

# **Growth and Reproduction of Hawaiian Kala, *Naso unicornis***

## FINAL REPORT

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## Executive Summary

Surgeonfishes (Acanthuridae) are a major component of Hawaiian reefs and as algal grazers, play an important role in structuring coral reef benthic communities. Declines in the overall catch and size of commercially targeted species have raised concerns about the long-term sustainability of local stocks. The bluespine unicornfish, *Naso unicornis* (or kala), is a large (up to 69 cm total length) surgeonfish that occurs in shallow habitats throughout the Indo-Pacific region and is extensively targeted by fishers. To support the sustainable management of Hawaiian kala we generated information on morphometric relationships and sex-specific size-at-maturity and growth rates.

A total of 197 kala were collected from four locations on Oahu (n = 137) and six locations within the Papahānaumokuākea Marine National Monument (n = 60). Collections were initiated in February and completed in June, 2009. Individuals ranged in size from 7.5 cm to 58.6 cm fork length with a mean fork length of 34.7 cm ( $\sigma = 12.83$ ). The length-weight relationship calculated from the total collection was:  $W = 0.026(FL)^{2.923}$ . Gonads from 182 individuals were examined histologically, from which we identified a sex ratio of 1.4:1 (M:F). Reproductive males were observed in all collections (n = 67) and we estimated the size at maturity ( $L_{50}$ ) for males to be 28.6 cm. Reproductive females were only recorded in May (n = 1) and June (n = 24). Because of an inability to distinguish between immature and non-spawning females, female  $L_{50}$  calculations were restricted to June collections. Female  $L_{50}$  based on June collections (n = 51) indicates sexual maturity at 37.8 cm.

Individual age was calculated from annual growth rings deposited within the sagittal otoliths. The annual deposition of alternating opaque and translucent bands has been repeatedly validated in the majority of reef fish families including confirmation of annual deposition in multiple species of surgeonfishes. Sampled fish ranged in age from 1 yr to 58 yrs with a mean age of 12.8 years ( $\sigma = 10.18$ ). There was no evidence of length-age sexual dimorphism or differences in growth between locations. The relationship between length and age for the full data set was described by the von Bertalanffy growth equation:  $l_t = 51.20(1 - e^{-0.167(t+0.5)})$ , with males maturing at 4.5 years (28.6 cm) and females maturing at 7.5 years (37.8 cm).

Hawaii's current legal size limit for kala is 35.6 cm (14 in) fork length resulting in nearly all male kala entering the fishery after maturation. For females, our observations indicate that approximately two thirds enter the fishery before maturation. Most surprising was our identification of 15 fish that were greater than 30 years old and two fish, collected off windward Oahu, that were more than 50 years old. Considering the well documented relationship between fish size and net fecundity, with larger, older fish producing exponentially more eggs and faster growing more starvation resistant larvae, the establishment of a maximum size limit would help to ensure the long-term sustainability of an ecologically important reef fish.

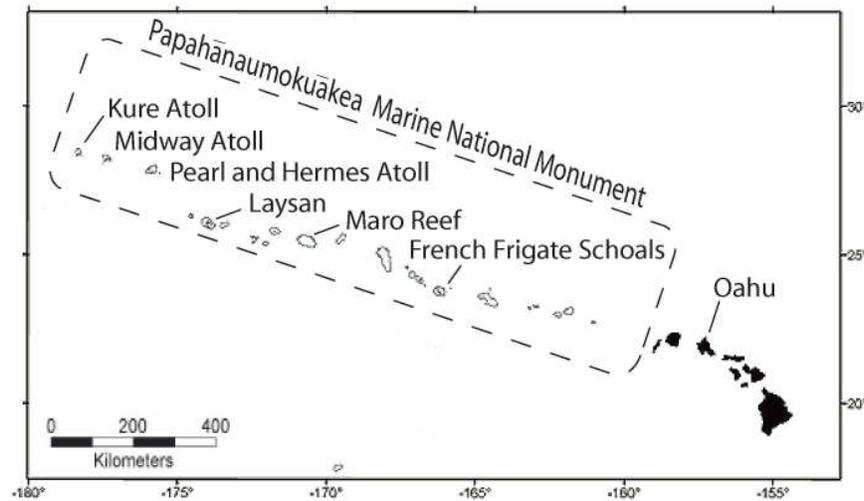
## INTRODUCTION

As a formerly abundant, large bodied herbivore, *Naso unicornis* (kala) has played an important role in structuring Hawaii's near shore benthic communities (Hixon and Brostoff 1996). Kala have been recorded down to depths of 200 m (Chave and Mundy 1994) yet it is primarily an inshore species that regularly moves into very shallow water to graze on leafy algae such as Sargassum species (Randall 2001); a point reinforced by the observation that some Hawaiian fishers bait their hooks with brown algae when fishing for kala (Titcomb 1972). Considering evidence of a clear connection between fishing pressure and target species abundance (Williams et al. 2008), a recent increase in commercial fishing pressure on kala ([http://hawaii.gov/dlnr/dar/fishing\\_commercial.html](http://hawaii.gov/dlnr/dar/fishing_commercial.html)) raises concerns about the long-term sustainability of local stocks. Effective fisheries management requires a robust understanding of target species life-history yet the paucity of published data for kala makes it difficult to predict the effectiveness of current or future management strategies. Herein we estimate the following life-history descriptors for Hawaiian kala: length-weight relationship, age structure, sex-specific size at 50% maturity ( $L_{50}$ ) and sex specific growth rates.

## METHODS

### STUDY SITES

Fish were collected by snorkelers using pole spears at four sites on Oahu and six sites in the Papahānaumokuākea Marine National Monument (PMNM) (Figure 1). Oahu collections were supplemented with the purchase of locally caught, commercially available fish. Unfavorable attention from predators prohibited sampling of the largest kala at PMNM collection sites.



**Figure 1.** Collection sites on Oahu (n = 137) and within the Papahānaumokuākea Marine National Monument (n = 60).

### LIFE HISTORY ANALYSIS

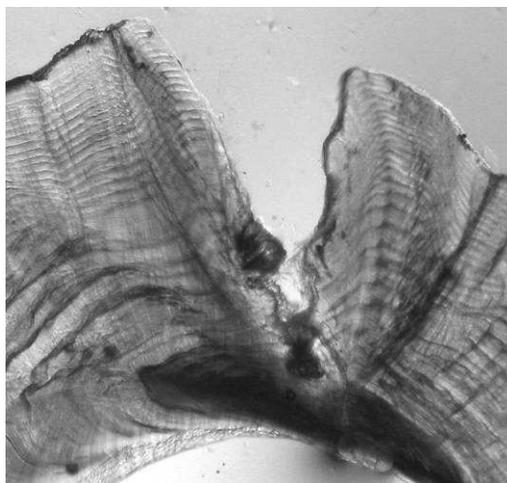
All fish were measured to the nearest 0.01 cm for total length, fork length (FL), standard length, body depth (distance between the origins of the dorsal and pelvic fins), and head length (distance

between the anterior-most part of the head to the posterior edge of the operculum). Fork length was used for all length dependant analyses unless otherwise noted. All fish were weighed to the nearest 0.1 g, sagittal otoliths were removed, and gonads were dissected, weighed to the nearest 0.01 g, and then preserved in Dietrich's fixative. Morphometric relationships were described using linear regression for lengths and a 2-parameter power function for length-weight.

### Growth

A single, transverse section of each sagittal otolith was prepared by mounting the otolith on a glass slide with thermoplastic glue (Crystal Bond #509 from Electron Microscopy Sciences, Hatfield, PA) so that 1/3<sup>rd</sup> of the otolith extended beyond one edge of the slide. The otolith was ground flush to the slide with 1000 grit wet sand paper. We affixed the remaining section, cut side down, and ground again to just above the primordium. The completed section was fully embedded in thermoplastic glue for otolith increment analysis and archival preservation.

Sectioned otoliths were examined at 20x magnification under a dissecting microscope using transmitted light. Total number of annual rings was estimated by counting the number of opaque increments occurring outside of an easily identifiable settlement mark (Figure 2). Because of time constraints, annual increment validation was not conducted. However, annual deposition of alternating opaque and translucent bands is a commonly used marker of individual age, with increment deposition rates having been repeatedly validated in numerous species from nearly all reef fish genera including: Acanthuridae (Choat and Axe 1996), Lutjanidae (Newman et al. 1996), Pomacentridae (Fowler and Doherty 1992), and Scaridae (Lou 1992). Each otolith was examined and read independently at least two times at a minimum of 1 week apart. Replicate annual increment estimates were averaged and this average was then used to construct a vonBertalanffy growth curve for males and females separately and for the full data set using Simply Growth version 2.1.0.48 (Pisces Conservation, Lymington Hampshire, UK).



**Figure 2.** Digital image of prepared *Naso unicornis* otolith. An age of 25 years was assigned to this otolith.

### Reproduction

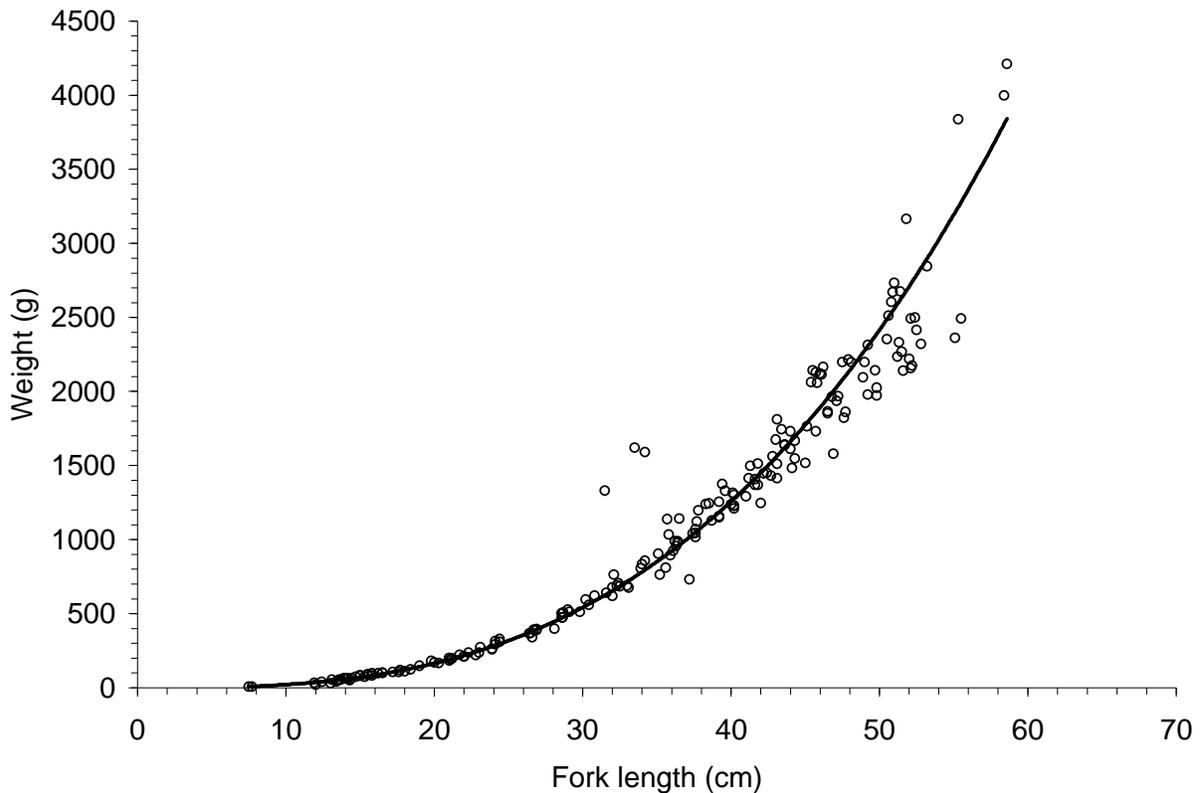
We identified individual sex and reproductive stage by removing a small biopsy from each of the preserved gonads, dehydrating the biopsy in an ethanol series with a final concentration of 95%, and embedding the sample in glycol methacrylate (JB-4 Embedding Kit from Electron Microscopy Sciences, Hatfield, PA). Embedded gonads were sectioned at 2 – 5  $\mu\text{m}$  on a rotary microtome (Sorvall Products, Newton CT) fitted with a glass knife. Sections were affixed on a glass slide, stained in toluidine blue or hematoxylin and eosin and examined for evidence of reproductive maturity. Ovaries were classified according to Wallace and Sallman (1981) and testes according to Nagahama (1983). The ovaries of inactive females consist primarily of primary growth oocytes and occasional yolk vesicle oocytes whereas the ovaries of reproductive

females can contain several size-classes of vitellogenic (yolked) oocytes that are characterized by the presence of small, lightly stained yolk granules. Inactive males are characterized by the presence of tightly packed gonial cells bound by stromal tissue while in reproductive males the testis consists of clearly-defined lobules containing spermatocysts in various stages of development. The size at sexual maturity was identified as the size at which a regression equation (3-parameter, sigmoidal) of percent mature individuals in each size class versus fork length indicates that 50% of individuals are mature.

## RESULTS

### MORPHOMETRIC RELATIONSHIPS

A total of 197 kala were sampled for life-history analyses (PMNM n = 60, Oahu n = 137). The length-weight relationship was best described by the two-parameter power function:  $W = 0.026(FL)^{2.92}$  (Figure 3;  $r^2 = 0.99$ ). Length-to-length relationships were linear (Table 1) with both standard length and body depth exhibiting a strong positive correlation with fork length ( $r^2 = 0.97$  and  $0.98$  respectively). Total length exhibited a relatively weak correlation with fork length ( $r^2 = 0.53$ ) due to the highly variable quality of the caudal fin filaments.



**Figure 3.** Length-weight relationship for *Naso unicornis*.  $W = 0.026(FL)^{2.92}$ ;  $n = 197$ ;  $r^2 = 0.99$ .

**Table 1.** Linear regressions predicting fork length (FL) of *Naso unicornis*.  $FL = a + (X)b$ , where X is a linear distance in mm. TL = total length; SL = standard length; BD = the distance between the dorsal and pelvic fin origins.

Variable	a	b	r <sup>2</sup>
TL	8.22	0.94	0.53
SL	0.06	0.88	0.97
BD	2.08	0.29	0.98

## GROWTH

We obtained 186 scorable otoliths preparations. There was no appreciable difference between the male and female vonBertalanffy growth equations (Figure 4a and b respectively), with both sexes exhibiting identical growth factor estimates ( $k = 0.17$ ) and asymptotic lengths of nearly 51 cm. Incomplete sampling of the larger size classes in the PMNM limited comparison of growth rates between locations. A scatter plot of the data indicates no effective difference in growth between Oahu and PMNM populations (Figure 4c). The vonBertalanffy growth equation for the full data set provided a good fit to the data (Figure 4c,  $r^2 = 0.70$ ), resulting in a growth factor estimate of 0.17 and an asymptotic length of 51.20 cm.

## REPRODUCTION

Gonads of 182 individuals were examined histologically. These were classified as mature or immature based on the stages of gametes present (Figure 5). Of these, 82 were ovaries and 100 were testes.

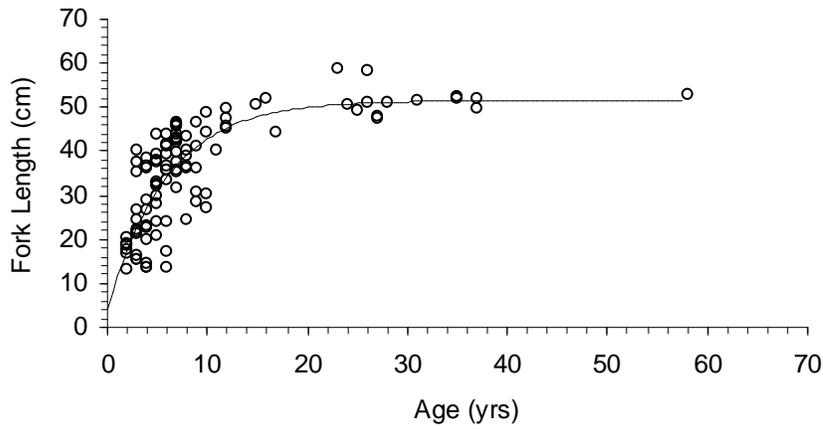
### *Females*

The ovaries of inactive females ( $n = 57$ ) consisted of tightly packed lamellae consisting primarily of primary growth oocytes (Figure 5a) and occasional yolk vesicle oocytes. Adjacent lamellae were separated by a narrow space which presumably extended into a central lumen. Primary growth oocytes were small (table 2) and stained darkly with toluidine blue or hematoxylin. Some contained a lighter-staining nucleus which contained visible nucleoli. Each was surrounded by a delicate ring of follicular cells.

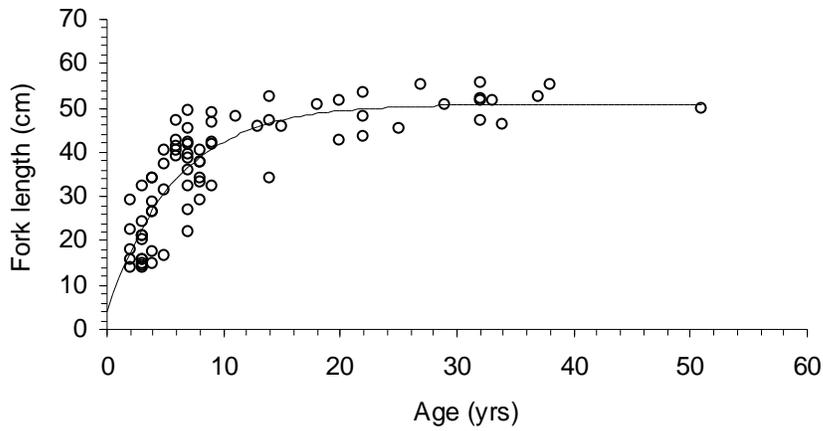
Yolk vesicle stage oocytes were, on average, twice the size of primary growth oocytes and contained multiple clear vesicles which were presumably lipid droplets, as they did not stain with hematoxylin nor eosin.

The ovaries of reproductive females ( $n=24$ ) contained various size-classes of vitellogenic (yolked) oocytes (Figure 5b), indicating that *N. unicornis* has group-synchronous oocyte development. This mode of oocyte development is typical of species that individually can spawn multiple times throughout the year, a common trait among coral reef fishes. Most oocytes undergoing vitellogenesis were  $> 200 \mu\text{m}$  in diameter. Small, light-staining yolk granules first appeared at the oocyte periphery. In larger oocytes, these migrated centrally and coalesced into larger yolk globules. All vitellogenic oocytes were bound by a prominent zona radiata.

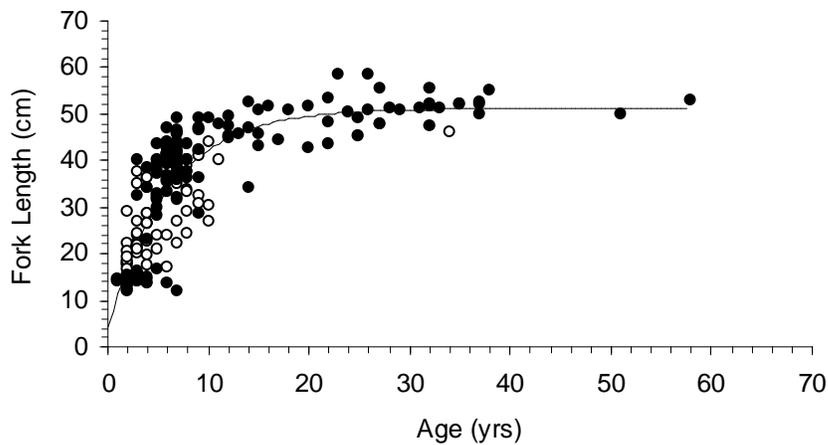
a.



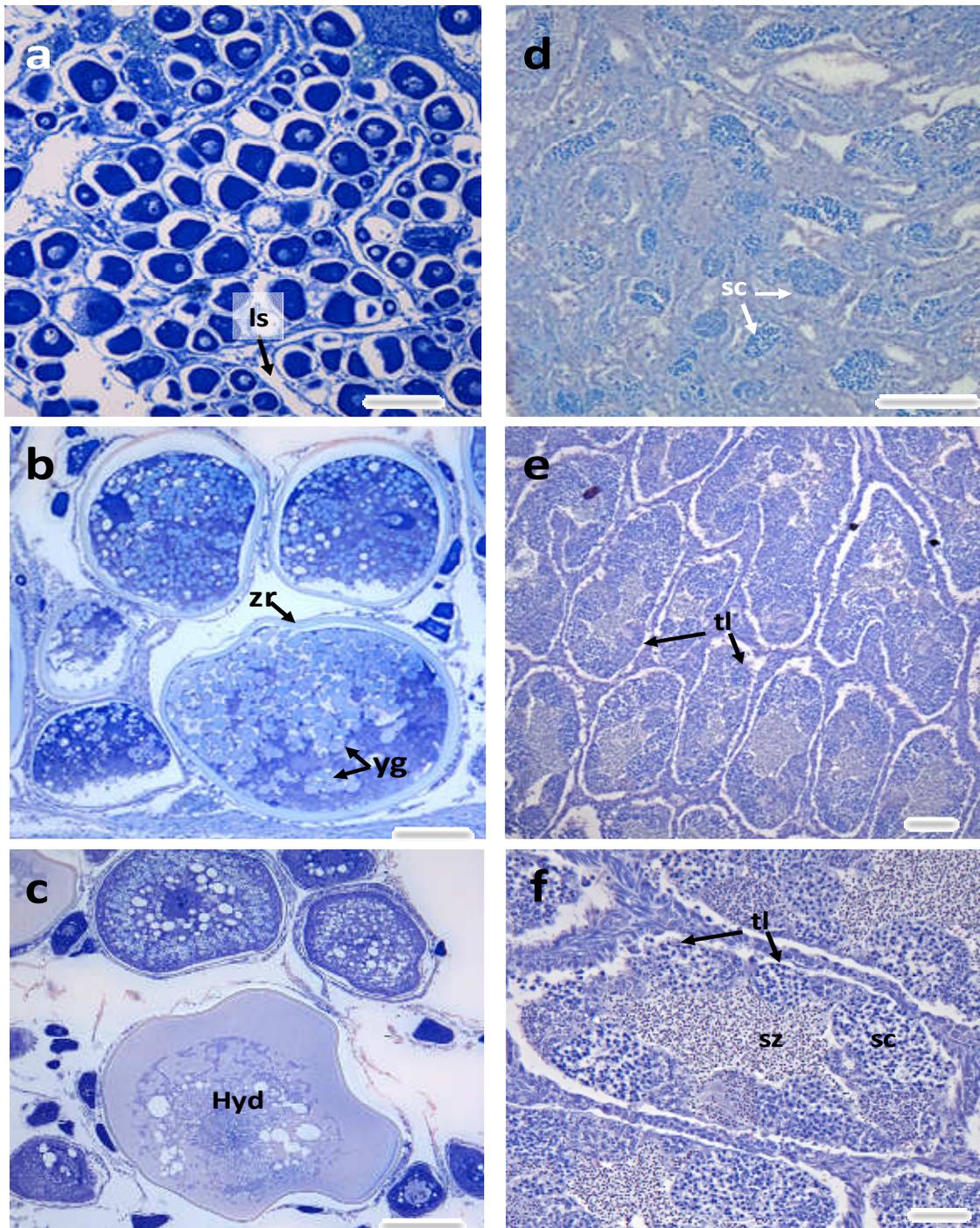
b.



c.



**Figure 4a-c.** A scatter plot of age versus fork length for *Naso unicornis*. The curves represent the resulting vonBertalanffy growth equations for: a) males,  $l_t = 51.53(1 - e^{-0.17(t+0.5)})$ ; b) females  $l_t = 50.87(1 - e^{-0.17(t+0.5)})$ ; c) full data set,  $l_t = 51.20(1 - e^{-0.17(t+0.5)})$ . Symbols indicate: open circles = PMNM, filled circles = Oahu.



**Figure 5a-f.** Histological sections of *Naso unicornis* gonads. a) Ovary of an inactive female containing primary growth oocytes b) ovary of a mature female containing vitellogenic oocytes c) ovary of a mature female containing hydrated oocytes d) testis of a inactive male containing undeveloped spermatocysts e) Transverse section of a mature testis showing testis lobule f) Close-up of a testis lobule. The lobule wall is composed of spermatocysts in different stages of development. Spermatozoa are clearly visible within the lobule lumen. ls= luminal space; Hyd= oocyte undergoing hydration; sc=spermatocyst; sz= spermatozoa; tl= testis lobule; yg=yolk granules; zr= zona radiata. Bar is 100  $\mu$ m. Section 5c is stained with Hematoxylin and Eosin. All others stained with toluidine blue.

**Table 2.** Oocyte diameters measured from histological sections of *Naso unicornis* gonads. Oocyte classification follows Wallace and Sellman (1981). Females were considered mature when their ovaries contained oocytes in stage III or later. n = # of oocytes measured.

Oocyte Stage	Size Range (µm)	Mean Size (µm)	Standard Deviation	n
I Primary Growth	18-81	47.0	13.5	78
II Yolk Vesicle Stage	63-187	119.6	34.6	60
III Vitellogenesis	175-460	296.1	78.8	46
IVa Maturation	340-500	392.8	39.5	21
IVb Hydration	425-500	450.0	35.4	4

Oocytes undergoing final maturation were, on average, 30% larger than those in mid-vitellogenesis. During early maturation, the yolk globules continued to coalesce into larger globules. Once yolk coalescence was complete, the result was a “hydrated” oocyte in which the ooplasm stained more lightly and uniformly than previous stages (Figure 5c).

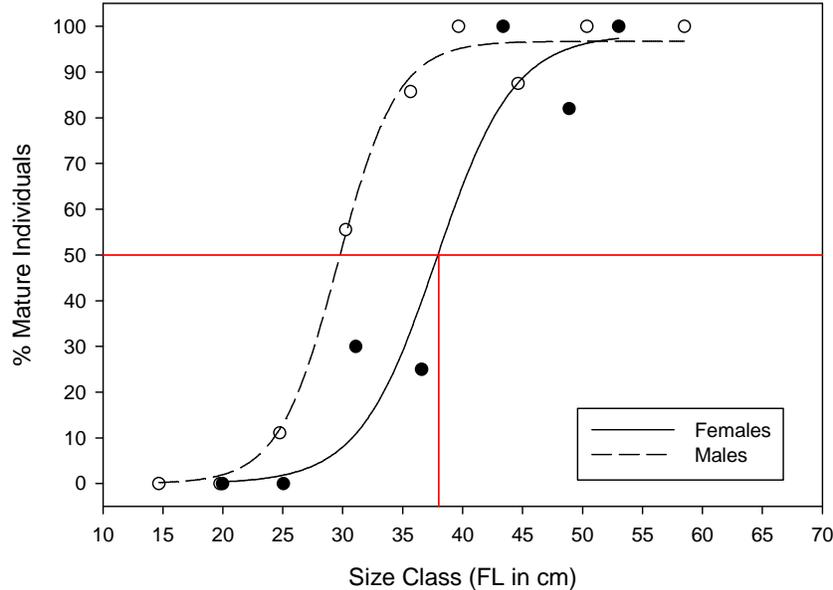
Reproductive females (those whose ovaries contained oocytes in stage II or beyond) were collected only in May (n = 1) and June (n = 24). The smallest individual with vitellogenic oocytes was 28.6 cm FL (Kure Atoll) whereas the smallest hydrated female was 34.2 cm FL (Oahu). Based on the June females, the  $L_{50}$  for *N. unicornis* is 37.8 cm FL.

### Males

Males have an unrestricted spermatogonial testis. In inactive males (n = 33), the testis consisted of clumps of tightly packed gonial cells or early spermatocysts bound by stromal tissue (Figure 5d). Discrete lobules were rarely evident.

In reproductive males (n = 67; figures 5e and f), the testis consisted of clearly-defined circular lobules separated by a lattice of stromal tissue (possibly connective tissue fibers and myoid cells). The walls of the lobules were composed of spermatocysts in various stages of development. Spermatozoa were readily evident within the lobule lumen.

The smallest male with sperm present was 26.6 cm FL. The size at 50% maturity ( $L_{50}$ ) was estimated at 28.6 cm FL. Unlike females, males with spermiated testes were present in all collections from March-June. A small number of males collected in January and February were not spermiated, but all were under 15 cm FL and thus were probably juveniles.



**Figure 6.** Size at maturity ( $L_{50}$ ) for kala, *Naso unicornis*. Open circles represent males, closed circles represent females. This graph is based on all males and females collected in June only (Combined Oahu and PMNM).  $L_{50}$  males = 28.6  $L_{50}$  females = 37.8 (based on 100 males and 51 females).

## DISCUSSION

There is compelling evidence that herbivore abundance can play an important role in maintaining the health and long-term productivity of coral reefs (McManus and Polsenberg 2004, McCook 1999). Repeated field experiments have demonstrated that a reduction in herbivore abundance results in an increase in the prevalence of algae (Smith et al. 2001) and a corresponding decrease in both coral growth (Lirman 2001) and the establishment of new coral colonies (Vermeij et al. 2009). Because of their tendency to graze on macro-algae, kala and other large-bodied herbivores play a particularly important role in limiting algal growth (Hixon and Brostoff 1996).

Incremental analysis of annually deposited rings in the sagittal otoliths of Hawaiian kala indicates the species to be long-lived, with rapid initial growth resulting in an asymptotic growth size being achieved relatively early in life. Sampled kala ranged in age from 1 to 58 years with the majority of growth occurring within the first 15% of the life span (Figure 5c). This pattern of rapid early growth and an extended life span is consistent with a similar study conducted on the Great Barrier Reef (GBR; Choat and Axe 1996). The GBR study examined longevity in four *Naso* species and this work, taken together with our survey of Hawaiian kala, indicates that *Naso* species in general exhibit life-spans in excess of 40 years.

The current minimum legal size limit of 14 in (35.6 cm) for Hawaiian kala effectively allows for male maturation prior to entering the fishery. However, our female  $L_{50}$  estimate of 37.8 cm FL indicates that a majority of females are currently entering the fishery prior to reproductive maturity. The removal of high numbers of immature fish has obvious implications for the sustainability of fished populations, most important being a reduction in spawning potential

through either decreased abundance (Lewin et al. 2006) or impacts on growth rates and the timing of maturation (Allendorf and Hard 2009). Nowlis (2000) describes a multi-strategy management model indicating that in cases where species are fished while immature, increasing minimum size limits can improve long-term fishery stability without requiring the establishment of additional no-take reserves.

For reef fishes, the relationship between size and fecundity is well documented, with larger fish producing exponentially more eggs (Birkley et al. 2004). Moreover, evidence from a diverse set of species indicates that older individuals produce larger, faster growing, and more starvation resistant larvae (Bobko and Birkley 2004). Maintenance and/or recovery of fished stocks is likely confounded by the selective targeting of the largest fish in a population, which results in poorer quality larvae and an exponential decrease in the total number of larvae produced (Birkeland and Dayton 2005). To protect the most reproductive adults, a number of coastal states have established maximum size limits for near-shore species including: surgeonfishes, parrotfishes, wrasses, and jacks. By protecting both maturing individuals and the most successful spawners, a combined minimum and maximum size limit can help mitigate growing pressure placed on Hawaii's economically and ecologically important coastal fisheries.

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