.

Further analysis of accuracy and precision was examined using Bland-Altman plots; these indicate that the reader estimates of age included simple random error related to the mean ages of all samples. The mean age estimates fall within the 95% confidence interval of the Bland-Altman plot. It is generally accepted that when the 95% confidence interval for the mean difference excludes the zero in the fit, there is an inherent bias in the age estimates being compared (McBride 2015). The primary reader appeared to have overestimated ages by half of a year for age classes 7-30 years (Appendix B2). However, the summary of the Generalized Additive Model (GAM) used to fit the Bland-Altman plot indicated no significant difference in the comparison of reader ages and mean ages (F=0.653, p = 0.42, df = 1), and the intercept term did not differ from zero. Therefore, the potential for a 0.5-year overestimate for larger age classes should not influence the age estimates of the primary reader that were used to describe growth in this study. To further evaluate potential 0.5-year ageing bias or error of the primary reader, and to validate the annulus counts, radiocarbon chronology was used on samples which fell within the most diagnostic portion of the reference curve.

Bomb radiocarbon validation

Most measured F^{14}C values aligned with the reported coral F^{14}C reference record from late 1950s to early 1970 (Table 2.2). The F^{14}C values ranged from near-bomb levels (mean 0.951) through the peak values (1.177) and ended with a recently collected individual in the decline period (1.093) (Fig. 2.8).

Table 2.2. Summary of pygmy ruby snapper (*E. carbunculus*) and associated fractionation corrected radiocarbon (F14C) data for archived otolith (sagittae) specimens from 1977-1980. Abbreviated names of NWHI reefs: FFS=French Frigate Shoals; P&H=Pearl and Hermes Atoll.

Sample # (NOSAM#)	Length (cm TL)	Otolith wt. (g)	Collection (location)	F^{14}C ± 1SD	δ^{13}C (‰)	Birth year	F^{14}C age (y)	Age (Sex)
E-04 (139338)	57.0	0.2677	1978.70 (Lisianski)	0.9582 ± 0.0024	–5.08	1944.5– 1945.7	34.20 (31.2-37)	33 (U)
E-11 (139343)	41.2	0.1491	1978.73 (P&H)	0.9530 ± 0.0037	–5.99	1939.3– 1953.5	32.73 (± 10)	30 (F)
E-15 (139348)	59.3	0.2016	1978.66 (P&H)	0.9742 ± 0.0032	–5.22	1958.0– 1959.0	20.16 (± 3)	21 (U)
E-16 (146610)	50.5	0.1280	1978.65 (P&H)	0.9995 ± 0.0038	–5.77	1960– 1962	17.65 (± 3)	18 (F)
E-17 (146613)	35.5	0.0731	1978.74 (P&H)	1.1198 ± 0.0044	–6.26	1965.5– 1967.3	12.28 (± 3)	12 (M)
E-18 (139351)	41.5	0.0892	1978.70 (P&H)	1.1464 ± 0.0035	–5.68	1968.9– 1970.3	9.10 (± 3)	8 (F)
E-20 (146615)	44.2	0.1111	1978.60 (Maro)	1.1145 ± 0.0036	–5.68	1965.5– 1967.2	12.24 (± 3)	11 (U)
E-23 (139350)	57.4	0.2655	1978.65 (P&H)	0.9740 ± 0.0029	–5.35	1939.7– 1948.3	35.15 (± 3)	30 (F)
E-25 (139342)	33.3	0.1528	1978.73 (P&H)	1.0892 ± 0.0026	–5.65	1958- 1959	20.23 (± 3)	21 (M)

E-27 (146611)	50.5	0.1525	1978.63 (Maro)	1.0849 ± 0.0063	–5.37	1964.1-1965.4	13.87 (± 3)	14 (F)
E-32 (139341)	31.6	0.0645	1978.66 (P&H))	1.1333 ± 0.0048	–5.91	1967.6–1968.6	10.92 (± 3)	11 (M)
E-35 (139352)	47.5	0.1424	1978.61 (P&H)	1.0608 ± 0.0033	–5.52	1963.5–1964.1	14.82 (± 3)	14 (M)
E-36 (139347)	45.1	0.1450	1978.67 (P&H)	0.9819 ± 0.0027	–5.59	1959.0–1960.0	19.17 (± 3)	19 (M)
E-38 (139340)	37.7	0.1127	1978.65 (P&H))	0.9855 ± 0.0030	–5.3	1967.3–1970.3	19.15 (± 3)	19 (M)
E-39 (139335)	60.3	0.2941	1978.70 (Lisianski)	0.9531 ± 0.0033	–5.64	1939-1953	32.70 (± 10)	32 (F)
U-03 (139334)	38.7	0.0661	1981.56 (FFS)	1.1454 ± 0.0053	–5.26	1967.4–1970.4	12.62 (± 3)	11 (F)
U-07 (139346)	31.0	0.0660	1977.44 (Necker)	1.1419 ± 0.0033	–5.31	1967.4–1968.5	9.46 (± 3)	9 (F)
U-10 (139336)	28.8	0.0494	1978.89 (NWHI)	1.1405 ± 0.0045	–5.71	1967.3–1970.1	10.20 (± 3)	5 (M)
U-12 (146616)	26.0	0.0288	1980.65 (FFS)	1.1532 ± 0.0023	–4.63	1964.1–1964.8	3.27 (± 3)	5 (U)
U-13 (146617)	26.9	0.0293	1978.71 (P&H)	1.1603 ± 0.0034	–4.25	1968.6–1972.8	7.95 (± 3)	5 (U)
U-15 (139339)	40.3	0.0915	1980.27 (P&H)	1.1532 ± 0.0023	–5.95	1968.9–1970.3	10.68 (± 3)	9 (U)
U-17 (149612)	50.0	0.0909	1980.24 (P&H)	1.1532 ± 0.0023	–6.01	1970.3–1973.9	8.11 (± 3)	7 (F)
U-18 (139337)	32.4	0.0570	1980.68 (P&H)	1.1532 ± 0.0023	–6.16	1968.3–1971.0	11.01 (± 3)	10 (M)
U-19 (139345)	58.4	0.2329	1979.36 (P&H)	1.1532 ± 0.0023	–5.18	1941–1955	31.26 (± 3)	29 (F)
U-20 (139333)	38.4	0.0998	1980.68 (P&H)	1.1532 ± 0.0023	–5.96	1960.4–1962.3	19.31 (± 3)	20 (M)
U-21 (139349)	52.0	0.1699	1978.84 (P&H)	1.1532 ± 0.0023	–5.85	1952–1955	25.34 (± 3)	26 (M)
U-22 (146614)	47.7	0.1443	1978.84 (P&H)	1.1532 ± 0.0023	–5.86	1956.0–1959.0	21.34 (± 3)	19 (M)
U-25 (146618)	55.0	0.1590	1978.66 (P&H)	1.1532 ± 0.0023	–5.58	1958.0–1959.0	20.16 (± 3)	19 (U)
Ehu-71 (139344)	71.0	NA	2015.57 (P&H)	1.1532 ± 0.0023	–5.22	1996.3–2000.1	18.00 (± 3)	22 (F)

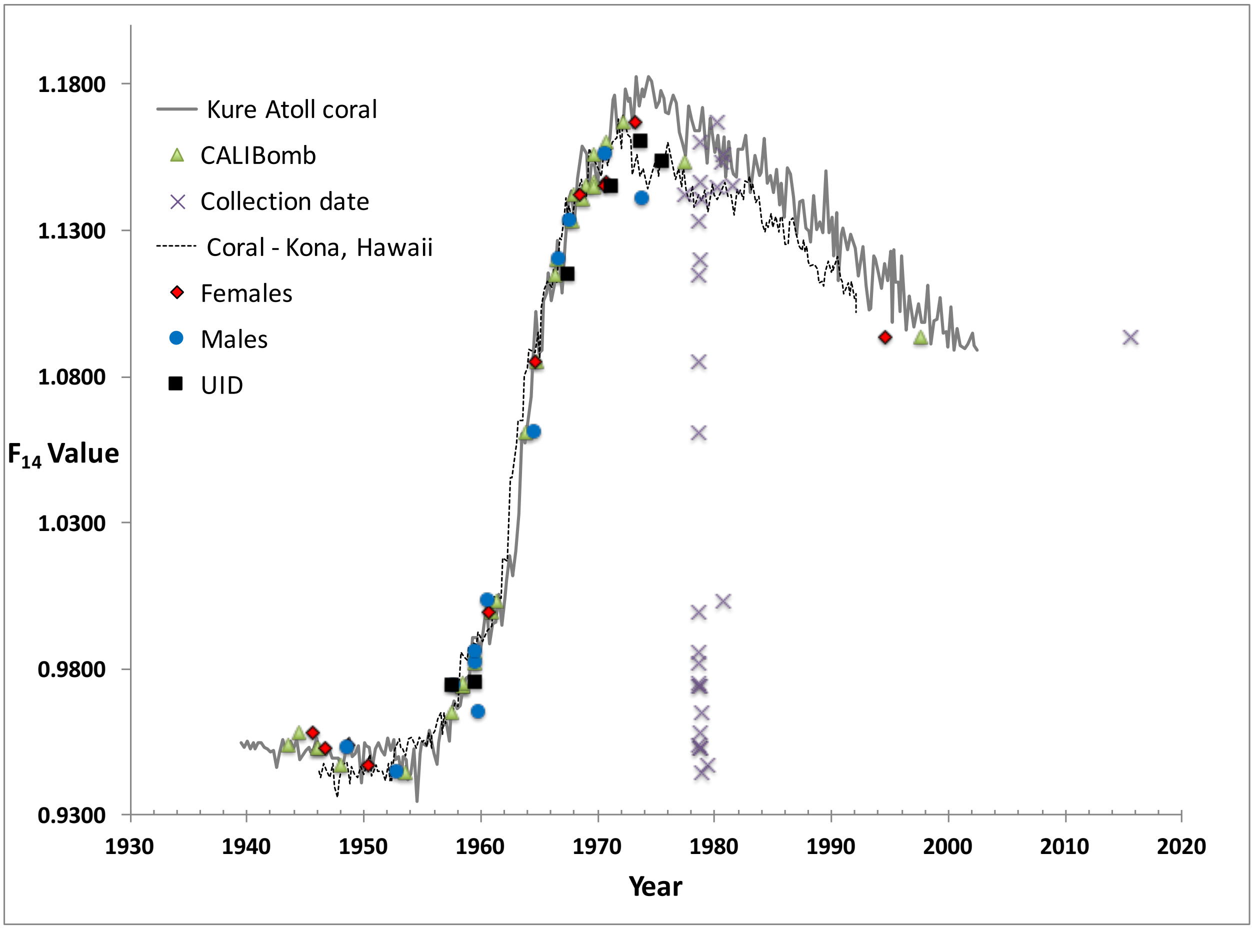

Figure 4.8. Plot of F14C fractional modern values from *E. carbunculus* otolith cores. Green triangles are otolith fractional modern values projected back to the corresponding Kure Atoll and Kona Hawaii Coral reference curves, Ages estimated using visual counts of annuli in cross-sections are plotted by sex (Red =Female, Blue = Male, Grey = unknown sex), “X” indicated collection date of individuals.

Overall, the good correspondence between otolith radiocarbon values and the coral reference curve should be interpreted as validation of estimated ages as well as the age reading criteria used in this study. The radiocarbon ages validated annulus-based ages that ranged from 9 to 25 years. Of the otoliths fit to the reference curve, 22 fell within the most diagnostic and sensitive years for $F^{14}C$ based ageing (years spanning 1958-1965). The annulus ages agreed by less than one year for 64% of the samples (14 of 22), and within three years for 95% (21 of 22). The remaining otolith aligned near peak levels, where variability in $F^{14}C$ values is commonly greater than what is reported in coral $F^{14}C$ reference record, due to variability across the archipelago and regional upwelling that may influencen some core values. These peak level specimen's annulus counts may still be valid ages, as they were collected further south-east along the archipelago, where regional influence may have grearter impact than the Kure location. The peak level variation begins around 1970, corresponding with a dip in the Kure reference curve values, and the same time as these peak level specimens were aligned. An additional test of symmetry between $F^{14}C$ age and annulus-based estimates indicates no systematic differences between the types of methods used and their respective ages (Bowker-Hoenig, *df=14,* chi-squared=14.0, *p=0.45).* Therefore, the differences observed between ageing methods should be considered simple random error.

Age composition

Mean ages (pooled samples) varied between regions and were 6.89 ± 0.20 years in the MHI and 12.79 ± 0.45 years in the NWHI (Table 2.3). The modal age was three and eight for females in the MHI and the NWHI, respectively. For males, the respective modal ages were three years old compared to nine in the MHI and NWHI. The maximum observed age in the MHI was 22 years compared to 33 years in the NWHI (Fig. 2.9). A greater number of younger fish (< 5 years) were present in the MHI samples because of targeting of smaller (< 26 cm FL) individuals in the MHI. Fish in the MHI were fully selected by sampling gear at an age of three years. In the NWHI however, there is a significant age deficit in fishes between 0+ and 8 years old, compared to MHI, which suggest that fish less than eight years old were not fully selected by the sampling gear there. Despite significant size and age differences between subregions, there was no significant difference in mean age between sexes within subregions (MHI: Female mean age = 7.68, SD = 4.68, Male mean = 7.46, SD = 3.97, KS test D= 0.067, *p* = 0.67; NWHI: Female mean age = 12.91, SD = 6.64, Male mean = 13.88, SD=5.7; KS test D = 0.198, *p* = 0.051; Table 2.4).

Table 2.3. Summary descriptive statistics of age distributions of *E. carbunculus* by NWHI and MHI subregion and collection period, for pooled and individual sexes. SD = standard deviation, Unkn sex = no sex data, na = no data avalable.

Subregion Time-series	Variable	Mean	SE	Median	Range	SD	No. of Samples
MHI Pooled	Age	6.89	0.20	5.72	0.67-22.17	4.49	495
Female 2008-2017	Age	7.7	0.36	6.5	0.7 - 22	4.89	190
Female 1993-1998	Age	5.05	0.37	4.0	2 - 20	3.24	88
Male 2008-2017	Age	7.64	0.37	8.0	0.8 - 22	4.42	141
Male 1993-1998	Age	5.82	0.34	5.0	2 - 15	2.77	69
Unkn sex 2008-2017	Age	1.98	0.23	2.0	0.7 - 4	0.93	16
Unkn sex 1993-1998	Age	1	na	3.0	3	na	1
NWHI Pooled	Age	12.79	0.45	11.0	3.67-33.17	6.21	192
Female 1993-1998	Age	11.63	0.63	9.0	4 - 31	5.64	81
Female 1977-1980	Age	14.96	1.75	11.0	6 - 33	8.77	25
Male 1993-1998	Age	13.67	0.75	13.0	5 - 29	5.7	58
Male 1977-1980	Age	13.15	1.06	11.5	5 - 24	5.41	26
Unkn sex 1993-1998	Age	na	na	na	na	na	0
Unkn sex 1977-1980	Age	6	1.53	9	4 – 9	2.65	3

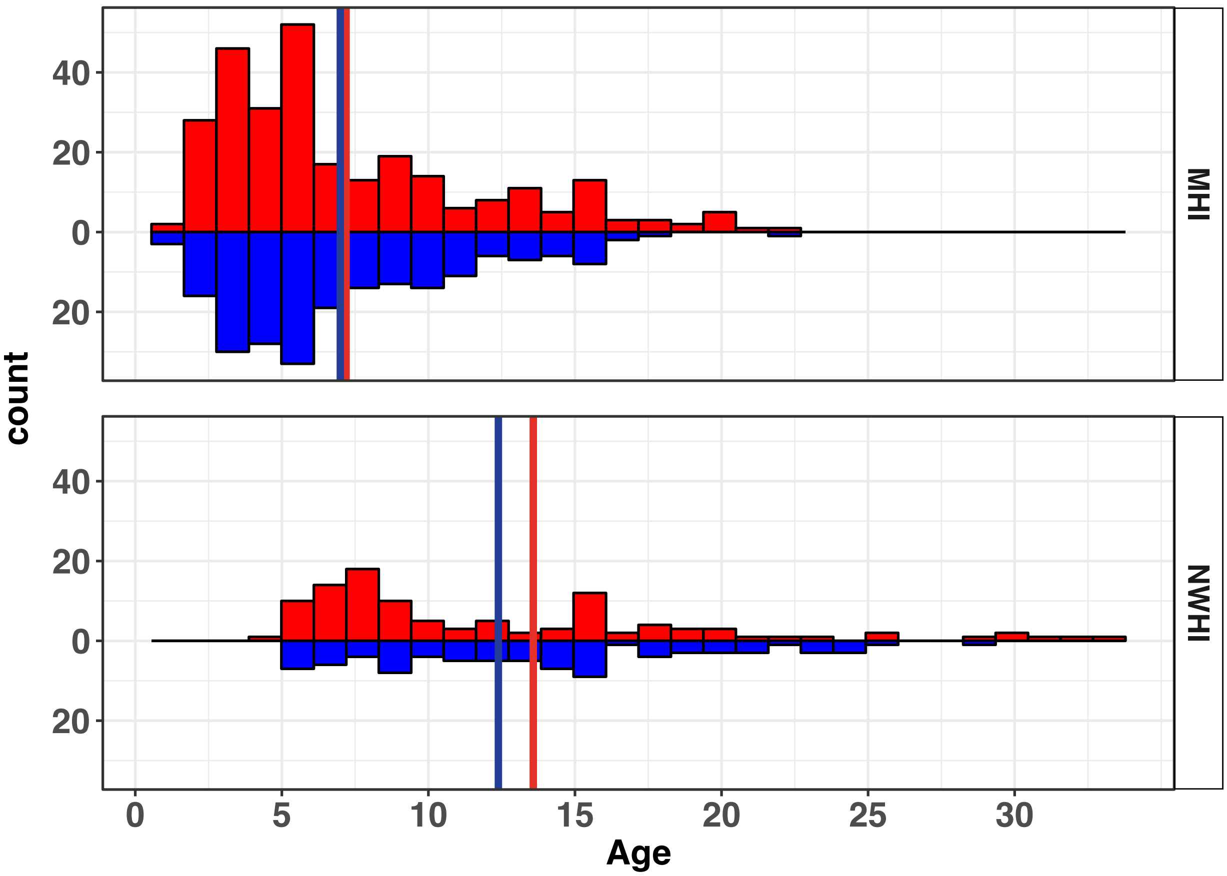

Figure 2.9. Age frequency distribution of *E. carbunculus* (pooled years) from the Hawaii Archipelago (subregion MHI on top, NWHI on bottom). The distribution of females is plotted above the line and males are below. Mean age for each sex and subregion is depicted by a vertical line (Red = female, Blue = male)

Table 2.4. Summary statistics of Kolmogorov –Smirnov (K-S) tests of sex-specific, size (FL cm) and age (years) for *E. carbunculus* collected from the two (MHI, NWHI) subregions of the Hawaiian Archipelago. Test results are a comparison of lengths or ages between subregions for sexes pooled, and between subregions for a given sex.

	Group	MHI		NWHI		D	P-value
		Mean	n	Mean	n		
Fork Length (cm)	Sexes pooled	33.37	793	43.59	541	0.499	<0.0001
	Males	32.66	239	40.88	148	0.266	<0.0001
	Females	34.61	493	45.07	351	0.121	<0.0001
Age (years)	Sexes pooled	7.4	509	13.2	192	0.459	<0.0001
	Males	7.5	212	13.8	83	0.493	<0.0001
	Females	7.7	280	12.9	106	0.121	<0.0001

Growth

The relationship between FL and W for pooled samples indicate that *E. carbunuclus* exhibit a positive allometric growth pattern that became linear on the double-log transformed scale, thus changing its mass proportionately as fish length increases (Table 2.5, Appendix B2, B3). Neither sex nor subregion had a significant effect on this relationship (ANCOVA; $p = 0.167$ and $p = 0.441$, respectively). However, a difference was seen in the corresponding intercept fits between the sexes (ANCOVA; $p < 0.001$). Equations of the best fit relationship with sexes and subregions pooled between FL (cm) and RW (Kg) are:

$$\log(RW) = -11.055\ x\ log(FL)^{3.02} (\text{Fig. 2.10,})$$

and on the original scale: and

$$RW = 2.14\ x\ 10^{-5}\ (FL)^{3.02} (\text{Appendix B4})$$

on the original scale.

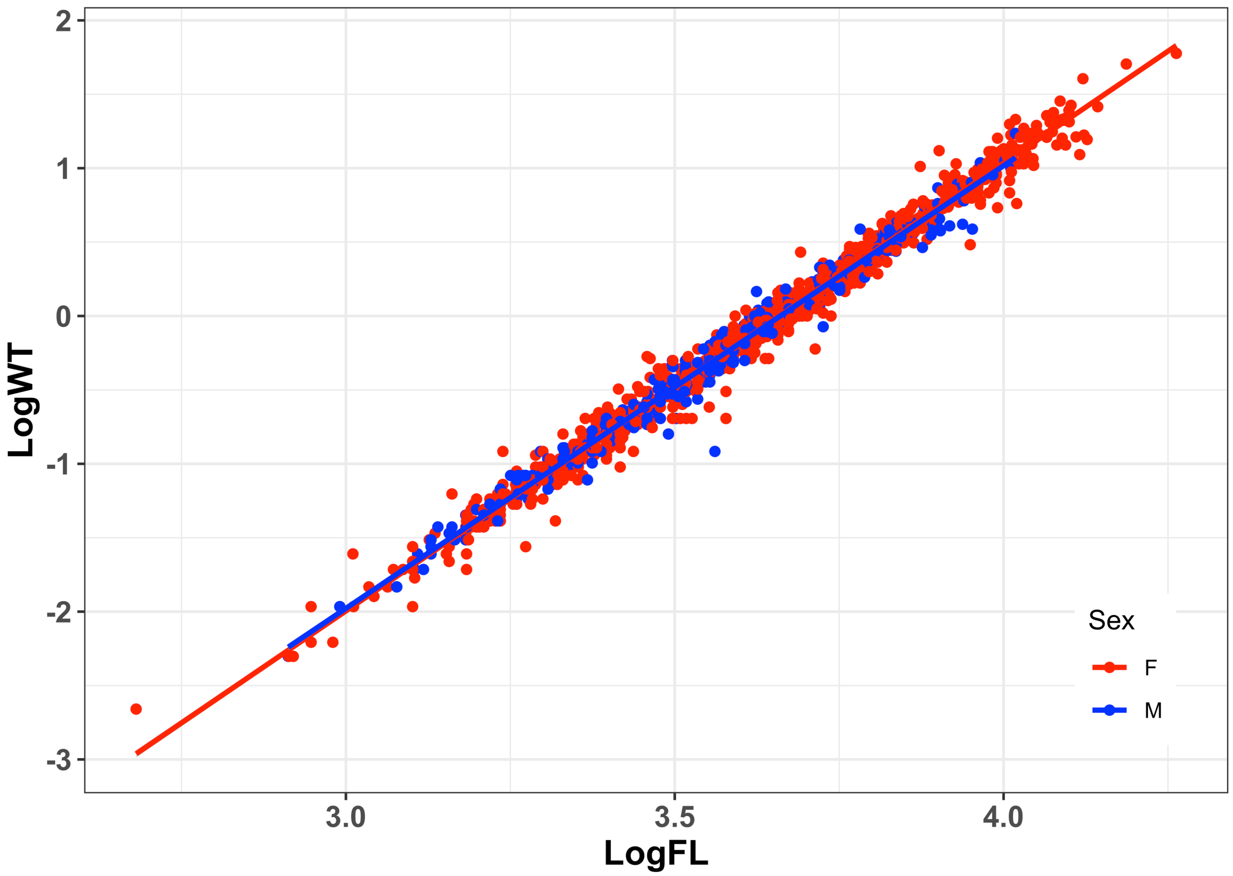

Figure 2.10. Log transformed weight-at-length regression and fitted curves for all subregions pooled (NWHI and MHI; (Red = female, Blue = male).

Table 2.5. Parameter estimates for length–weight relationships of *E. carbunculus*, by subregion and sex; a and b: parameters of the linear model of the log-transformed relationship between fork length (FL, cm) and weight (RW, Kg).

Weight-Length	*log(a)*	*b*	r^2	n
Pooled Regions				
Pooled sexes	-11.055	3.02	0.98	1231
Female	-11.084	3.03	0.98	844
Male	-10.962	2.99	0.98	387
MHI all years				
Pooled sexes	-10.868	2.96	0.98	732
Female	-11.017	3.01	0.98	493
Male	-10.868	2.96	0.98	239
NWHI all years				
Pooled sexes	-11.046	3.03	0.97	539
Female	-11.135	3.04	0.97	351
Male	-10.796	2.93	0.96	148

Overall, *E. carbunuclus* has moderately slow growth, attaining an average observed size of 16, 30, 40 and 56 cm at ages 1, 5, 10 and 20 years, with longevityy to 22 in the MHI and greater than 30 years in the NWHI. The fitting of length-at-age data, using both the unconstrained (3-parameter) and constrained (2 parameter, t_0= 0) von Bertalanffy growth function (VBGF), provided reasonably good fits for pooled subregions (Fig. 2.11, Fig. 2.12). However, when evaluated by subregion, the limited number of smaller individuals overall and especially in the NWHI made the unconstrained models a less realistic fit (large negative t_0), especially in the NWHI (Fig. 2.12, Appendix B5, B6). The constrained VBGF model (pooled across Hawaiian Archipelago), illustrates that both sexes reach an asymptotic length within 15 - 20 years (Fig. 2.13, 2.14). The growth profiles illustrated that female *E. carbunculus* attained asymptotic sizes (L_∞) on average at 10 cm (20%) greater lengths and growth coefficients (k) approximately 0.10 (20%) less than males and in both regions (Table 2.6). Females differed between subregions in asymptotic sizes (L_∞) (p= 0.002), but only moderately for growth (k) (p=0.047). Likewise, males differed moderately between the MHI and NWHI in asymptotic sizes L_∞ (p= 0.043), but not growth (k) (p=0.365) (Table 2.6).

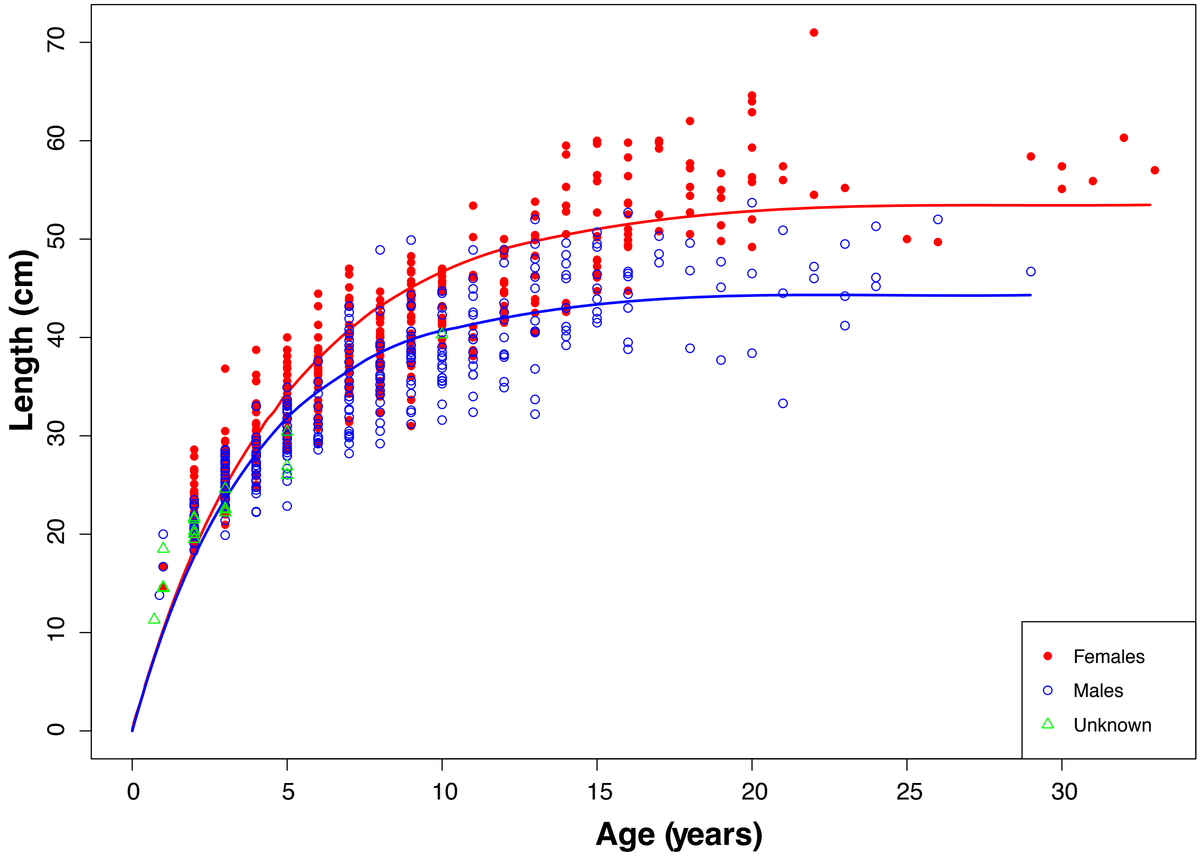

Figure 2.11. Length-at-age data and fitted constrained von Bertalanffy model (VBGF) showing growth profiles for all *E. carbunculus* (pooled across Hawaiian Archipelago). (Red = female, Blue = male, Green = unknown sex).

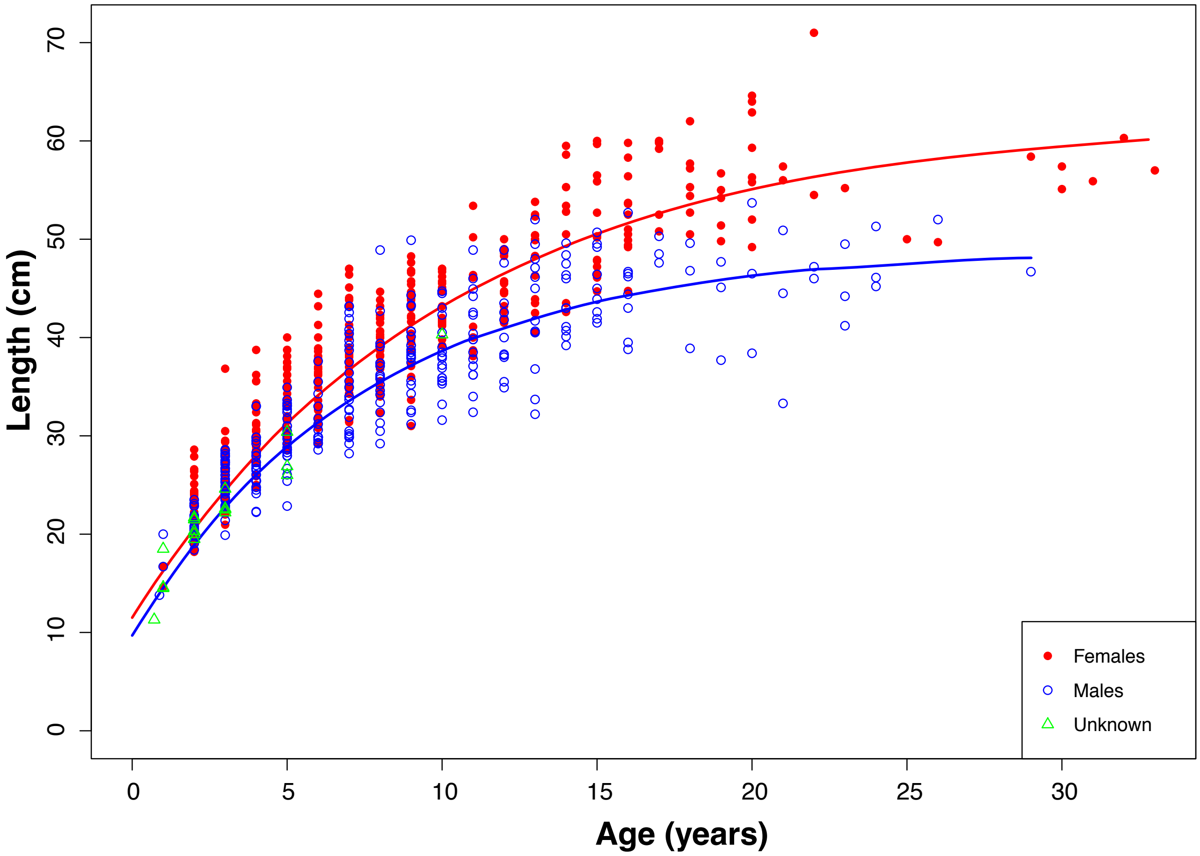

Figure 2.12. Length-at-age data and fitted unconstrained von Bertalanffy model (VBGF) showing growth profiles for all *E. carbunculus* (pooled across Hawaiian Archipelago). (Red = female, Blue = male, Green = unknown sex).

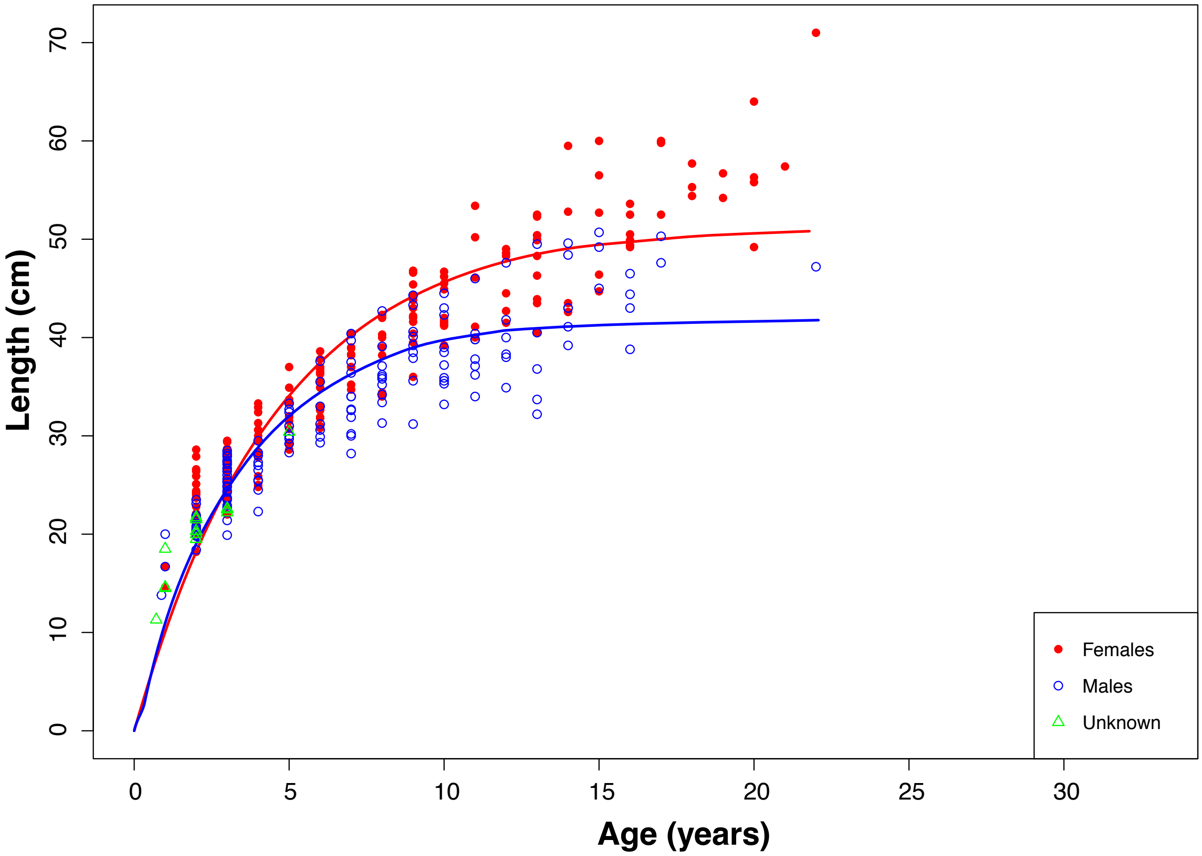

Figure 2.13. Length-at-age data and fitted constrained von Bertalanffy model showing growth profiles for all *E. carbunculus* collected from the MHI (pooled time series; Red = female, Blue = male, Green = unknown sex).

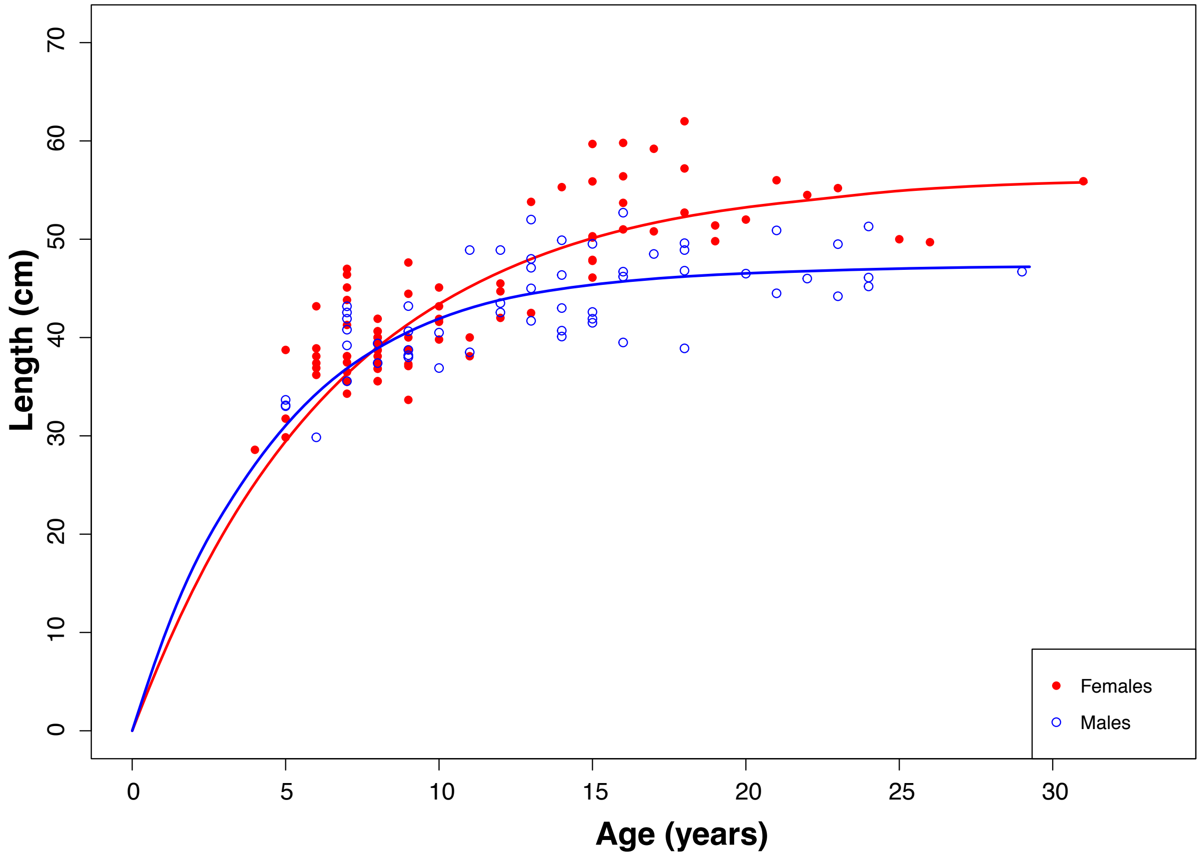

Figure 2.14. Length-at-age data and fitted constrained VBGF model showing growth profiles of *E. carbunculus* collected from the NWHI during Period 2 (1997-1998; Red = female, Blue = male).

Table 2.6. Parameter estimates (± SE) of the unconstrained and constrained von Bertalanffy growth function (VBGF) for *E. carbunculus* collected from the Northwestern Hawaiian Islands (NWHI) and Main Hawaiian Islands (MHI); L∞: mean asymptotic fork length (FL, mm); k: growth coefficient (yr–1); t0: theoretical age (yr) at length 0 for the VBGF

Subregion	**Model**	L_∞	**k**	t_0	**N**
POOLED	Constrained	50.87 (0.52)	0.17 (0.005)	0.0	701
	Unconstrained	56.62 (1.12)	0.11 (0.01)	-2.02 (0.2)	701
NWHI 1997-98	**VBGF**				
Pooled sexes	Constrained	51.12 (0.91)	0.18 (0.011)	0	138
Female		56.23 (1.44)	0.15 (0.010)	0	81
Male		46.92 (0.90)	0.22 (0.021)	0	58
MHI 1997-98	**VBGF**				
Pooled sexes	Constrained	45.99 (6.44)	0.21 (0.002)	0	143
Female		54.00 (2.01)	0.18 (0.13)	0	76
Male		42.50 (1.8)	0.21 (0.02)	0	67
NWHI 1997-98	**VBGF**				
Pooled sexes	Unconstrained	52.61 (1.91)	0.13 (0.03)	-2.73 (1.91)	138
Female		59.62 (3.79)	0.10 (0.03)	-3.24 (2.10)	81
Male		46.63 (1.4)	0.25 (0.08)	-0.14 (2.01)	58
MHI 1997-98	**VBGF**				
Pooled sex	Unconstrained	57.23 (6.45)	0.07 (0.03)	-3.65 (1.13)	143
Female		59.22 (4.45)	0.13 (0.03)	-1.52 (0.62)	76
Male		43.55 (2.43)	0.28 (0.09)	-0.78 (0.76)	67
MHI 2008-2018	**VBGF**				
Pooled	Constrained	50.33 (0.75)	0.07 (0.01)	0	343
Female		54.92 (0.92)	0.17 (0.006)	0	186
Male		42.21 (0.74)	0.23 (0.01)	0	141
MHI 2008-2018	**VBGF**				
Pooled sex	Unconstrained	67.26 (4.00)	0.07 (0.01)	-3.76 (0.47)	343
Female		68.87 (3.38)	0.08 (0.01)	-3.10 (0.39)	186
Male		48.70 (1.94)	0.13 (0.02)	-2.23 (0.42)	141

DISCUSSION

Despite the cultural and economic importance of deep-water snappers across the Indo-Pacific and Hawi‘i, the biology of these snappers are poorly understood. This study provides new information on deep-water snapper biology, a first validation of the ageing criteria for *E. carbunculus*, and highlights the age truncation and significant variability in the life history parameters of this species within the Hawaiian Archipelago.

Using marginal edge analysis, DGI counts, and bomb-radiocarbon dating we validated the formation of a single opaque zone per year in *Etelis carbunculus* sagittal otoliths. While some information suggest that growth increments can be difficult to detect in the otoliths of other tropical species (Fowler 1995), there is no evidence to conclude that these increments are other than annual in most species (Choat et al. 2009) or in *E. carbunculus* (this study). The marginal edge analysis herein clearly demonstrated that there was periodic growth on the edge of the otolith, and mean DGI counts reaffirmed that the first annulus is fully formed at an age of one year. The deposition of an opaque zone in winter-spring, as found in this study, has been observed previously for other deep-water species of the family Lutjanidae. For example, Newman and Dunk (2003) and Lloyd (2006) found the formation of the translucent zone (the period of faster growth) was formed during the summer months and the opaque zone (slower growth) formed during winter-spring months in otoliths of goldband snappers (*Pristipomoides mulitidens*). Additionally, Williams et al. (2017) indicated that opaque zone dilineation begins during the winter – spring months (October) for *E. carbunculus*. Both of these southern hemisphere studies concluded that the onset of opaque zone formation was related to declining sea-surface temperatures at depth (corresponding to late in the calendar year at Hawaii in the northern hemisphere) and not by reproduction, because spawning occurred later in the year in the

Indian Ocean. However, seasonality of growth zone formation can vary between populations of the same species due to combination of factors. Reproduction may influence the onset of translucent zone formation of *E. carbunculus* in Hawaii, because these fish are actively spawning primarily during July-October (DeMartini 2016). There are likely other associated factors besides temperature and reproduction that influence the formation of annual growth increments but these hypotheses cannot be resolved without further research.

The bomb ^{14}C dating analysis successfully validated annual growth zones as representing absolute ages for 22 of the 29 individual *E. carbunculus* specimens. Radiocarbon studies to date suggest that bomb radiocarbon can confirm the accuracy of an age and utility of an ageing method to within 2-3 years (Kalish 1993; Hamel et al. 2008; Andrews et al. 2016a). Melvin and Campana (2010) target a single year-class, collected during the steepest and most diagnostic portion of radiocarbon chronology (~1962). Melvin and Campana's (2010) methodology eliminated the constrains of either evaluating across the entire chronology of the test species (both rise and decline of post-bomb period) or estimating the initial year of increase. Their approach found the uncertainty was reduced to 0.66 years. Our study, found similar accuracy (± 1-3 years) and validated the annulus-based methods and ages estimated for 92% (21 of 23 diagnostic targets) of *E. carbunculus* examined. The 33-year estimate of observed maximum age, one that extends into the pre-bomb period with a minimum validation of 25 years, is comparable to the maximum reported age estimates of 32 years from *E. carbunculus* in Australia (Williams et al. 2015; Wakefield et al. 2016), and is similar to the validated maximum age of the cryptic species, *Etelis sp.*, at 35 - 43 years (Andrews et al. 2011; Williams et al. 2015).

Six otoliths had F^{14}C values that were not in the diagnostic rise portion of the reference curve. These six annulus-based ages ranged from 26 to 33 years, and were less than the diagnostic $F^{14}C$ values at the initial rise of the reference curve. Therefore, these six aligned on the pre-bomb portion of the reference curve. Because tropical marine waters with $F^{14}C$ values greater than 0.95 did not generally exist prior to the late 1950's, otolith cores of fishes with values less than this must have formed before the late 1950's (Campana 2001). However, the minimum ages that could be considered validated for these six otoliths would be set at 25 years, coinciding with the rise of post-bomb $F^{14}C$ values. Although these specimens provide some insight to minimum ages, these six individuals are less diagnostic in their overall usage because the upper age limit is undefined given the general trend in onset, bomb-produced $F^{14}C$ values (Andrews et al. 2016). The single individual specimen that was not well defined on the post-peak side of the reference curve was much younger (both using bomb-radiocarbon and traditional aging methods) than anticipated given the correlations between size and age of other *E. carbunculus* (Williams et al. 2017). This discrepancy may reflect subtle differences in the shallow slope associated with the post-peak decline period, explained by Andrews et al (2016) as being related to large-scale ocean circulatiosn patterns of the North Pacifig Gyre (NPG). This individual was collected from further south along the Hawaiian Archipelago. However, this individual was the largest (71 cm FL) *E. carbunculus* collected in the Hawaiian Archipelago or central Pacific and indicates the potential disconnection between size and age (Newman et al. 2015, Williams et al. 2017).

The *E. carbunculus* collected in the Hawaiian Archipelago have evolved a life-history characterized by moderate longevity (20 – 30+ years), maturity at a relatively young age and small sizes (~ 3.5-5 years for 23.4-27.2 cm FL, MHI and NWHI, respectively DeMartini 2016), and age composition that vary considerably between regions, but not necessarily between sexes. The causes of greater mean and maximum length of female vs male *E. carbunculus* across both sub-regions of the Hawaiian Archipelago are unknown. They are; however, consistent with observations for this species across the Central and Western Pacific and for *E. occulatus* in the Atlantic Ocean (Gobert et al. 2003; Wakefield et al 2016; Williams et al. 2017), which suggest that environmental, genetic, anthropogenic factors (or some combination of these) gennerally influence the demographic variabiloty of this species.

Additional intra-specific shifts in size and age distributions could result from varying interactions involving latitude, phenotypic expression, and fishing pressure (Cossington et al. 2010; Wakefield et al. 2017). Although similar temperature dynamics occur at comparable depths in the NWHI and MHI (Rooney et al. 2010; Steve Scherrer pers. comm). During winter annual range in temperature at mesophotic depths is greater in the northern portion of the Archipelago (colder at depth in NWHI). Since growth and temperature are often positively correlated, whereby reduced temperature results in lower initial growth rate and delayed maturation at larger body sizes, the larger $L_\infty$and smaller k obswerved in *E.* carbunculus in the NWHI could be predicted (Atkinson 1994; Williams et al. 2017). Although evidence exists for habitat preference as a driver leading to sex-related differences in size and age in some reef species (Garcia-Charton et al. 2001; Gobert et al. 2003), it is implausible for *E. carbunculus* in Hawai‘i given the lack of evidence for size- and presumably, age- related changes in habitat (Misa et al. 2013; Sackett et al. 2017). Secondly, distinguishing phenotypic from genotypic response in size and age is difficult. However, genotypic responses are unlikely based on recent results by K. Andrews et al. (2014) indicating that *E. carbunculus* exists as a single genetically homogeneous population throughout the Hawaiian Archipelago.

Finally, exploitation can have a profound effect on age and size distributions of fisheries resources, especially in slow-growing and long-lived species like deep-water snappers (Newman et al. 2015). Fish populations with long histories of exploitation are subject to removal of older age classes, lowered resiliency to environmental changes, and shifting age distributions towards younger, smaller, and more quickly maturing individuals (Walsh et al. 2010; Kuparinen et al. 2016). Such age truncation has been a documented pattern of both commercially and recreationally exploited, long-lived fish populations worldwide (Fergusson et al. 2008). Fishing effort across the two sub-regions of the Hawaiian Archipelago varied considerably over time. Only commercial vessels would venture to fish the distant NWHI grounds (offically closed on June 15, 2011), whereas the MHI has been subject to both short-range commercial and non-commercial fishing for over a century. Total annual landings (1988-2007) were at least 100,000 lbs greater in the MHI compared to the NWHI, and in some years the MHI landings were more than twice those in the NWHI (Brodziak et al. 2010). The greater duration of time and increased fishing pressure within the MHI (prior to 2011 and the NWHI samples examined in this study), may partly explain the age truncation, and greater apparent prevalence of younger individuals in the MHI. However, the lack of very young individuals (<5 yrs) in NWHI was more likely due to sampling bias, rather than exploitation, as these smaller individuals are usually not retained, not provided to us or go unreported (Zeller et al. 2006). Despite this, the same methods are utilized in each sub-region and these are capable of catching small individuals of young age classes of *E. carbunculus* (Ralston 1990), Thus the apparent absence of small, young fish in the NWHI may be a true reflection of the demography of these populations at specific habitats or reef features and not gear selectivity.

In Hawaii, long term exploitation also may have also led to the preponderance of females in the catch, and the fish populations deviation from an assumed 1:1 adult sex-ratio. Female biased sex ratios for *E. carbunculus* in the Pacific and Indian Oceans were similar to populations in Hawaii, where ratios were at unity or female biased for all length classes other than 38 cm FL (Williams et al. 2017). Williams et al. (2017) proposed that a diviation from the 1:1 sex ratio towards females suggests a more active foraging behavior when compared to males, and thus a higher propensitory for females to take baited fishing gear. Other life history traits of *E. carbunculus* differed between the two Hawaiian subregions, with a 4-cm smaller size-at-maturity for females in the MHI compared to the NWHI (DeMartini 2016). DeMartini (2016) further postulated that either environment, fishing pressure, or both could affect spatial differences in size-at-maturation but distinguishing evidence was lacking. Exploitation can impact both the size- and age-structure of fish populations (Ricker 1981). The smaller size-at-maturity in *E. carbunculus* in the MHI has not been directly attributed to either the prolonged impact to its population dynamics, or phenotypic plasticity (Kuparinen and Merilä 2007). Changes in fecundity, egg production, or sperm limitation, however, have been identified in other long-lived deep-water species and atributed to exploitation (Beamish et al. 2006; Stares et al. 2007; Pittman et al. 2015).

The maximum age of *E. carbunculus* observed in this study were much greater and growth much slower for the species compared to earlier studies from the central Pacific and Hawaiian Archipelago (Ralston and Williams 1988; Smith and Kostlan 1991). Our maximum ages were more than a two-fold increase from the previous estimates for *E. carbunculus* in the NWHI and central Pacific (Ralston and Williams 1988; Smith and Kostlan 1991). This substantial is attributable to differences in the methodology used to determine ages in the earlier studies. The previous work, which incorporated the Ralston and Williams (1988) numerical integration techniques, significantly underestimates ages and consequent life history parameters of growth and longevity (Newman et al. 2017; Williams et al. 2017). Additionally, the discrepancies between age estimates of the present study and earlier work in the central and western Pacific are most likely influenced by the inclusion of the sympatric *E.* sp., presumably with different life history traits, in earlier work (Wakefield et al. 2014).

Longevity estimates reported in recent work using similar methodologies, indicate that maximum ages for Eteline snappers were characteristic of most deep-water snapper species with extended longevities across the Pacific. A direct comparison of longevity for *E. carbunculus* in the Western Pacific denote maximum ages as 25 – 30 years and 21 – 32 years in the Indian Ocean (Williams et al. 2017). Other eteline snappers, notably *Pristipomoides flavapinnis* was recvently estimated to reach 28 years in the Samoan Archipelago, while *P. auricilla* reached 32 years in the Mariana Archipelago, using otolith thin sections (Joseph O'Malley pers comm). Similarly, Newman and Dunk (2003) estimated the maximum age for *P. multidens* to be 32 years, and *P. typus,* also collected from waters off Western Australia, was estimated to reach 21 years by Newman (2001). While more closely related geographically, *P. filamentosus* from the NWHI was estimated to reach 43 years by A. Andrews et al. (2012) using bomb radiocarbon dating. The bomb radiocarbon age results of A. Andrews et al. (2011) for *E. carbunculus* reflect similar extended ages to 35 years; however, the *E. carbunculus* incorporated in their study may in fact be the sympatric *E.* sp., as indicated by the much larger fork lengths attained in their specimens compared to *E. carbunculus* in the present study. Although these studies all document snapper species with extended longevities, they also highlight their intraspecific and sex-specific demographic variabilities. Given this variability and concern, future studies on eteline snappers should utilize standard methodologies for ageing, incorporate sex-specific evaluations in any age structure, and consider spatial variation when evaluating age-specific demography.

Table 2.7. Summary of von Bertalanffy growth function (VBGF) for this study and compared to other studies of *E. carbunculus* in the Hawaiian Archipelago and across the Indo-Pacific. The size and age distributions, and consequential growth parameters from other previously reported studies.

Region	**Size range** **FL cm**	**Age range** **Years**	**L_{inf}**	**K**	**t_0**	**Study**
Hawaii (MHI)						This study
Female	14 - 71	1 - 22	54.9	0.17	0	
Male	13 - 50	.8 - 22	42.2	0.23	0	
Hawaii (NWHI)						This study
Female	26 - 62	4 - 33	56.3	0.15	0	
Male	28 - 53	4 - 29	46.9	0.22	0	
Pacific Ocean						Williams et al. 2017
Female	26 - 62	3 - 25	51.4	0.14	0	
Male	28 - 51	5 - 32	45.4	0.15	0	
Indian Ocean						Williams et al. 2017
Female	22 – 44	3 - 22	38.1	0.38	0	
Male	22 - 42	5 - 33	37.8	0.27	0	
Hawaii (NWHI)	17 - 65	5 - 14	58.3	0.06	2.06	Smith et al. 1991
Marianna Islands	15 - 57	5 - 14	71.8	0.16	-1.06	Ralston et al. 1988

The growth patterns of *Etelis carbunculus* in the Hawaiian Archipelago are characteristic of most deep-water snapper species (e.g., A. Andrews et al. 2012; Newman et al. 2016); however, differences in methodology and recent evidence of age truncation in the MHI, suggests that caution is warranted when comparing our results with those of other studies in the region. Smith and Kostlan (1991), concluded that the best estimates of the VBGF growth coefficient (k) ranged between 0.064-0.19 and those for asymptotic mean length (L_∞) ranged from 71.8-118.3 cm. Uchida et al. (1982) estimated (k) at 0.36 and Ralston and Kawamoto (1987) used length-frequencies to estimate (k) at 0.15-0.17, The latter being more reasonable compared to the current estimates. However, these earlier works lacked smaller sized fish, and did not evaluate sex-specific growth for *E. carbunculus*. Additionally, Williams et al. (2017) and Newman et al. (2016) stated that a direct comparison of any growth parameter estimates to previous estimates in the Hawaiian Archipelago are not warranted, based on significant biases inherent to the previous methodology.

A direct comparison is nonetheless possible for a handful of studies in the Indo-Pacific regions that incorporated methods similar to our study. Our fitting of the VBGF curve to length-at-age assumed that growth was adequately described when growth was constrained to $t_0 = 0$, this assumption is resonable for maintaining consistency with other studies (i.e., Wakefield et al. 2015; Williams et al. 2017), as juveniles were not available across the Indo-Pacific distribution of *E. carbunculus* for comparison. Williams et al. (2017) showed that growth of *E. carbunculus* varied significantly between oceans and sexes, and across latitudes. They observed that the spatial variation was larger than the variation between sexes and attributed this to consistencies with the temperature-size rule associated with latitude, based on the metabolic theory of ecology (MTE, Atkinson, 1994). Since strong correlation exist between the growth coefficient (k) and asymptotic mean length (L_∞), it should not be expected that growth models of *E. carbunculus* estimated in the Hawaiian Archipelago should mirror results across the South Pacific by Williams et al. (2017); where the strength of the temperature gradient, exploitation rates, and sampling of smaller (<20 cm FL) individuals are not of similar magnitude. However, the sex-specific variation found between this study and those in the Pacific Ocean (Williams et al. 2017) do reflect a similar trend in that, at higher latitudes, VBGF models predicted larger L_∞, smaller k values and length-at-age in cases where females attained a larger L_∞ than males (Cossington et al. 2010; Wakefield et al. 2016; Williams et al. 2017). The results from the Indian Ocean did not display this exact trend; instead *E. carbunculus* from the Indain Ocean exhibits a relative protracted asymptote in length-at-age, with less prominent L_∞ projection and larger k values for both sexes, and estimated k values greater for females than males. Growth patterns were similar for both sexes in the Indian Ocean; rather than those based on observations at Hawaii and elsewhere in the Pacific Ocean (Grimes 1987; Newman et al. 2000; Williams et al. 2017).

Sex-specific variation in growth of *E. carbunculus* in Hawaii, slower growing and ultimately larger females, could be explained by cost associated with maintaining reproductive potential, or by exploitation. Differences in growth could also reflect the influence of key reproductive events of sub-adults (e.g., onset of oogenesis and spermatogenesis) or a tradeoff between reproductive potential and mortality (Malison et al. 1985; Grimes 1987; Kritzer 2004). It is generally accepted that energy diverted to reproduction to benefit offspring, ultimately detracts from the overall energy available for maintenance and somatic growth of the parent (Wootton 1985). It has been suggested, in other populations of *Lutjanidae* species, that fecundidity selection has contributed to the sex-specific variabilty in size and growth, and indeed female grow more slowly and larger than males (Roff 1983; Newman and Dunk 2003). For many Lutjanid species, males are reported to be significantly larger after the first few years and this is consistent with energy expenditure being greater for female than males (Newman et al. 1996; McPeherson and Squire 1992). For female *E. carbunculus*, the reproductive potential was postulated to be the driver for growth variation in the Pacific and Indian Oceans, based on the significant increase in female gonad weight with whole fish weights (Williams et al. 2017). As discussed above, if increased energetic cost is placed on females, a greater mortality risk may be incurred from predators and disease associated with foraging, to support the higher caloric demand of female reproductive cost (Roff 1983; Williams et al. 2017).

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CHAPTER 3: A non-invasive method for external sex determination of the pygmy ruby snapper *Etelis carbunculus*.

ABSTRACT

The ability to provide an effective, non-invasive and non-lethal method for collecting sex-specific information on deep-water snapper (Lutjanidae, Etelinae) species could provide direct benefits to current fisheries conservation and management throughout the Indo-Pacific. Utilizing external sex identification of the urogenital region of the pygmy ruby snapper or ehu, *Etelis carbunculus*, we accurately determined the sex of 96% of 157 specimens ranging between 18.7 – 45.7 cm fork length (FL) for females and 16.7 – 49.7 cm FL for males from the main Hawiian Islands. Comparably-sized mature males and females differed in the length of the papilla septum distance (PSD) of the urogenital region with the measure significantly larger for females. Since sexually-dimorphic differences in life history traits are non-commonly present in Lutjanidae snappers, the application of external sex determination facilitates rapid collection of sex-specific data of *E. carbunculus* and other similar species for fisheries research and management of these fishes.

INTRODUCTION

Various methods have successfully identified sex in marine teleosts for species without obvious external sexually-dimorphic characteristics, but these vary in cost and ease of application in the field. The use of histology may provide the most accurate means of determining sex and maturity; nonetheless, the cost (e.g., processing time, effort and financial cost per specimen) can be exorbitant (Brown-Peterson et al. 2011). Many developing countries or smaller scale studies (e.g., market sampling, mark-recapture studies) may not have the finacial capability to develop this kind of rigorous study and would require simple methods. The use of non-lethal, and external sex identification methodologies (e.g., cytogenetic, catheterization and ultrasonic technology) have shown sucess for certain species (Galetti et al. 2000; Coyle et al .2004; Newman et al. 2008). Despite the applicability in the field these methods still retain a high cost because of the amount of time, money or effort involved. This predicament leaves some studies with a data poor situation for evaluating sex-specific variation or presenting appropriate management recommendations (Newman et al. 2017).

In most field based research settings the assigning of sex or reproductive state typically invovles the use of secondary characteristcis. The examinations of gondas generally forces the sacrifice of the specimen, unless the species exhibits a high degree of sexual dimorphism (e.g., secondary characteristic of external color, size, behvior, or morphological modifications). Many species do not exhibit conspicuous secondary sexual characteristics. However, it is not uncommon in marine fish: as seen in species of wrasses and greenling (Choat 1965, DeMartini 1985) as sex dichromatism, in marlin (Makaira sp., Wilson et al. 1991) as size differences, and in weakfish (Cynoscion sp., Tellechea and Norbis 2012) as behavior of sound production. Yet, these secondary sexual dimorphims characteristics are not widely applicable (Breder and Rosen 1966). But inconspicuous sex-specific differences have been recognized as present in the urogenital regions of marine fish that did not display obvious secondary sexual dimorphism characteristics (Smith et al. 2014; Luers et al. 2018).

Unlike other more expensive methods, the best application of an external method should be inexpensive, relatively simple (i.e., quick to perform), and be minimally invasive or inflict negligible stress (Smith et al. 2014). To date the visual examination of external urogenital features have satisfied the above needs and have been incorporated into creel surveys and aquaculture practices (Vecsei et al. 2003; Smith et al. 2014; Leurs et al. 2018). These methods, which utilize different characteristics of the urogenital region (e.g., shape and number of urogenital pores, Nichols 1954; Casselman 1974; Noltie 1985) of teleost's, have made it possible to assign sex to commercial landings and recreational catches, even for species that do not exhibit conspicuous morphological sex-specific differences, including deep-water species of Lutjanidae (Everson 1988; Smith et al. 2014; Luers et al. 2018).

The pygmy ruby snapper, is a deep-water snapper (Lutjanidae) inhabiting the tropical and subtropical Indo-Pacific distribution (Williams et al. 2017). *E. carbunculus* is a slow to moderatly fast growing, long lived snapper (30 + years) that inhabits mesophotic depths (100-300m) of slope waters, and is associated with benthic structures (Randall 2007; Misa et al. 2013; Sacket et al. 2017; see Chapter 1, Fig 1.3). It has been exploited for several centuries as an important component of the demersal deep-water snapper fishery, especially in Hawaii, where *E. carbunculus* is managed as part of a bottomfish complex of seven species (WPRFMC 2009). Previous studies on *E. carbunculus* that focused on their reproductive biology have indicated a preponderance of females in catches across the Indo-Pacific, and sex ratios of sampled populations across oceans are significantly female-biased, ranging between 1.7:1 to 2.54:1 from the Indian Ocean to the Hawaiian archipelago, respectively (Everson 1984; Williams et al. 2018). Sexual dimorpismresult (size, color, behavior, etc) is rare among the Lutjanidae family, especially in the eteline snappers (Kami 1973; Grimes 1987; Luers et al. 2018). However, recent studies on *E. carbunculus* have reported sexual size dimorphism in maximum length, with females tending to be of lengths 20% longer than males (Williams et al. 2017; Chapter 2: Results).

The collection of sex-specific data is needed for appropriate fisheries conservation and management, it is also important for basic fisheries research. Age-based demography, reproductive biology, and sex ratio are critical to a comprehensive and effective fisheries management plan for marine fish; including deepwater snapper species (Newman et al. 2017; DeMartini 2016). Disproportionate catches of one sex over the other, if they actually represent an unbalanced adult sex ratio, could indicate potential impact on population dynamics of the stock that might result from possible egg or sperm limitation arising from low numbers of mature individuals of a particular sex (Alonzo and Mangel 2005; Molloy et al. 2009; Smith et al. 2014). Information on sex ratio, paired with species-specific size-at-maturity and age-specific demographic parameters, is required to provide the comprehensive input to management necessary for understanding the impacts of exploitation on recruitment overfishing and disproportionate sex ratios. To better provide sex-specific information for Lutjanid snappers the objectives of this study were (1) to confirm that sex of *E. carbunculus* can be identified using external urogenital characteristics and (2) to develop criteria for quantifying these characteristics to distinguish the sexes using metrics describing the urogenital region.

METHODS

Sample selection

Specimens of *E. carbunculus* (*n* = 157) were collected from October 2013 to September 2018, which included months prior to and during peak spawning periods so as to ensure that observations were not limited to periods of spawning. Multiple methods were used to collect specimens, including both fisheries dependent and independent surveys across the main Hawiian Islands (MHI), spanning 18°-22° N latitude (see Chapter 1, Fig. 1.1). Fish were collected using vertical dropline, consisting of multiple baited hooks, fished to depth of 100–300 m. Fish of a broad size range were sampled that included both mature and immature individuals.

All fish utilized in this study were either processed on the same day that they were collected or placed in coolers with saltwater and ice mix and processed as soon as the cooperative fisher was available to deliver (usually within 1-2 days). Whenever possible the following metrics were recorded for each fish: collection date, capture location (general island or specific GPS data), collection depth (meter or fathoms), fork length (FL, to nearest 0.1 cm), round body weight (RW, to nearest 0.01 kg), and, after examination of external characteristics, gonad weight (GW, to nearest 1 g).

External sex characteristics and accuracy of identification criteria

As these fish are collected from depths of 200-300m, the effects of barotrauma (e.g., prolapsed gonads or extruded reproductive material) could bias the determination of sexual identity of some specimens. Therefore, fish were wiped clean to remove any expelled waste material. Further, any fish that still had visial evidence of sex such as oocytes extruding through the genital pore were not used. Fish that were selected for this study had sex determined using methods similar to those used by Smith et al. (2014) and Luers et al. (2018). Briefly, the fish was examined with a small probe to locate the urogenital pore(s) and anus of both sexes (Appendix B7). Criteria were noted for possible sex-specific variations in general appearance, color, spacing or location of urogenital pore(s). Then the fish was scored as a male or female, without *a priori* knowledge, based on the location of each pore in relation to the other and the appearance of the pores and surrounding tissue. Digital photographs were taken of each individual for later reference and, whenever possible, photos were taken of both sexes, side by side, of fish with comparable lengths (Fig. 3.1).

Sexual identity was verified by direct visual inspections of the macroscopic appearance of gonads after dissection or by expression of milt from insertion of a probe into the genital pore. The sex of a subset of fish ($n = 60$) was confirmed by histological preparation of a gonad sample, followed by microscopic examination of that sample based on methods in DeMartini 2016). Breifly, DeMartini (2016) methods included a cross-section of the ovary or testis that contained the gonad wall and were taken from the central portion of the gonad. These were preserved, then processed by a contracted vendor: specimens were dehydrated, embedded in paraffin, sectioned, and mounted on microscope slides. Mounted sections were stained with hematoxylin and counterstained with eosin. The processed histological slides were evaluated using a compound microscope at 60-400X using the histological criteria of Brown-Peterson et al. (2011).

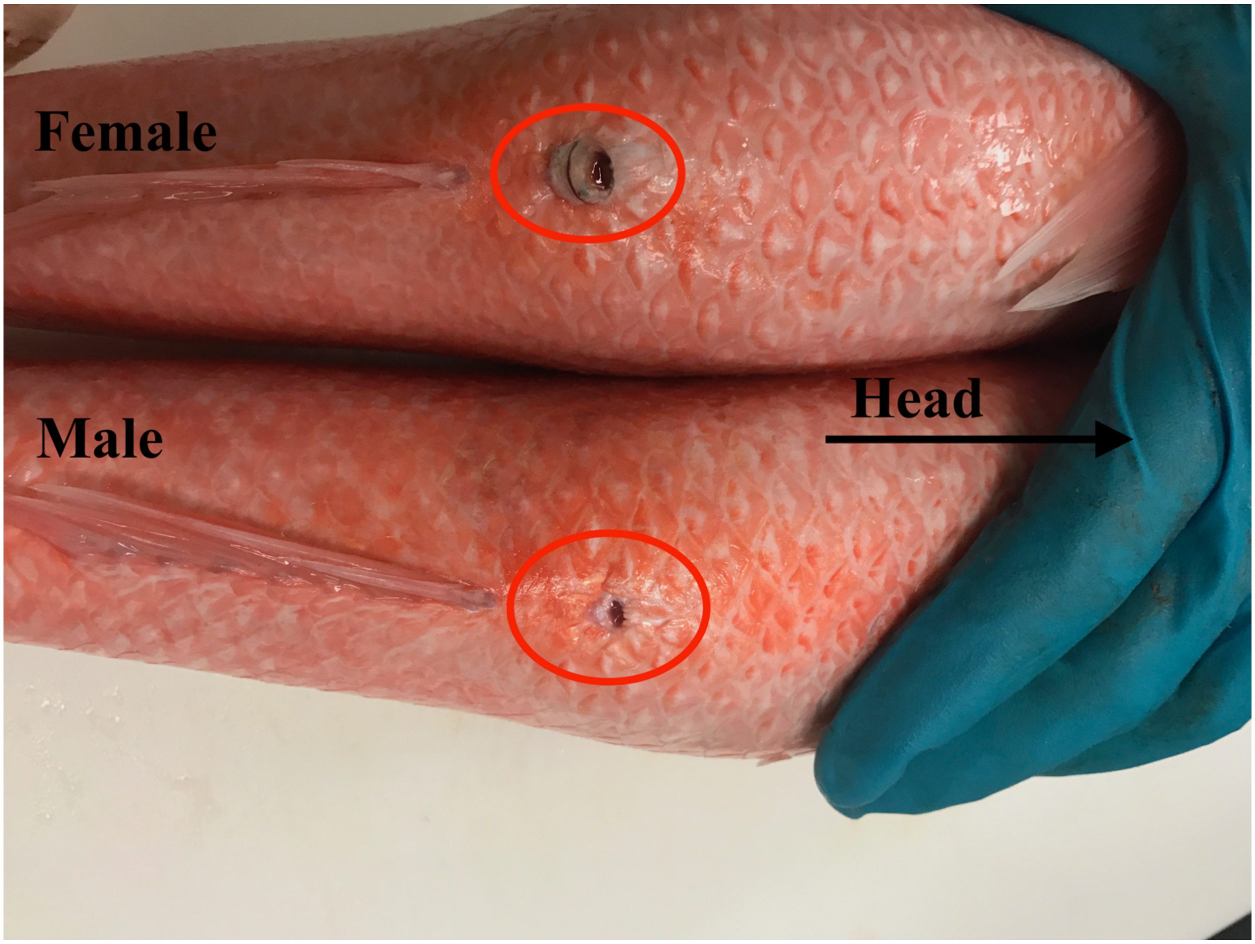

Figure 3.1. Ventral view of female and male *E. carbunculus* showing general location and external characteristics of urogenital regions of comparably sized individuals (Top: Female 32.4 cm FL; Bottom: Male 33.4cm FL).

Urogenital measurements

For a subset of fish (n = 88), urogenital regions were measured (0.01 mm) using digital calipers; optivisor headband magnification lenses up to 3x magnification were used to ensure accuracy (Appendix B7). The urogenital distance (UD), defined as the entire distance spanning the urogenital region, was measured from the posterior-most edge of the urinary pore to the anterior-most edge of the anus (Fig. 3.2). The papilla septum distance (PSD), defined as the region between anus and urinary pore which includes the opening of the female oviduct, was measured from the anterior-most edge of the urinary pore to the posterior-most edge of the anus. (Fig. 3.2).

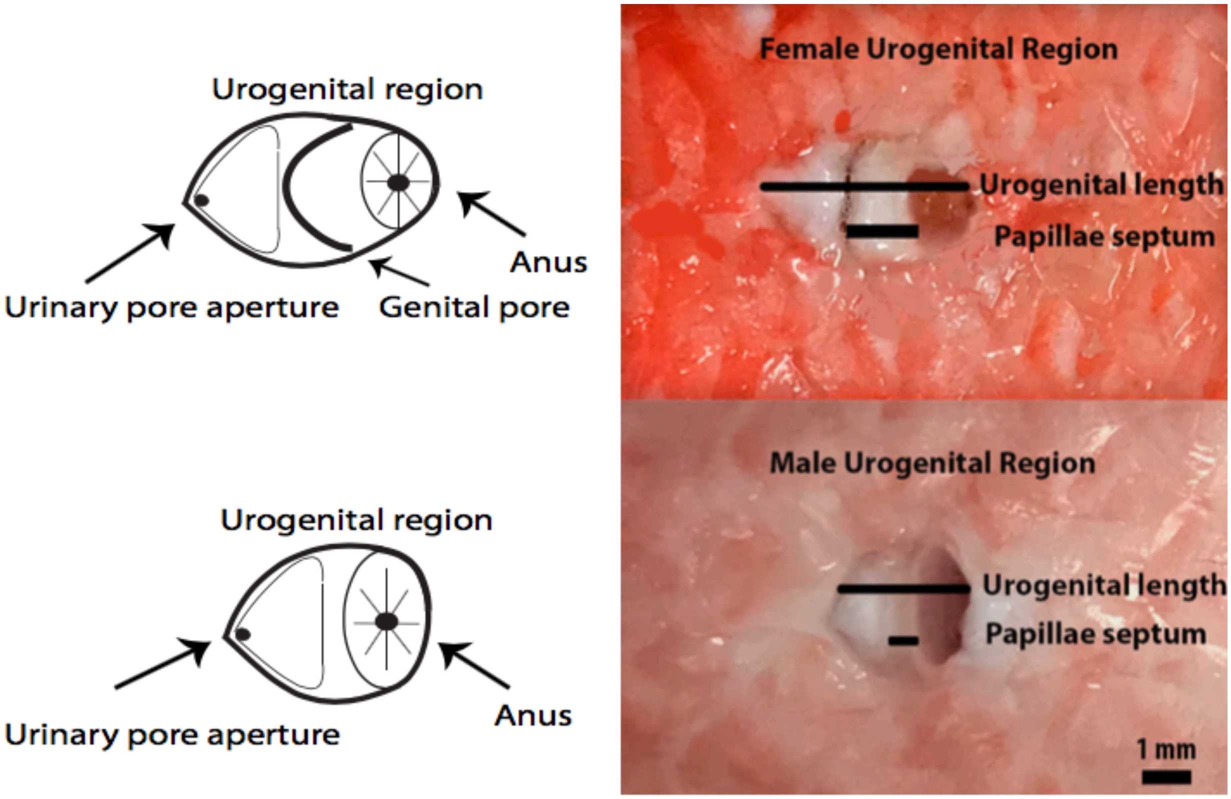

Figure 3.2. Photographs (external view) of urogenital regions of E. carbunculus (right), with anus, genital pore, and urinary pore depicted diagrammatically (left). Top: female 35.6 cm FL; Bottom: Male 35.9 cm FL. Scale bar = 1 mm.

Statistical analyses

Urogenital metrics relationships to fork length were evaluated using linear regression. Papillae septum distance (PSD) relationships were fitted with the following allometric equations:

$$PSD = \alpha + FLb$$

where *PSD* was measureed in millimeters, FL was measured in centimeters and the parameters *a* and *b* were estimated using the available data for measured individuals of both sexes.

Simple logistic regression was used to model and predict the successful identification of gender using a general linear model (GLM) framework implemented in R with the stats package (glm(), Chambers and Hastie 1992). The glm() model was fit with binomial error (link = “logit”), while the response value was Sex identification (M = 0, F =1) and interaction terms was based on an index (PSFL) of the log transformed urogenital region lengths and fork length.

$$PSFL = \log(PSD)/FL$$

where *PSFL* is equal to the ratio of log tranformed PSD relative to FL.

All statistical analyses were conducted in statistical program R version 3.1.1 (R Core Team 2016) and R studio version 1.3.05 (Rstudio, Inc 2015).

RESULTS

Specimen collections and macroscopic identification

All of the 157 *E. carbunculus* were examined using the visual examination of the external sex characteristic criteria; of these sampled fish, 94 (60%) females and 63 (40%) males provided quantitative metrics for sex identification. Fish of a broad size ranging from 16.7 - 49.7 cm FL were sampled (Table 3.1, Fig. 3.3). Neither length distributions nor mean lengths (cm FL) differed between the two sexes (K-S test on length: D=0.1614, p=0.337; t-rest on mean length t = 0.971, p = 0.334). All 157 of the fish were sexed. Of these, 60 individuals had their sex validated by histological examination (35 females and 25 males), three males were verified by their expression of milt, and the remainder were verified by dissection. In total, 151 were correctly sexed for an overall accuracy of 96.2. % (Fig. 3.4). All but one immature female (22.7 cm FL) was correctly sexed and this female was verified through histology. Five males in total were incorrectly sexed, and of these, three were verified through histology and two by dissection. Sex-specific accuracies were 98.9 % and 92.0% for females and males, respectively.

Table 3.1. Descriptive statistics for female and male *E. carbunculus* examined for urogenital sex determination. SE = standard error, SD = standard deviation, GW = gonad weight (g), No. = number sampled.

Sex	Variable	Mean	SE	Median	Range	SD	No.
Female	FL (cm)	33.8	0.73	33.9	19.4-45.9	6.96	94
Male		32.6	1.09	32.4	16.7-49.7	8.03	63
Female	Wt (g)	376.7	25.45	322.0	102.0-940.0	244.16	94
Male		394.5	36.75	304.0	60.0-948.0	270.04	63
Female	GW (g)	27.21	4.02	15.26	0.15-262.3	37.25	86
Male		2.34	0.32	1.74	0.03-11.75	2.33	54
Female	Urogenital	6.07	0.33	6.35	3.0-12.02	2.09	47
Male	dist. (mm)	4.24	0.17	4.10	2.26-6.04	1.02	36
Female	Pap.	2.07	0.11	3.05	0.87-3.94	0.74	47
Male	septum dist. (mm)	0.45	0.03	0.44	0.15-0.77	0.16	36

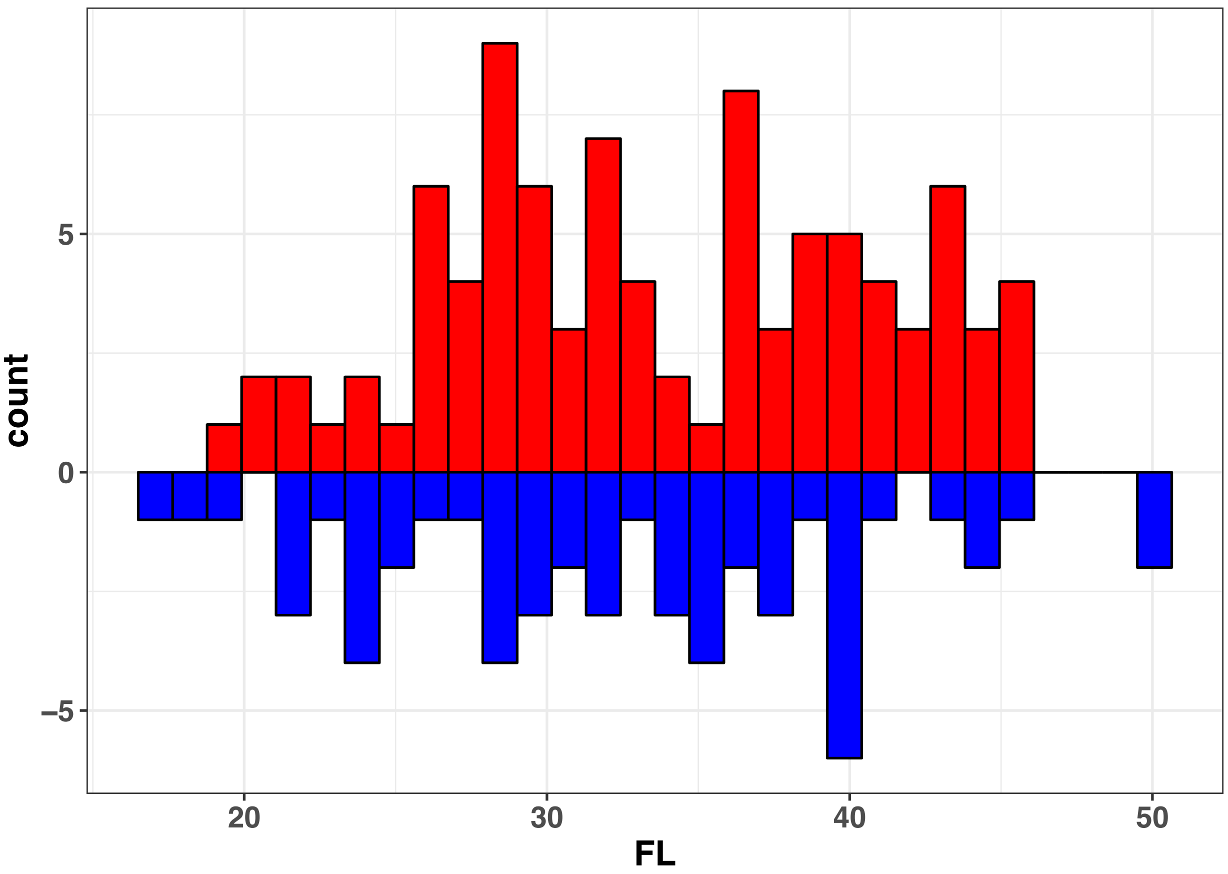

Figure 3.3. Length frequency distributions of all *E. carbunculus* examined for sex determination. All fish were collected in the MHI subregion (Red = female, Blue = male).

Sex differentiation using external characteristics

The accuracy of visually determining the sex of females was greater than that of males, especially in individuals greater than the median size-at-maturity (23.4 cm FL, DeMartini 2016). As overall body length increases in *E. carbunculus*, the sex-specific differences (visually or papillae length measurement) increases. This was because of the difference in urogenital appearance for each sex. The urogenital pore(s) of both female and male *E. carbunculus* were surrounded by white papilla tissue (see Fig. 3.2). These pores all lie along the ventral midline, although there is a visible distinction between the two sexes. In females the anus is farthest anterior, followed by the genital pore, and the urinary pore is located directly posterior to it. The female genital pore is enlarged and crescent shaped, and the septum tissue is papillae-like and divides the two pore openings. The female urogenital papillae is dark and more crescent shaped, and the papillae septum is whiter in color than in males. Males lack a distinct separation between the urogenital pore and urinary pore at the epidermal layer. Further investigation of males indicated that the sperm duct joins with the urinary bladder duct just interior of the urogenital pore. Males therefore appear to have a single pore opening that serves both reproductive and excretory functions.

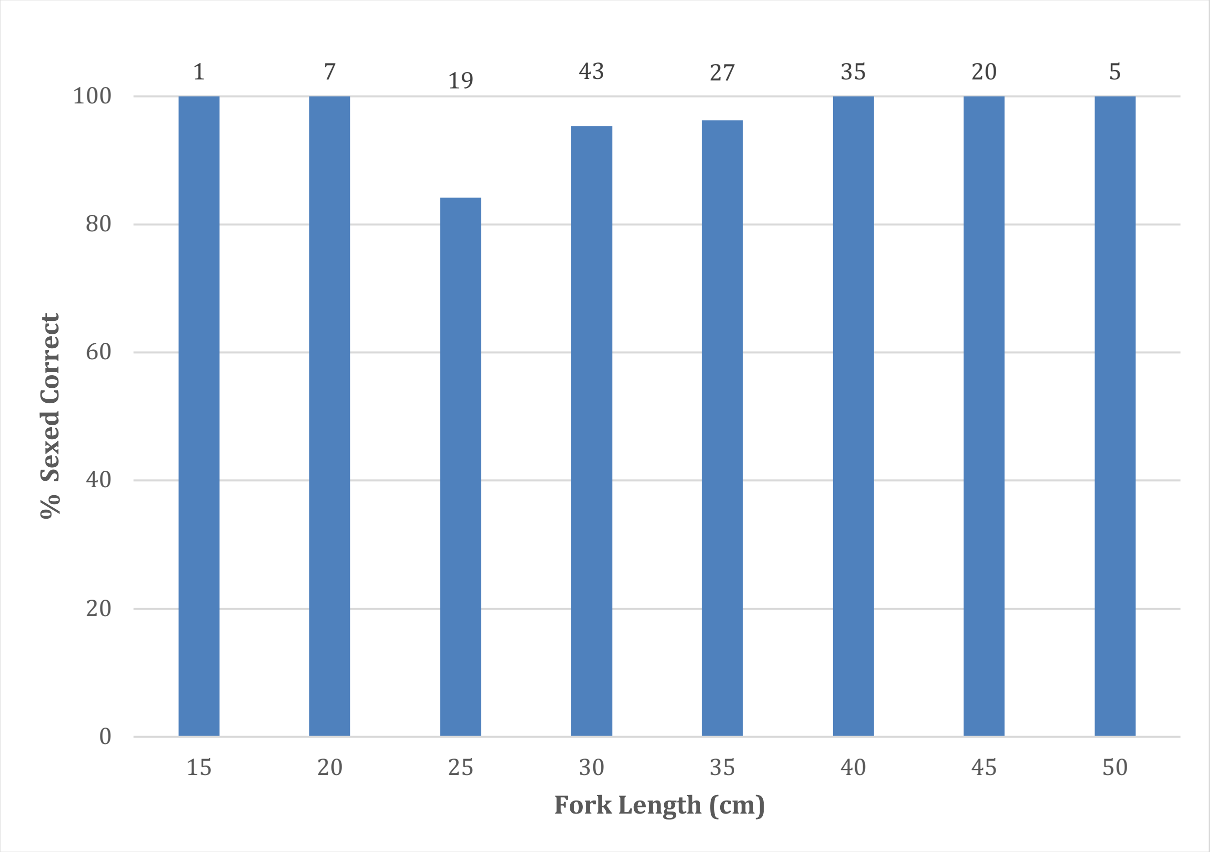

Figure 3.4. Percent accuracy by fork length of external sex characteristics of 157 E. carbunculus from MHI. Samples sizes are indicated above respective histogram bars.

Urogenital measurements

A subset of 95 *E. carbunculus* were evaluated for sex-specific morphometry in urogenital measurements. The length distributions of the subset were similar for the 50 females and 45 males examined (K-S test on length: D = 0.1779, p = 0.372). The two sexes differed significantly in UD (D = 1, p < 0.001); both mean and maximum UDs were greater for females than males (Table 3.1). Fork length was significantly correlated with UD for each sex (females: product-moment correlation coefficient $r^2 = 0.574$, males: $r^2 = 0.478$). For size classes greater than the size-at-maturity (23.4 cm FL, MHI), the UDs of females averaged twice those of males (Appendix B8). Similary, papillae septum distance (PSD) exhibited distinct differences between sexes yet there were no overlaps in the length ranges (D = 0.6667, p < 0.001; Fig. 3.6**)**. As overall body length increases in *E. carbunculus*, the sex-specific differences (visually or papillae length measurement) increases. This was because papillae septum length tends to be larger (>1.0 mm) to accommodate the two separate female urogenital pores (urinary and oviduct). There was a strong relationship between fork length and papillae septum length in females ($r^2 = 0.582$); however, there was not as strong of a correlation for males ($r^2 = 0.083$). The PSD-fork Length realtionship was best described as follows:

$$PSD\ (female) = -0.9996 + FL * 0.08$$

$$PSD\ (male) = 0.304 + FL * 0.005$$

Analysis of covariance (ANCOVA) indicated there was a significant difference between regression slopes for female and male fish ($F_{1,91} = 51.4,\ p < 0.001$). The PSD of females were greater than 1.0 (mm) in all fish examined, while the maximum urogenital length of the male was never larger than 0.75 (mm) (See Table 3.1).

Prediction of sex using papillae septum index

Urogenital measurements showed a strong relationships between fork length and papillae septum distance and the logistic regression model also accurately predicted the response variable (Sex) of *E. carbunculus* based on independednt predictor (index PSFL) at p = 0.025.

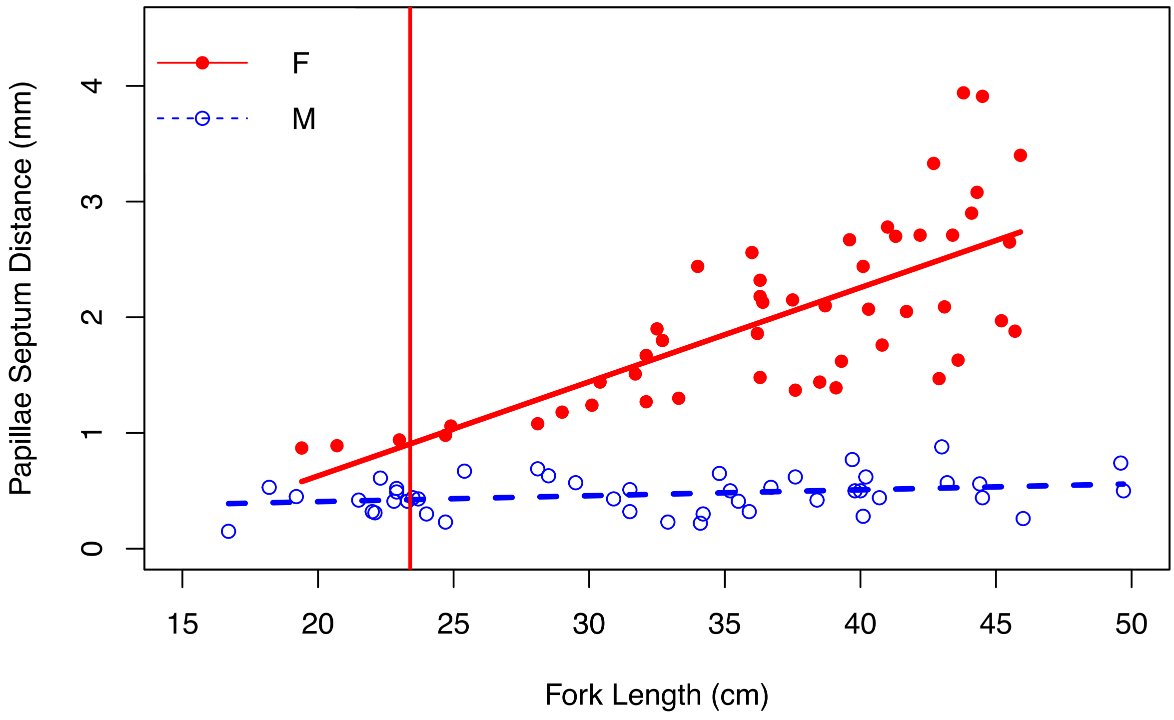

Figure 3.5. Scatterplot and linear relationships between papillae septum distance (PSD; mm) and fork length (cm) of E. carbunculus by sex (Red = female, Blue = male). Red line indicates the median size-at-maturity (23.4 cm FL) of females in the MHI. The female regression equation was Papillae Septum Distance = -0.9996+ FL(0.08) (R^2= 0.58; n = 50), while the male was Papillae Septum Distance = 0.304 + FL(0.005) (R^2= 0.08; n = 45).

DISCUSSION

The use of papillae septum characteristics to distinguish sexes of *E. carbunculus* has proven to be an effective method with high accuracy overall (96%). For the first time, evidence of significantly distinct morphology has been quantified to show sex-specific genital papillae lengths and corroborate both sexual dimorphism and the visual identification of papillae septum when determining sexes in *E. carbunculus*. The use of urogenital characteristics has similar accuracies across the size distributions of both sexes, including immature and mature individuals (98.9% and 92.1% for female and male, respectively). These methods, adapted from a previous study on other pelagic (Smith et al. 2014) and deepwater species (Luers et al. 2018 unpublished data) for which comparable accuracy rates were reported, clearly demonstrate sexual dimorphism in the urogenital region of the eteline snapper *Etelis carbunculus*.

Sexual dimorphism has been reported as rare amongst snapper species (Lutjanidae), (Grimes 1985; Maritnez-Andrade 2003). Yet, Kami (1973) indicated that two species (genus *Prisitpomoides)* were found to exhibit differnces in on the coloration of the ventral lobe of the caudal fin (*P. auricilla)* and anal or pelvic fin (*P.filamentosus*) but these findings were not confirmed during a separate studies on these same species (Luers et al. 2018). Recently, the application of urogenital characteristics were effective when applied to other deep-water eteline snappers in Hawaii (*P. zonatus, P. seboldii, Apharerus rutilans* and *Etelis coruscans,* M.A. Luers and R.Nichols unpublished data). However, the use of urogenital distances were not evaluated and further work would be required to determine of these were sexually dimorphic. Nonetheless, the success of this study using urogenital characteristcs to determine sex in *E. carbunculus*, and anecodtal evidence for more than one species of Lutjanid snapper may warrant further investigations of these methods across the entire family of Lutjanidae.

One potential bias, of this study's sampling methodology was the lack of females greater than 50 cm FL. This study and others have reported preponderances of large females in the catch and no males being obtained greater than 54 cm FL (Everson 1984; DeMartini and Lau 1999; DeMartini 2016; William et al. 2017). This study has excluded the evaluation of females greater that the maximum length threshold of males (~50 cm FL), as there is no evidence that these individuals of greater length would be males, regardless. This was reinforced by the significant difference of papillae septum length, which indicated that, by the median size-at-maturity (23.4 cm FL, DeMartini 2016), the papillae septum length was at least twice that of males and simultaneously displays a visually distinct dimorphism between sexes. Any future work utilizing these methods should not exclude size ranges unless sexual size dimorphism is evident in the length distributions as seen in *E. carbunculus*.

There are some valid limitations to this methodology, especially in sex determination of smaller fish, detecting barotrauma, and in detecting reproductive maturity. Smith et al. (2014) reported higher instances of incorrectly sexed fish in smaller size classes (< 500 mm) for *Seriola dumerili*, this was based on difficulty identifying the urogenital pores on these smaller individuals and the corresponding misidentification of female or male genital pore in an individual. The present study attempted to minimize misidentification of sex by the use of headband magnification lenses— a recommendation made by Smith et al. (2014). By including magnification, successful determination was made on the smallest individuals of both sexes (19.4 cm FL and 16.7 cm FL, a female and male, respectively) and for the measurement of papillae septum distances < 1.0 mm. Presumably, there would be an increase in overall measurement error associated with methodologies if no magnification aid was used, especially on smaller individuals.

Additional concerns may arise with individuals that express physical signs of barotrauma. The expansion of the swim bladder from barotrauma is known to prolapse reproductive organs, stomach, and other internal organs (Peregrin et al. 2015; Newman et al. 2017). These could distort or rupture urogenital pores of individuals and cause misidentification. However, if an individual is suffering these impacts from barotrauma, most likely they will not be released and a more thorough evaluation of the gonad could be used to determine the sex of these sacrificed individuals. The most concerning limitation of these methods is determination of the maturity stage of an individual. At present, these methods alone can only determine sexual identity, not maturity, unless an individual is spawning and expressing milt or eggs. The ability to distinguish maturation stage would require either the use of ultrasonic technology, urogenital catheterization, or the sacrifice of the individual for removal of gonad tissue and histological examination.

The inclusion of additional methods of external sex determination would surely increase the accuracy of sex determination and support the staging of maturity (Ferraz et al. 2004; Newman et al. 2008); however, they all suffer from increased cost (greater processing time and effort per specimen). In many cases (data poor and/or developing fisheries), the cost benefit may not merit the more detailed reproductive data generated by including additional methods to determine reproductive maturity. Size-at-maturity of *E. carbunculus* was not investigated as part of this study because it has been found to be similar in the South Pacific and NWHI (~ 27.6 -27.9 cm FL), although slightly smaller in the MHI (23.4 cm FL) (Williams et al. 2017; DeMartini 2016). Nevertheless, as mentioned in Newman et al. (2015), the added value of including sex-specific data (length and age) would, at the very least, be highly beneficial to any data-poor or developing fishery examining demographic variation for fisheries management.

Here we have shown a simple and reliable method to visually determine sexual identity in *E. carbunculus* in the field using external characteristics. The sexual identity data that this approach can provide could readily be incorporated into a stock assessment or fisheries management plan. Expanded applications of the method will hopefully contribute to a broader understanding of sex ratios in Lutjanidae species, especially those deepwater snapper species throughout the Indo-Pacific, thereby enabling improved management through estimation of sex-specific exploitation in the fisheries. Application of this method to market studies, movement studies and others can also now be facilitated.

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CONCLUSION

Given the prolonged exploitation of deep-water snapper species (commercially and recreationally) reducing the paucity of information on the age-based life-history of deep-water snapper species is paramount for providing a comprehensive basis to stock assessments, management decisions and understanding the impacts of exploitation of deep-water snappers. The *E. carbunculus* collected within the Hawaiian Archipelago exhibit patterns in growth and longevity that are comperable to other deep-water snapper species with extended longevities, yet this study highlights two important points. First, the variability in demography, across the spatial distribution, and second that lifespan does not appear to be linked with size for *E. carbunculus*. This study presents a better understanding of a fish populations response via life history over the course of a century of exploitation and comparisons to less exploited population across regions of the Indo-Pacific. The potential impact to the resiliency of *E. carbunculus* in the Hawaiian Archipelago, as seen with age truncation, could make the population less durable to perturbation in the environment or fishing pressure. The increase in evidence of spatial variation in age-based demography support the reliance on age-based life history information, rather than length-based, and the inclusion of sex-specific life history when providing direction to fishery management. Both demographic variation and age-truncation should be of particular note, as the impact is rarely considered in management plans; however, this information is advantageous to developing countries and data-poor regions who will benefit from this nascent research on sex-specific life history. The hope is that this study may facilitate the development of more robust datasets and reduce the number of data-poor regions actively targeting and currently exploiting deep-water snapper species, in particular *Etelis carbunculus*.

APPENDIX A: Additional Tables

Table A1. Sex ratios and parameter estimates for age-specific sex change from Hawaiian Archipelago. X^2: test of significance; n: number of samples used to estimate each parameter.

Function	Parameter	Pooled	NWHI 1978-1980	NWHI 1997-1998	MHI 1997-1998	MHI 2008-2017
Sex ratio	M:F	1:2.12	1.0:1.25	1:2.6	1:1.41	1:2.54
	n	1380	42	194	162	577
	X^2	181.15	39.92	39.92	7.76	109.19
	p	< 0.001	<0.001	<0.001	<0.005	<0.001

APPENDIX B: Additional Figures

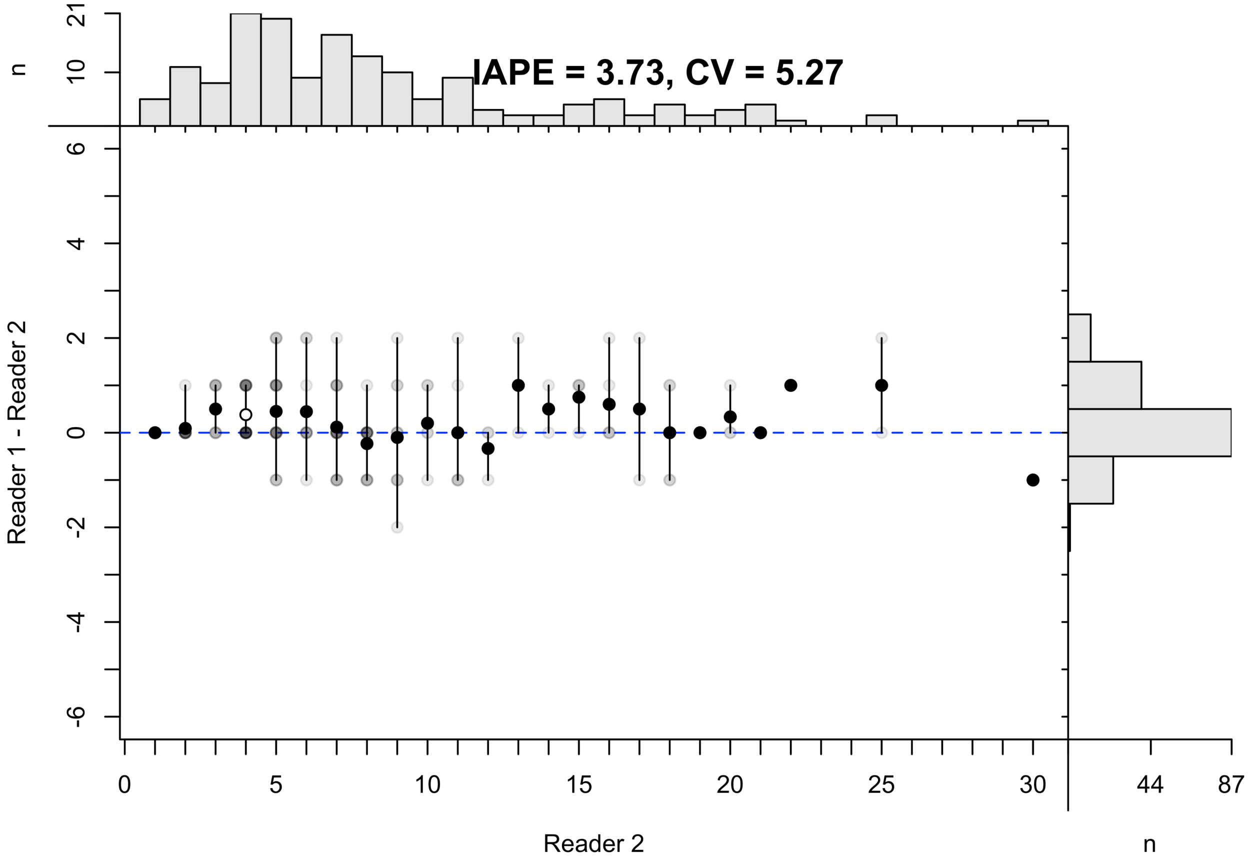

Figure B1. Bland-Altman Plot between readers. Index of Average Percent Error (IAPE) and Coefficient of Variation (CV) listed on top. Reader 1 = Author; Reader 2 = experienced reader.

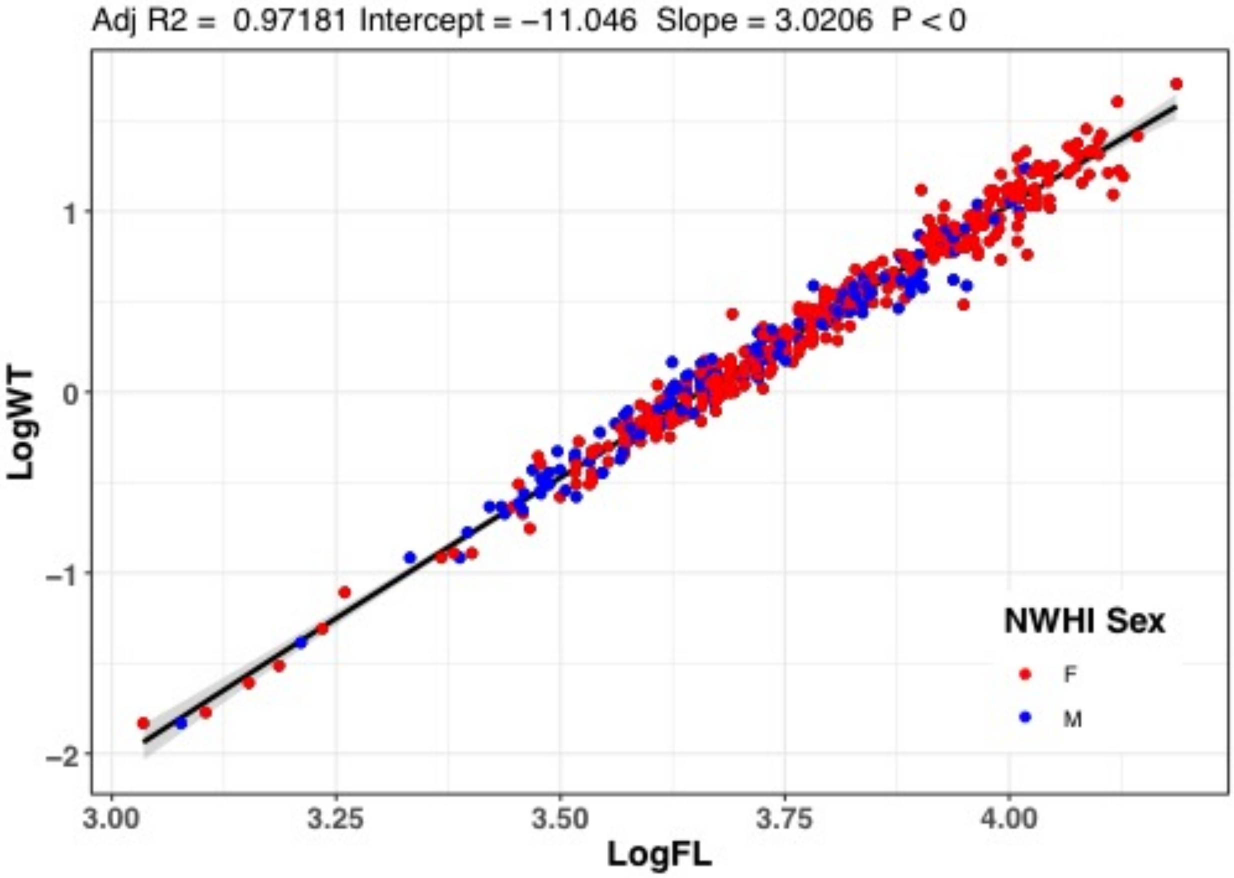

Figure B2. Log transformed Length-Weight Regression for NWHI regions (pooled sampling years). (Red = female, Blue = male)

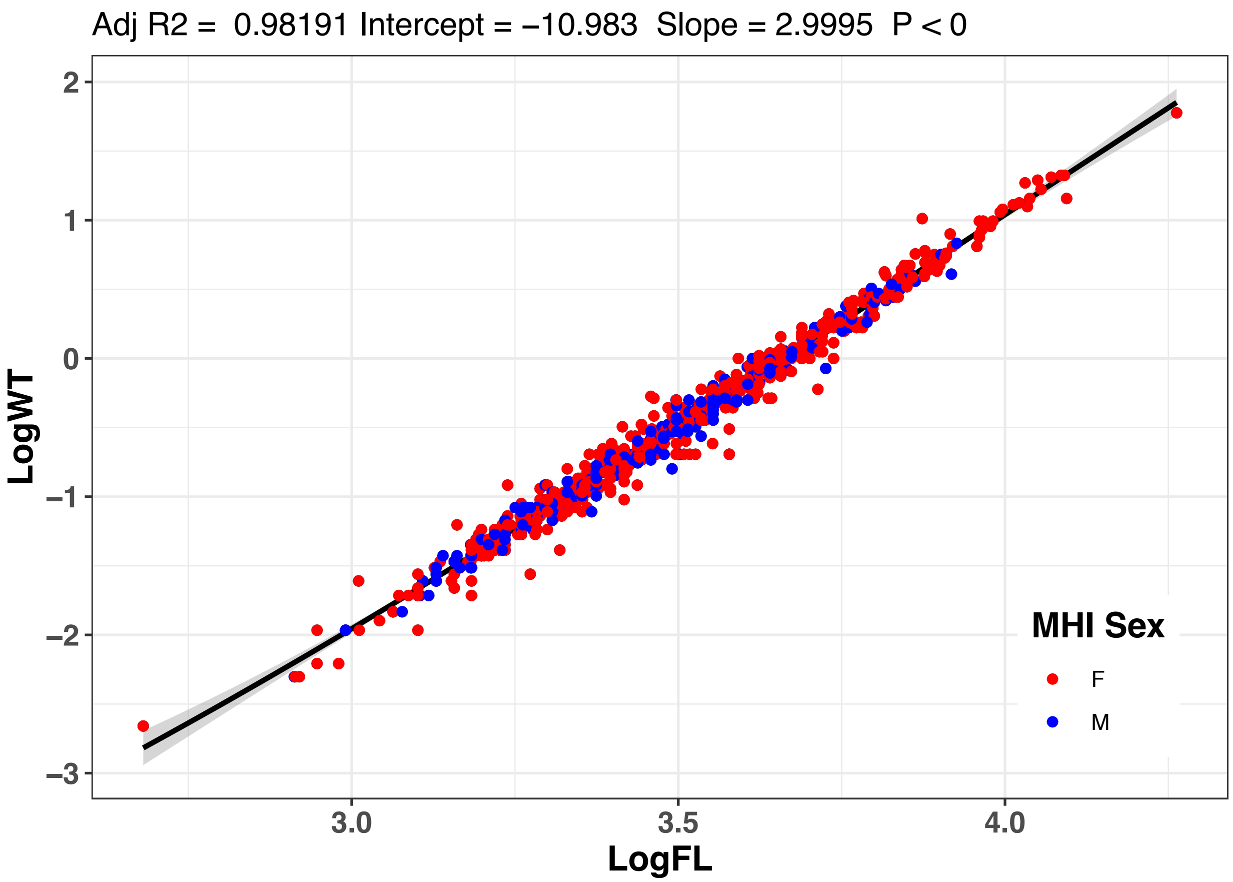

Figure B3. Log transformed Length-Weight regression for MHI regions (pooled sampling years). (Red = female, Blue = male)

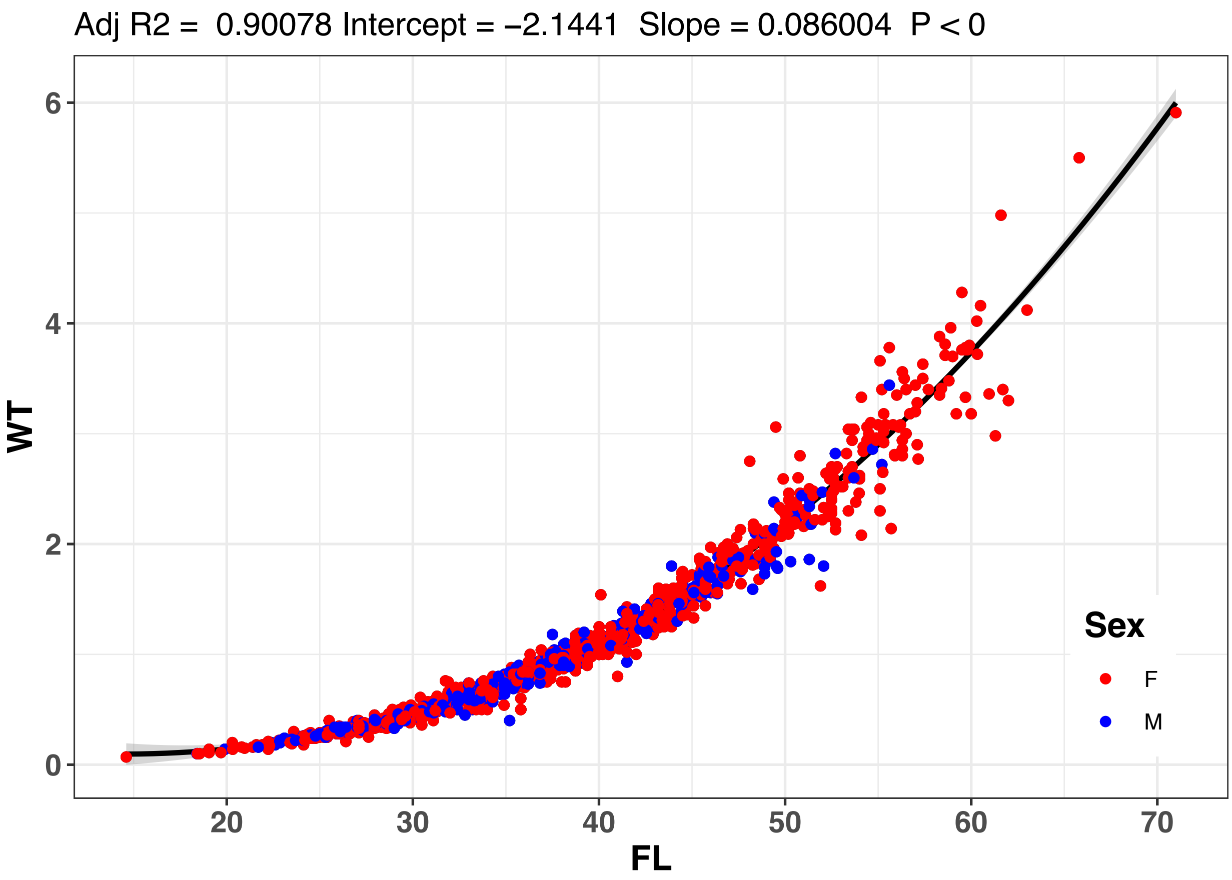

Figure B4. Two-parameter power function for Length-Weight relation of pooled regions.

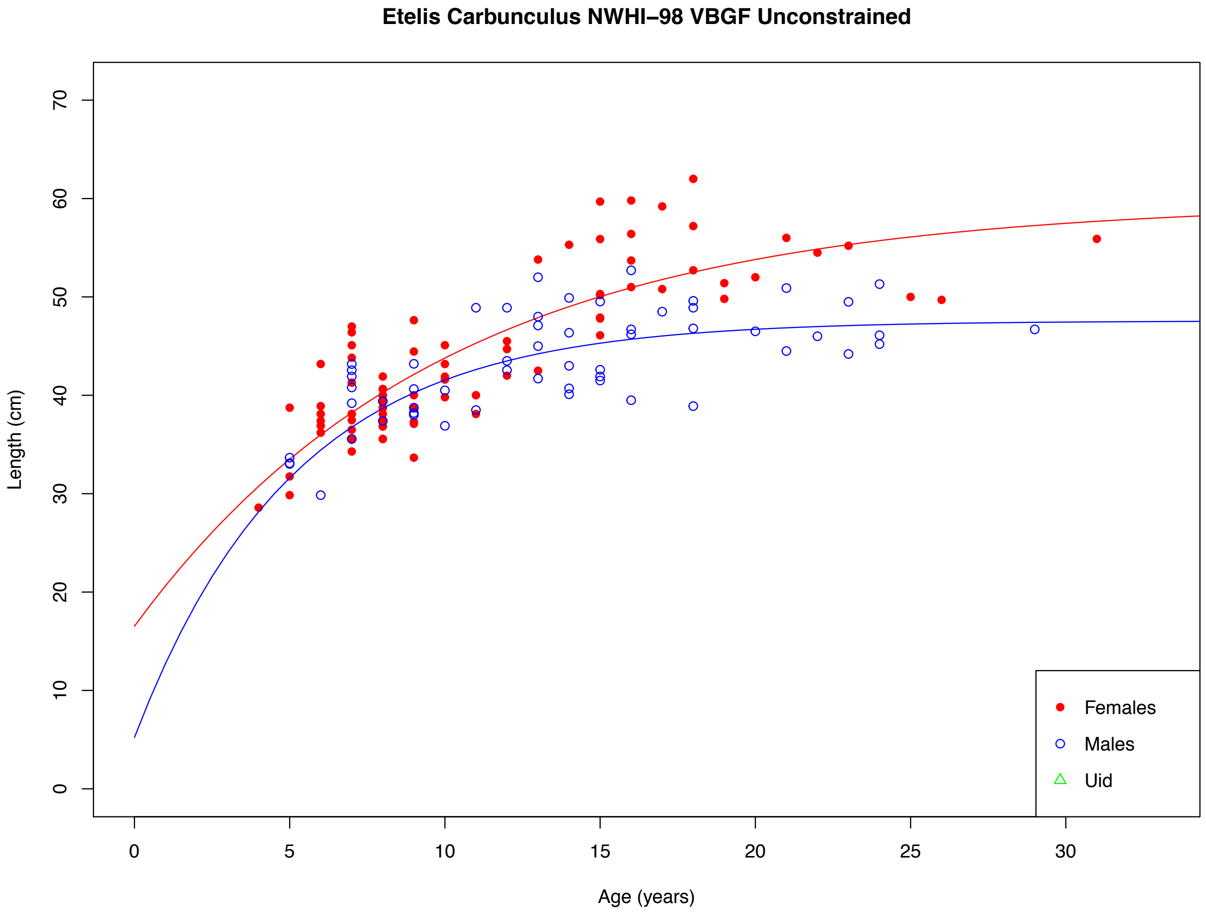

Figure B5. NWHI unconstrained VBGF model showing growth profiles of females (Red) and males (Blue) from Period 2 (1997-1998). Green triangles indicate individuals with un-identified sex.

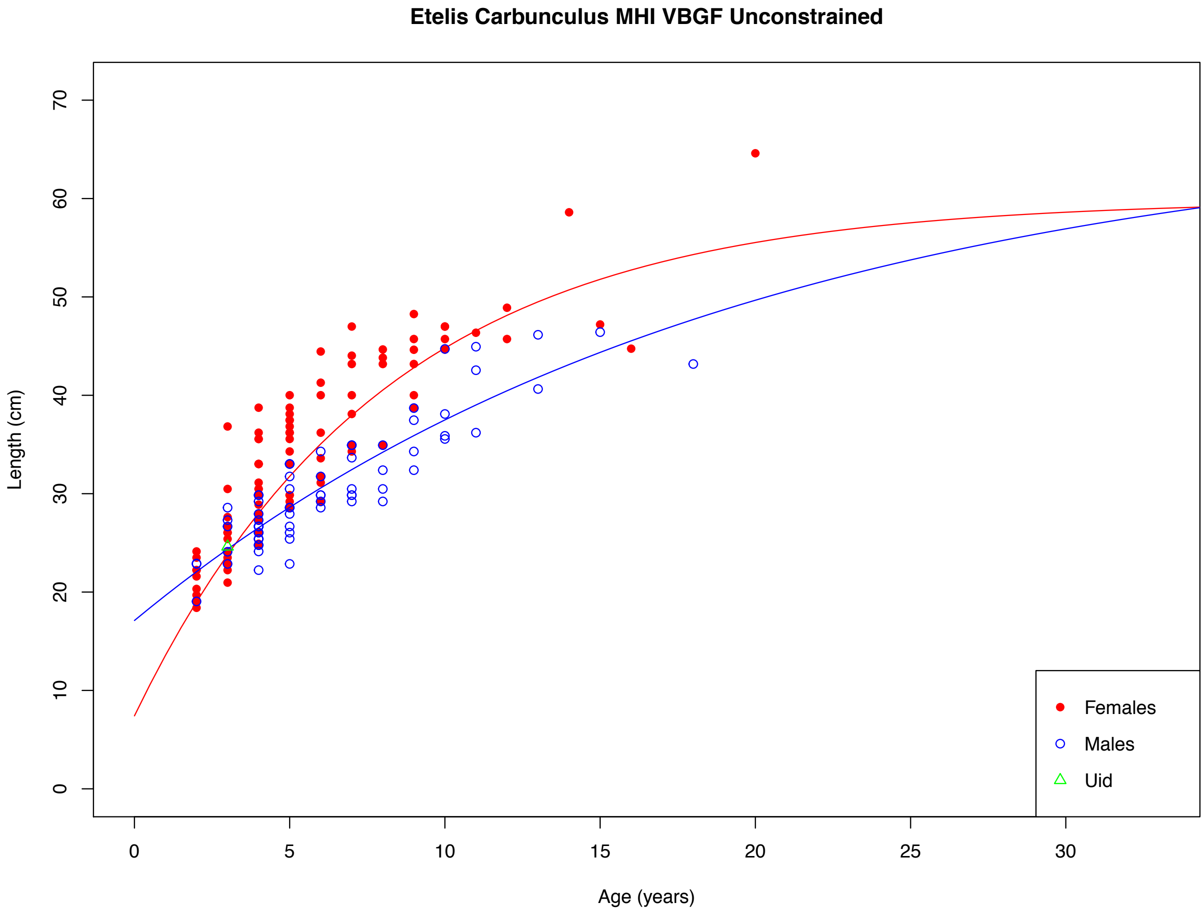

Figure B6. MHI unconstrained VBGF model showing growth profiles of females (Red) and males (Blue) from Period 2 (1997-1998). Green triangles indicate individuals with un-identified sex.

Figure B7. Method of recording urogenital measurements of *E. carbunculus* with digital calipers and optivisor 3X magnification lenses. Photo courtesy of E. Reed.

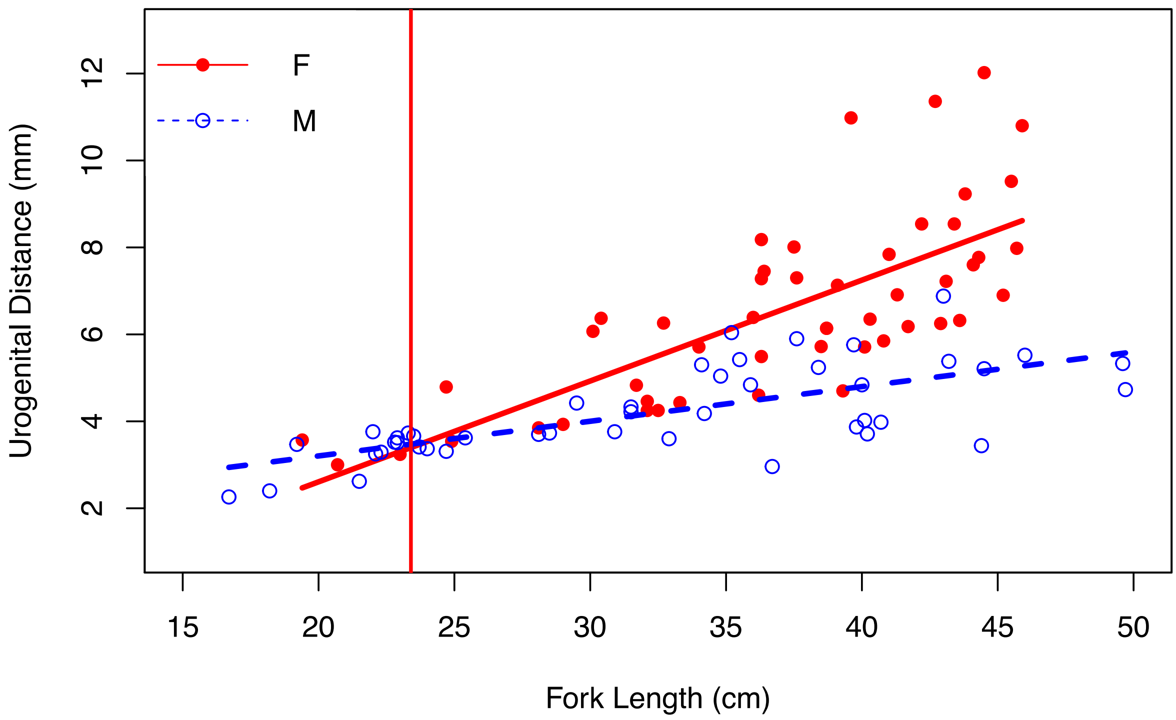

Figure B8. Scatterplot and linear relationships between urogenital distance (UD, in mm) and fork length (cm) of *E. carbunculus* by sex (Red = female, Blue = male). Vertical red line indicates the median size-at-maturity (23.4 cm FL) of females in the MHI. The female regression equation was UD = 0.213(FL) – 1.211 (R^2= 0.49; n = 50), while the male was UD = 0.077(FL) + 1.661 (R^2= 0.48; n = 45).