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A Preliminary Study of the Age and Growth of Paddletail Snapper *Lutjanus gibbus* (Forsskål 1775) in Bunaken Marine Park, North Sulawesi, Indonesia

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Abstract

Paddle tail snapper *Lutjanus gibbus* (Forsskål 1775) is an important target species of commercial, recreational, artisanal and indigenous fisheries in tropical Indo-Pacific waters, but away from the centre of its distribution it is often avoided as being a high risk for ciguatera poisoning. This study investigated the age and growth of a tropical population of *L. gibbus* in Bunaken Marine Park, Indonesia. A total of 95 specimens were sampled with fork lengths between 151-312 mm. From growth increments on sectioned otoliths, ages ranged from 1-9 years old, with ages 3 and 4 years being the most common. Growth in length was described by the von Bertalanffy growth function with parameters L_∞ = 274 mm (fork length), K = 0.78 and t_0 = -0.24. The population of *L. gibbus* in Bunaken Marine Park consists of fast-growing fish that attain smaller size than populations at higher latitudes, which may possibly contribute to a lower risk of ciguatera poisoning. The fast population turn-over characteristics of *L. gibbus* indicates the potential of the population to sustain high harvest pressure. However, the lack of management of the fishery could still lead to over-exploitation.

Introduction

The family Lutjanidae comprises a substantial proportion of many tropical and sub-tropical reef-associated fisheries, whether artisanal (Kamukuru et al. 2005; Grandcourt et al. 2011), commercial (Cowan et al. 2011) or recreational (Russell et al. 2003). However, several *Lutjanus* species including the paddletail snapper *Lutjanus gibbus* (Forsskål 1775) have been recognised as being an unacceptable risk for human consumption due to the risk of ciguatera poisoning in parts of their range (Grant 2014) and are avoided by fishers or rejected by marketing authorities (e.g. Sydney Fish Market 2013).

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Despite the risk in the southern part of its range, *L. gibbus* remains a popular fish in the markets of northern Sulawesi, Indonesia (C. J. Holloway, pers. obs.) where it is caught in a largely unregulated artisanal fishery.

Accurate and reliable length-at-age data are an important component of the demographic information required for stock assessment and estimating growth and mortality rates when developing a sustainable harvest strategy (Campana 2001). Piddocke et al. (2015b) noted a tendency for lutjanids to show latitudinal gradients in growth and other life history characteristics, with those further from the equator living longer and growing more slowly to larger asymptotic size. Therefore, growth models for populations in different parts of the species' range are not transferable. Growth of *L. gibbus* has been studied using annual growth rings in cross sections of sagittal otoliths in both the northern (Nanami et al. 2010) and southern (Heupel et al. 2010) parts of its range, but has not been studied near the geographical centre of its range in the Indo-Pacific.

Ageing of tropical fishes using otolith growth bands has sometimes proven more difficult than for temperate species (Fowler 1990). This has been attributed to a lack of discernable rings in tropical otoliths, which has been connected to more equitable seasonal growth in the tropics (Heupel et al. 2010). Although difficulties in the analysis of tropical fish otoliths can prove challenging, they seldom prove insurmountable (Marriott and Mapstone 2006). Larger sample sizes are often needed to provide sufficiently readable otoliths, and strict protocols must be developed for each population to provide consistency in age estimates from multiple readers when growth checks are less distinct. The annual periodicity of otolith growth marks has been well-established for lutjanids, in general (Piddocke et al. 2015a) and for *L. gibbus*, in particular (Nanami et al. 2010; Heuple et al. 2010). This study reports the age and size relationship for *L. gibbus* caught in the coastal waters of Bunaken Marine Park, North Sulawesi, Indonesia.

Materials and Methods

Study site and sample collection

Sulawesi is the northernmost island of the Indonesian archipelago. North Sulawesi is surrounded by the tropical waters of the Celebes and Moluccas Seas (Lewis 1991). These seas are known to be productive environments, supporting commercial, artisanal and subsistence fishing (Pet-Soede et al. 2006). The 80 ha Bunaken Marine Park (Fig. 1) was established in the early 1990's to protect the diverse marine ecosystem that was experiencing increasing pressures from tourism and fishing (Leisher et al. 2007).

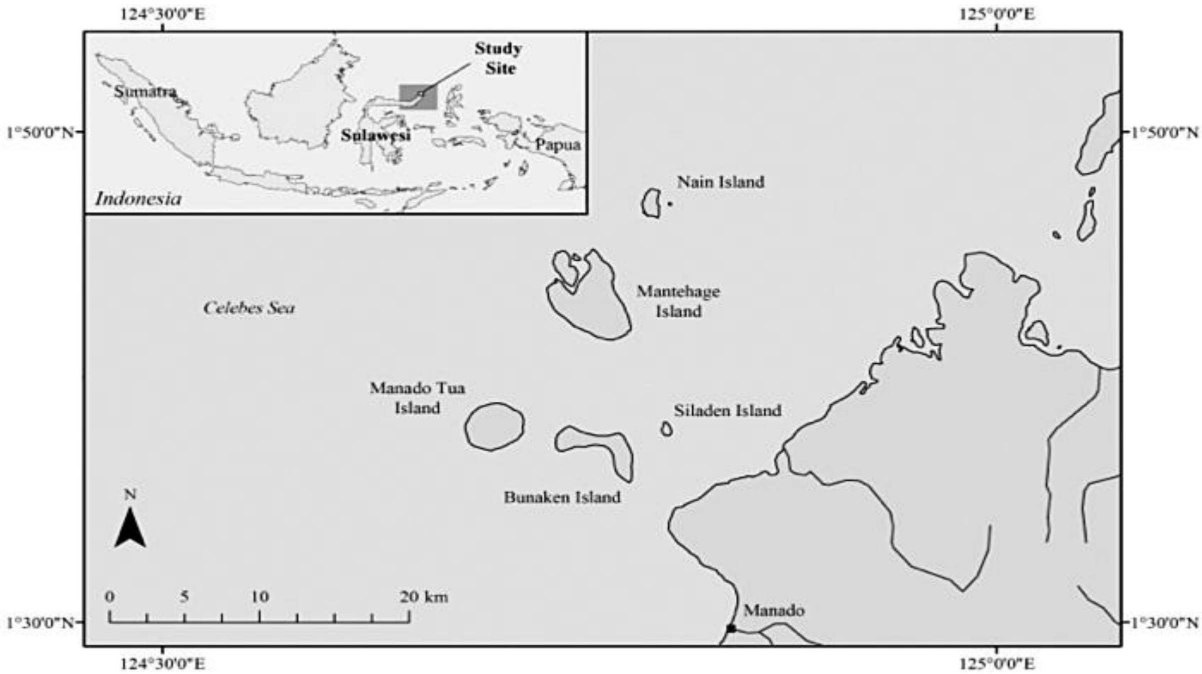

Fig. 1. Location of the study site for the fishery dependent collection of *Lutjanus gibbus* in Bunaken Marine Park North Sulawesi, Indonesia.

Specimens of *Lutjanus gibbus* (n=95) were purchased over a 5 day period from 19-24 January, 2014 at Manado fish market in North Sulawesi, Indonesia. All specimens of *L. gibbus* that were presented for sale on these days were purchased. Fork length (FL) was measured to the nearest millimetre using a metric tape measure, and whole weight was measured to the nearest gram on electronic scales (Beurer KS28). Sex was determined by visual examination of the gonads.

Otolith preparation and analysis

The sagittal otoliths were removed from each fish through the brain cavity by removing the top of the cranium. The otoliths were briefly washed in hypochlorite bleach to remove any residual tissue and to meet import requirements for transport of biological material into Australia for subsequent processing. The wash was unlikely to penetrate the internal structure or affect opaque zone readability in sectioned otoliths. The otoliths were then rinsed in water, dried and stored in individually labelled envelopes until age estimation processes were applied. All otoliths (left and right) were embedded individually in polyester resin and sectioned using a Buehler Isomet low-speed diamond saw. After initial trials of different thicknesses transverse sections (106-110 μm) were cut through the centrum of each otolith. The sections were then polished with 1,200 mm wet and dry sandpaper and rinsed in distilled water. When dry, a thin film of vegetable oil was applied to both sides of the section before viewing and photomicrography to enhance the clarity of opaque and translucent banding (e.g. Piddock et al. 2015a). The sections were examined under a compound microscope at 40× magnification using transmitted and reflected light.

Digital photos were taken using a Micropublisher 3.3 camera (Q-imaging Pty Ltd.). Contrast between opaque and translucent zones in each image was optimised using Adobe Photoshop v.12 (Adobe Systems Inc.). Where unambiguous opaque bands could not be distinguished in photographs the original specimen was re-examined. In all cases no greater clarity could be determined, so all counts are based on enhanced digital images.

Age determination

All fish were allocated an assumed birthday of January 1, reflecting the spring-summer spawning season identified for *L. gibbus* by Nanami et al. (2010). As all fish were collected in January, all ages were estimated in whole years as the number of opaque zones in the otolith sections (Heupel et al. 2010; Nanami et al. 2010). Irregular width or positioning of opaque zones were considered as stress marks and excluded from counts (Marriott et al. 2007). Age marks were often associated with distinct crenulations of the dorsal margin of the section (Fig. 2). The opaque zones were counted by three readers without knowledge of sample characteristics such as weight, length and sex. The average distance to the first opaque mark was established at 800-1,200 μm and used to justify some age estimates where a first mark was indistinct. Opaque zones were counted along the ventral axis that extended from the primordium to the proximal surface of the otolith with further reference to opaque zones in the sulcal axis of the sectioned otolith (Fig. 2). Otoliths for which counts of opaque zones differed between readers were read a second time. Otoliths for which a consensus of age estimation could not be reached or that were unreadable due to diffuse or faint opaque zones were omitted from the data set.

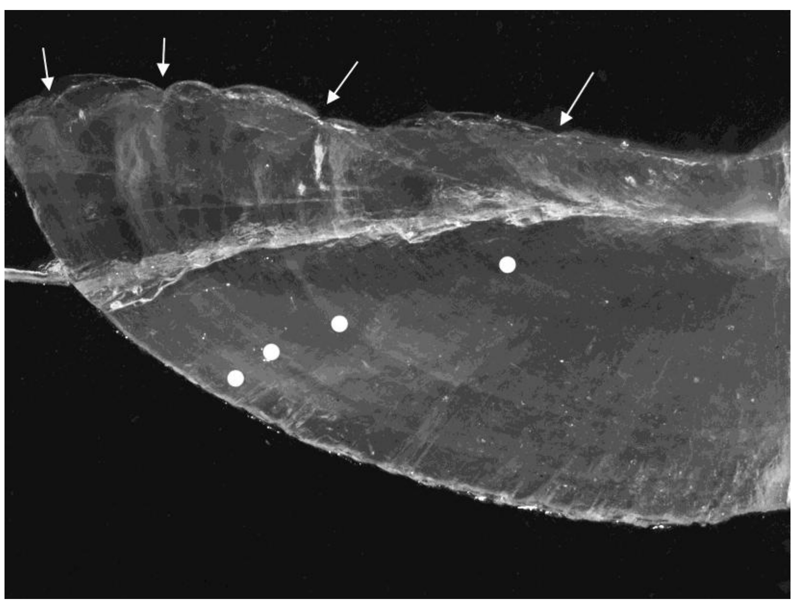

Fig. 2. Ventral margin of a transversely sectioned otolith used as the reading plane for age estimations. Arrows indicate the crenulations corresponding with opaque zones. White filled circles indicate the positions of the opaque zones (note the very faint appearance of the first zone in this specimen – a 4 year-old male, fork length 267 mm, weight 362 g). Multiple finer opaque rings are present within each annulus, but the ages are determined from the presence of broader translucent zones between clusters of opaque marks.

The length-weight relationship for *L. gibbus* was obtained by fitting the power relationship $W = a[FL]^b$, where W is the weight of an individual fish (g), FL is the fork length (mm) and a and b are empirically-derived constants, to observed lengths and weights of 56 males and 39 females using Microsoft 'Excel for Mac 2011', version 14.4.1 (Microsoft Corporation 2010).

Growth modelling

Growth was modelled by fitting the von Bertalanffy growth formula (von Bertalanffy 1938) to observed length-at-age data. The von Bertalanffy growth parameters were derived by using an iterative non-linear least-squares estimation procedure in the statistical package SPSS v.22 (IBM Corporation 2013). The von Bertalanffy growth formula is described by the equation:

$$L_t = L_\infty [1-e^{(-K(t-t_0))}]$$

Where L_t = mean FL (mm) at age t years, L_∞ = asymptotic length, K = the Brody growth coefficient, describing the rate of growth towards L_∞ and t_0 is the theoretical age at which the fish would have at zero length, had it grown in the manner described by the equation throughout its early life.

Results

Sample collection

Over the 5-day sampling period, 95 *L. gibbus* were purchased from commercial landings at Manado markets. Fork lengths ranged from 151-312 mm with averages similar for both sexes (Fig. 4). Of the 95 fish collected, 56 were identified as males ranging from 160-312 mm FL and 83-544 g fresh weight while 39 were females ranging from151-289 mm FL and 53-456 g (Table 1.).

Table 1. Summary of fork length and fresh weights for male and female *Lutjanus gibbus*.

	Mean fork length (FL)±S.D. (mm)	**Mean weight±S.D.** (g)
Female (n=39)	242.76±106.37	272.84±38 00
Male (n=56)	247.36±109.81	286.32±37.23

Age and growth

Growth increments in the sectioned otoliths varied in definition between individuals and were generally difficult to read with opaque bands often thin, faint or consisting of multiple fine lines.

Of the 95 otolith sections, 19 were deemed unreadable, or age estimations between the readers varied by more than 1 year and were therefore omitted from analyses. Age estimates ranged from 1-9 years, with the two most common age classes being 3 and 4 years (Fig. 3A.). These two age classes accounted for 47% of the total sample, and 76% of all fish sampled were under 4 years old. The distribution of fork lengths in the sample was bimodal with modes at 170 and 260 mm (Fig. 3B) with no samples in the range 190-210 mm.

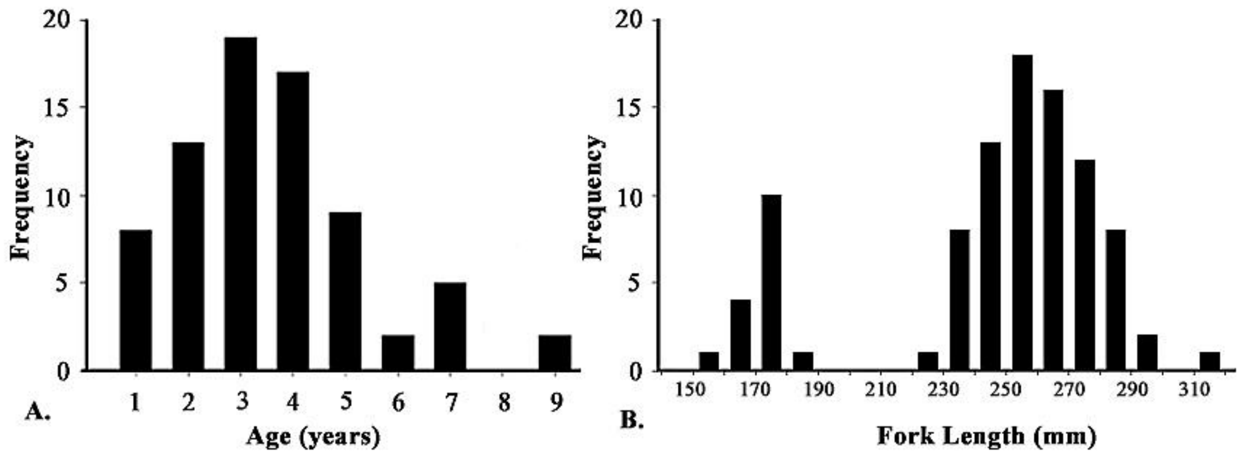

Fig. 3. (A) Age frequency distribution of *Lutjanus gibbus* sampled from the local markets in Bunaken Marine Park, North Sulawesi, Indonesia. (B) Length frequency distribution for all *Lutjanus gibbus* used in age estimation in Bunaken Marine Park, North Sulawesi. (*n* =95).

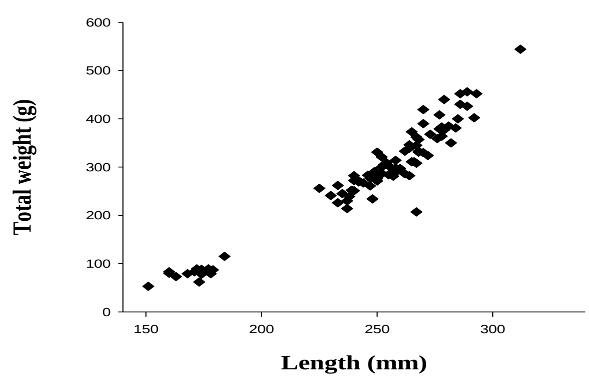

Fig. 4. Relationship between fork length and total weight of *Lutjanus gibbus* (male and female combined, n = 95, R^2 = 0.94).

As there was no significant difference in weights at selected lengths between males and females the length-weight relationship was calculated for both sexes combined (Fig. 4). The length-weight relationship is represented by the equation $W = 8{\times}10^{-6}(FL)^{3.14}$ Overlaying scatterplots of length at age for males and females showed no obvious divergence of the two, so the sexes were pooled for determination of von Bertalanffy parameters (Fig.5). The resulting parameters were L_∞ = 274 mm FL, K = 0.78 and t_0 = -0.24.

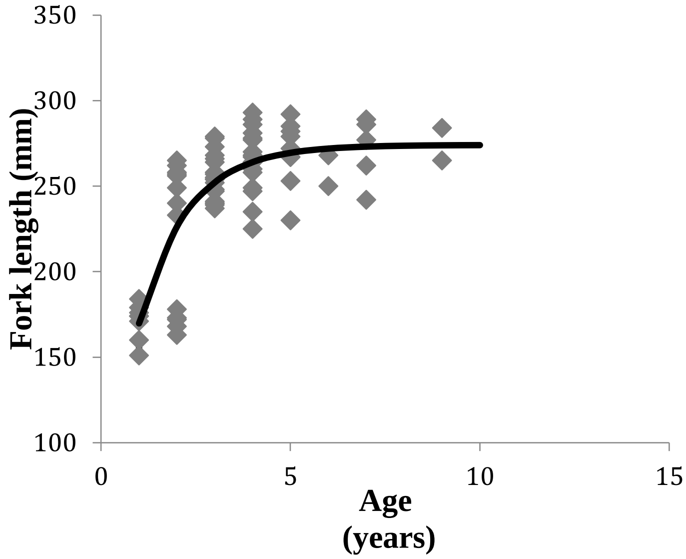

Fig. 5. Length-at-age and von Bertalanffy growth curve of *Lutjanus gibbus* (male and female combined).

Discussion

Direct aging of tropical fish species can often be difficult due to the reduced seasonality of growing conditions compared to higher latitudes (Fowler 1990; Pilling et al. 2000). Defining the first annulus can be particularly problematic as it occurs when the fish is still rapidly growing and may not have reached sexual maturity, so metabolic resources are not being diverted from growth to reproduction (Fowler 1990; Allman et al. 2005). In this study, a high proportion of otolith sections (20%) could not be assigned a reliable age due to the lack of contrast between seasonal bands when viewed by direct microscopy or digital imagery. By establishing a distance range to the first annulus in the clearest specimens, together with correspondence with surface crenulations, the position of the first annulus could be determined in most sections, even when it was faint or diffuse. The position of the opaque bands suggests winter deposition, although water temperatures in the region vary by only 2 °C throughout the year (Eco-Divers 2014). Reduced growth possibly coincides with gamete maturation leading to the spring-summer spawning. Diversion of energy to reproduction may have had a greater influence on growth (and subsequent otolith band formation) than temperature, as has been assumed for the Okinawan population of *L. gibbus* (Nanami et al. 2010) and other lutjanids (e.g. Garcia-Contreras et al. 2009).

Due to the logistical constraints associated with the remote location this study did not attempt validation for this population. However, annual increment formation has now been validated for many tropical lutjanid species including populations of *L gibbus* (see review by Piddocke et al. 2015a). The von Bertalanffy parameters for the *L. gibbus* population of Bunaken Marine Park indicate a short lived, fast growing fish, a result that is in contrast to many other lutjanid populations (Piddocke et al. 2015b).

The maximum age in this study of 9+ years continues a trend for faster growth to smaller size with shorter longevity of *L. gibbus* (Newman et al. 2000a; Heupel et al. 2010; Nanami et al. 2010) and lutjanids generally at low latitudes (Newman and Dunk 2003; Shimose and Tachihara 2005; Groundcourt et al. 2006, Garcia-Contreras et al. 2009, Piddocke et al 2015b). In the artisanal fishery of the Mazatlan coast, Mexico, *L. argentiventris* (Peters1869), aged four or less dominated the catch by 81% (Garcia-Contreras et al. 2009), which is similar to this study where 76% of the fish sampled were under 4 years of age. This could possibly reflect the nature of the local commercial fishing techniques such as mesh net size, schooling characteristics of younger cohorts or ontogenetic migration of larger fish to offshore reefs beyond the reach of small vessels (Garcia-Contreras et al. 2009). These dynamics could also affect the Bunaken population of *L. gibbus*. The limitations posed by fishery-dependent data collection must be considered when analysing the biological characteristics of *L. gibbus* (Heupel et al. 2010).

In this study, no individuals below 151 mm FL were collected possibly contributing to an overestimation of mean length at ages 1 and 2 as only the faster-growing individuals of the younger age classes may be vulnerable to the fishery. Lutjanids are known to be fast growing in their first year of life (Grandcourt et al. 2006) and missing this crucial life stage information can restrict model forecasts. Observations of other fish available for sale at the Manado markets indicate that numerous large fish from families such as Lethrinids and Scarids were caught by the fishery, suggesting that if large lutjanids were available for capture then they would be present at the local market. The popularity of small *L. gibbus* in Manado may in part be the result of its rapid growth and consequent lower risk of ciguatera. It is known that larger, older individuals of carnivorous species have a higher risk of containing ciguatera toxin (Lehane and Lewis 2000).

In South Africa, Papua New Guinea and the Great Barrier Reef *L. gibbus* is regarded as a high-risk species and eating it is discouraged (Grant 2014). The nature of the fishing techniques used in Bunaken Marine Park, such as small boats suggest that larger lutjanids (regardless of age) are possibly inaccessible and not common in the local fishery. The gear used to catch the fish sold at Manado (i.e. mesh nets such as gill and trammel nets) may also be strongly size-biased (Clay 1981). As such, only the smaller individuals of older cohorts may be sampled, resulting in a potential underestimate of L_∞ (Marriott and Mapstone 2006). Further studies should investigate whether larger specimens are available from other parts of the fishery that may land their catch at other ports.

The L_∞ value calculated for this study is almost two-fold lower than that recorded from the Great Barrier Reef and also less than studies undertaken in Japan (Table 2). In addition to the potential sampling bias mentioned above, genetic and environmental variation may explain the different L_∞ values. Environmental variables such as warm water and enriched ocean currents at Bunaken Marine Park may also influence the relatively high K value. Bunaken Marine Park is a highly diverse region influenced by naturally enriched currents that promote high productivity (Pet-Soede et al. 2006). K values within species have been shown to differ along latitudinal gradients (Russell et al. 2003).

Lutjanids at higher latitudes generally have slower growth rates to reach larger sizes (Piddocke et al., 2015b). This appears to be true of *L. gibbus* when comparing the Sulawesi population with Okinawa and the Great Barrier Reef (Heupel et al. 2010; Nanami et al. 2010; Newman et al. 2010).

Table 2. Comparison of L_∞ values for *Lutjanus gibbus* in the Indo-Pacific region.

Author	**Area of Study**	L_∞ **(mm)**	***K***
Heupel et al. 2010	Great Barrier Reef, Australia Latitude 14-22°S	544	0.06
Nanami et al. 2010	Ishigaki Island, Okinawa, Japan Latitude 24°N	Male 390.5 Female 303.4	0.21 0.25
This study	Bunaken Marine Park, Sulawesi Latitude 1.5°N	274	0.78

The bi-modal length frequency distribution in the fish available at the markets at the time of sampling (Fig. 5) is not solely the result of discrete age cohorts in the population as the smaller group (150-180 mm) includes both 1 and 2-year-old fish. The lack of fish in the length range 190-220 mm may therefore be the result of selectivity of different fishing gears, or different habitats used by fishers rather than an absence of those sizes in the population (Heupel et al. 2010). Had this group of smaller individuals consisted entirely of 1-year-olds then it may have been possible to examine early growth rates through progression of the modal length of the cohort and thereby validate the rapid growth constant estimated in this study.

The smaller group did include all the 1-year-old fish in the sample, so further sampling of otoliths from this size range at different times of the year would provide validation of the periodicity and seasonal timing of opaque band formation if necessary. Studies have shown that the lutjanids in the Great Barrier Reef undergo initial rapid growth in the first two years of their life (Newman et al. 2000b), before stabilising and diverting energy into reproduction (Martinez-Andrade 2003). This form of growth pattern leads to length-at-age curves reaching asymptote at comparatively young ages. The findings of this study support this conclusion, as all individuals reached 50% of L_∞ within 1 year (Fig. 7).As well as growing quickly, many lutjanid species are known to reach sexual maturity early (Heupel et al. 2010). In this study, mature gonads were present in all individuals allowing for sex determination, even in the smallest individuals.

The fast growth rate indicates that the Sulawesi stocks of *L. gibbus* can potentially sustain high levels of harvest. However, the scarcity of large, older fish in the catch suggest that overfishing is likely and with no minimum size regulations enforced, there is also substantial pressure on the younger stocks. Minimum size limits should ideally be promoted to allow the 1+ and 2+ cohorts to add biomass to the stock during their phase of very rapid growth and ensure sufficient recruitment into the spawning stock.

Conclusion

This study has demonstrated that *L. gibbus* in Bunaken Marine Park are a fast growing fish with shorter life span than populations studied elsewhere. The potentially fast population turnaround for *L. gibbus* indicates the species' suitability to commercial, subsistence and artisanal fishing pressure in Bunaken Marine Park. However, the population is subject to high levels of fishing and, adequate fishery regulations to ensure sustainable management of this resource presently do not exist. The issue of ciguatera toxicity is also a priority if the fishery is to develop.

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