

## Population variables and life-history characteristics of the alligator pipefish *Syngnathoides biaculeatus*, in Papua New Guinea

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Population structure and life-history variables of the widely distributed alligator pipefish *Syngnathoides biaculeatus* were characterized in Bootless Bay, Papua New Guinea over the course of 11 months. There was little evidence of seasonality with four focal populations showing no significant change in abundance. Similarly, the sex ratio remained 1:1 for all but 1 month. Reproductive males carrying eggs (148–278 mm in total length,  $L_T$ ) were found in all months. Brood size was significantly, positively related to male  $L_T$  for newly laid broods only. Maximum observed brood size was 351 and mean  $\pm$  s.d. brood size was  $238 \pm 57$  for newly laid broods. Juveniles and males showed no change in mean  $L_T$  over the year while slightly smaller females were captured in November 2006 and September 2007. Males were significantly longer than females so von Bertalanffy growth coefficients were estimated separately for each sex: males  $L_\infty = 285$  mm,  $K = 0.82 \text{ year}^{-1}$  and females  $L_\infty = 261$  mm,  $K = 1.10 \text{ year}^{-1}$ . These estimates suggest that this species grows rapidly and has a short-life span. In the context of growing concern about overexploitation of syngnathids, a rapid growth rate combined with year round reproductive activity suggests that the tropical *S. biaculeatus* may be relatively resilient with regard to fishing pressure.

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## INTRODUCTION

Syngnathids (seahorses, pipefishes and their relatives) have been recognized as a potential focal group for marine conservation, acting as either ‘umbrella’ or ‘flagship’ species (Foster & Vincent, 2004; Shokri *et al.*, in press). Umbrella species are those whose conservation will also conserve other species or habitat incidentally, while flagship species are those which are charismatic and attract increased attention and funding from the general public and the policymakers

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(Caro & O'Doherty, 1999; Zakarias & Roff, 2001). Although possessing some of the characteristics of putative umbrella species, syngnathids are probably more useful as flagships as they are easily recognizable and generally evoke public support and affection (Zakarias & Roff, 2001). Seadragons and seahorses have been successfully adopted in some Australian states as emblems of the marine environment and are the focus of a number of community conservation initiatives (Martin-Smith & Vincent, 2006).

In marine systems, flagship species may draw attention to issues such as unsustainable exploitation, habitat loss or incidental by-catch. For example, concerns over the number of seahorses traded internationally for traditional medicine, curios and the aquarium trade led to their listing on Appendix II of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) in 2002 (Foster & Vincent, 2005).

Syngnathids are an important component of seagrass ecosystems, which have undergone major loss from anthropogenic impacts (Duarte, 2002; Orth *et al.*, 2006). Syngnathids are often dominant components of the fish fauna in seagrass, representing up to 80% of the total number of individual fishes captured in some instances (Pollard, 1984; Hindell *et al.*, 2000; Kendrick & Hyndes, 2003). As ubiquitous residents they probably perform an important trophic role as prey for mobile, non-resident species linking seagrass beds with adjacent ecosystems (Dorenbosch *et al.*, 2005). Shokri *et al.* (in press) provide convincing evidence of a spatial correlation between the presence of syngnathids and other fish taxa in Australian estuarine seagrass habitats and thus their utility as flagship species.

Little is known about the population status or life-history variables of seagrass-associated syngnathids, particularly throughout the Indo-Pacific where their species diversity is highest (Dawson, 1985) and exploitation pressures are greatest (Vincent, 1996). These data are fundamental inputs when assessing the vulnerability of species to exploitation and their resilience to disturbance both natural and anthropogenic.

The alligator or double-ended pipefish *Syngnathoides biaculeatus* (Bloch) is widely distributed throughout the tropical Indo-Pacific with records from seagrass habitats extending from the northern Red Sea and the eastern coast of Africa, eastward to Japan, Samoa, the Tonga Islands and Australasia (Dawson, 1985). It is a large species growing to c. 280 mm in total length ( $L_T$ ). As with other syngnathids, male *S. biaculeatus* become 'pregnant', i.e. ovigerous, and incubate the eggs (Kuiiter, 2000). *Syngnathoides biaculeatus* displays simple 'pouch' morphology whereby the embryos are attached to the ventral surface of the male abdomen which has no flaps of surrounding tissue (Dawson, 1985; Kuiiter, 2000).

*Syngnathoides biaculeatus* is thought to be the most heavily exploited pipefish in traditional Chinese medicine (TCM), but there are few estimates of trade volumes. Vincent (1996) reported records of 1600–16 500 kg year<sup>-1</sup> of dried pipefishes into Taiwan over the period 1983–1993 while Martin-Smith *et al.* (2003) reported records of 7500–21 300 kg year<sup>-1</sup> of dried pipefishes into Hong Kong from 1998 to 2002 although in both instances these were a mixture of species. Recently, *S. biaculeatus* has also appeared in the aquarium trade where current trade volumes are thought to be low (S. Bartnik, pers. comm.). The recognized lack of information for *S. biaculeatus* is reflected in its 'Data Deficient'

listing in the IUCN Red List (IUCN, 2007). The only population and life-history data for *S. biaculeatus* are from a subtropical site in Australia (Takahashi *et al.*, 2003). This species, however, has not been exploited from Australia historically, but rather trade has occurred from tropical areas particularly in India, Malaysia, the Philippines and Thailand (Martin-Smith *et al.*, 2003; Martin-Smith & Vincent, 2006), where population variables may differ significantly (Pauly, 1998).

The aim of this study was to provide estimates of the population and life-history variables of unexploited populations of *S. biaculeatus* in Papua New Guinea. The specific objectives were: (1) to determine the  $L_T$  and the mass ( $M$ ) relationship, (2) to estimate population density and seasonal changes, (3) to characterize population structure in terms of sex ratio and size frequency, (4) to assess reproductive output and seasonality, (5) to estimate growth rates and (6) to compare these variables with the species from elsewhere in its geographic range. These data can then provide a basis for developing models of population dynamics and thus contribute to management and conservation planning.

## MATERIALS AND METHODS

Populations of *S. biaculeatus* were sampled from seagrass areas in Bootless Bay, Central Province (9° S; 147° E) in Papua New Guinea. Four monitoring sites were established and sampled monthly or bi-monthly from October 2006 to July 2007 along with a further eight sites that were sampled opportunistically (Fig. 1). All sampling took place in relatively close proximity to mangrove habitat. Sampled locations encompassed sparse to continuous seagrass beds, predominately *Thalassia hemprichii*, over sediment ranging from fine to coarse sand with algae present at most sites. Mean water temperature was 27.5° C (range 24.0–32.0° C). Fish were caught using a 20 × 2 m seine incorporating a bunt with a stretched mesh-size of 5 mm. The net was extended perpendicularly from a small boat by one person until the full length was in the water whereupon a second person took hold of the other end. Both people then dragged the ends of the net parallel to each other for *c.* 5 m, and then converged on the boat which had been moved in the interim. The net was then hauled into the boat with care taken to keep the footrope closed. Sampling was usually undertaken within 4 h either side of low tide, typically on the outgoing tide. Each monthly sample at each site consisted of 10 hauls, separated by at least 10 m from each other, covering a total area of *c.* 1000 m<sup>2</sup>, conducted over a period of *c.* 2 h.

All *S. biaculeatus* caught were sexed and  $L_T$  was measured (tip of snout to tip of tail ±1 mm). Females were identified by the presence of a white zig-zag pattern on the abdomen accompanied by 15–25 blue dots, often interspersed with red patches (Takahashi *et al.*, 2003). Distinguishing between non-pregnant males and females for individuals <155 mm was often not possible, and in such cases the individual was recorded as juvenile. Egg-bearing status was recorded for male fish. All individuals were released at the capture site once all the hauls had been completed.

In August and September 2007, 129 specimens of *S. biaculeatus* were collected from north-east Bunamotu, south-east Mirigeta and north-east Motupore and transported to seawater aquaria at the Motupore Island Research Centre (MIRC). The  $L_T$  and  $M$  for 41 individuals (18 male, 21 female and two juveniles) were measured to determine their  $L_T$  and  $M$  relationship. All pregnant males ( $n = 55$ ) were measured, and the ventral abdominal surface of each was photographed using a high resolution (seven megapixel) digital camera. Photography was used rather than direct counting *in situ* to minimize handling time for the animal and provide a data archive. The brood size for each male was counted from the resulting image using the particle analyser plug-in for ImageJ software to ensure accuracy (Abramoff *et al.*, 2004). Brood developmental stage for

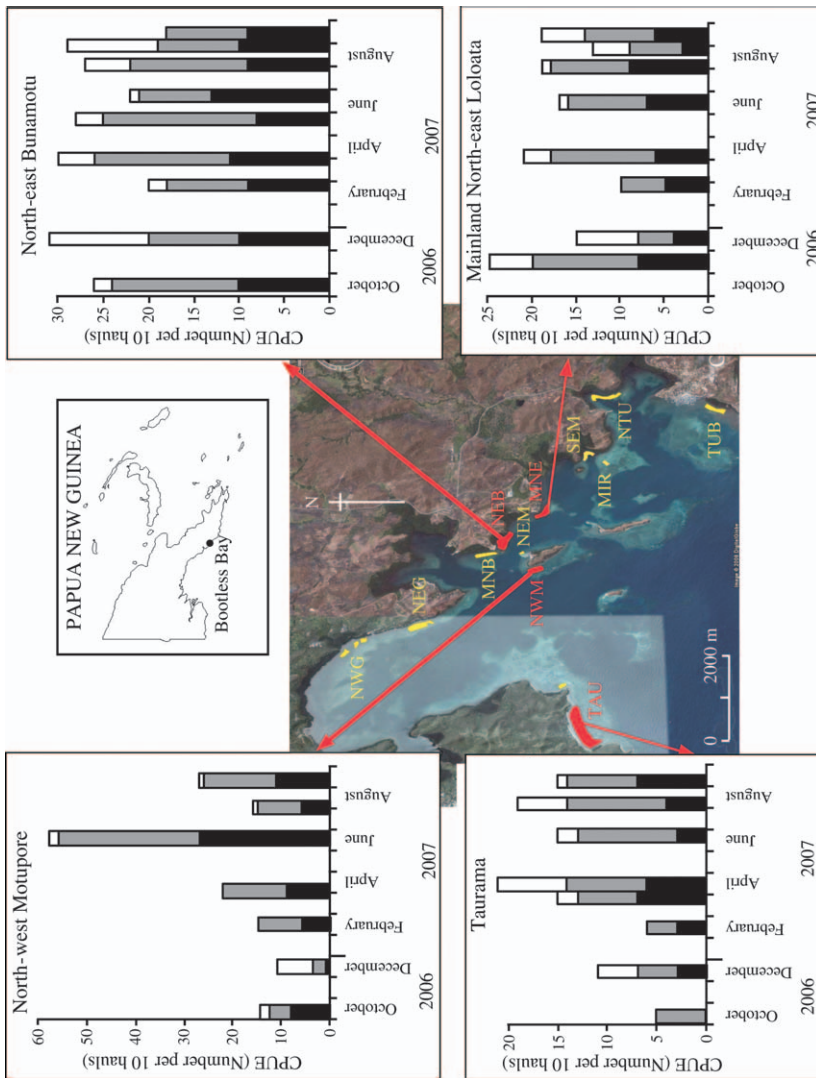


FIG. 1. Location of sampling sites in Bootless Bay, Papua New Guinea (MNB, Mainland north Bunamotu; MNE, Mainland north-east Loloata; MIR, Mirigeta; NEB, north-east Bunamotu; NEG, north-east Gerekia; NEM, north-east Motupore; NTU, north Tubuseria; NWG, north-west Gerekia; NWM, north-west Motupore; SEM, south-east Mirigeta; TAU, Taurama; TUB, Tubuseria). Insets show catch per unit effort (CPUE) of *Syngnathoides biaculeatus* (■, males; ▒, females and □, juveniles) at four monitoring sites from October 2006 to September 2007.

each was assessed from the colour of the eggs and assigned to one of three classes (in order of increasing age): clear (recently laid), white or brown (Takahashi *et al.*, 2003; Dhanya *et al.*, 2005).

Brood development was observed directly in two male *S. biaculeatus* that were caught with eggs in the earliest developmental stage (clear). These individuals were maintained in shaded, flow-through aquaria at MIRC and fed daily on wild caught zooplankton. Water temperature was *c.* 25° C, fluctuating slightly with the tides, and the aquaria experienced ambient day and night cycles (9° S). Digital photographs of the ventral abdominal surface of each male were taken daily and egg counts undertaken as above.

## DATA ANALYSES

The  $L_T$  and  $M$  relationship for *S. biaculeatus* was determined by least-squares linear regression on  $\log_{10}$ -transformed variables with  $M$  as the dependant variable and sex as a covariate. Sexual dimorphism in size between males and females was analysed using a Kolmogorov–Smirnov test on pooled size frequencies distributions for all individuals. Abundance data were standardized to catch per unit effort (CPUE), where unit effort was defined as 10 seine hauls (*c.* 1000 m<sup>2</sup>). Abundance data were normally distributed. Changes in abundance over the course of the study were analysed using general linear models (GLM) with CPUE as the dependant variable and sampling occasion as a random factor. Sex ratios for each month were assessed for departure from a 1:1 ratio using Yates-corrected  $\chi^2$  tests. Relationships between brood size for each developmental stage and male size were determined by least-squares linear regression with brood size as the dependant variable. To determine if there was a relationship between male size and probability of pregnancy all data were pooled, and a logistic regression performed with egg-bearing as a dichotomous response variable and size as a continuous predictor variable. Within each month egg-bearing data were pooled to provide a measure of reproductive seasonality. Changes in size over the study for juveniles, males and females were analysed using GLM with  $L_T$  as the dependant variable, site as a fixed factor and sampling occasion as a random factor. All statistical analyses were performed using SPSS 11.0.4 (www.spss.com). Mean values are presented  $\pm$  s.d.

Growth ( $L_T$  at age  $t$  years,  $L_t$ ) was assumed to conform to a specialized von Bertalanffy model (von Bertalanffy, 1938), *i.e.*  $L_t = L_\infty[1 - e^{-K(t-t_0)}]$ , where  $L_\infty$  is the theoretical maximum  $L_T$ ,  $L_T - K$  is the von Bertalanffy growth coefficient and  $t_0$  is the theoretical age at zero  $L_T$ . frequency data were pooled by sex (male, female and juvenile) into 10 mm size bins for each month, and these pooled data were used in an ELEFAN length-based analysis of growth (Brey & Pauly, 1986) as implemented in the software package FiSAT II (FAO, 2005). Powell–Wetherall plots were used to estimate  $L_\infty$  separately for males and females. These were then used as fixed values in subsequent ELEFAN scans for  $K$ .

## RESULTS

### TOTAL LENGTH AND MASS RELATIONSHIP AND SEXUAL DIMORPHISM

There was no significant difference in the  $L_T$  and  $M$  relationship between males and females (ANCOVA, d.f. = 1,39,  $P > 0.05$  for slopes and d.f. = 1,40,  $P > 0.05$  for intercepts). Therefore, the data were pooled to give an overall  $L_T$  and  $M$  relationship as follows:  $M = 3873 \times 10^{-5} L_T^{4.074}$  ( $r^2 = 0.929$ , d.f. = 1,39,  $P < 0.001$ ). Males were significantly larger than females (Kolmogorov–Smirnov, d.f. = 1,  $P < 0.001$ ), mean  $\pm$  s.d.  $L_T$  for males =  $222 \pm 18$  mm and for females =  $201 \pm 21$  mm.



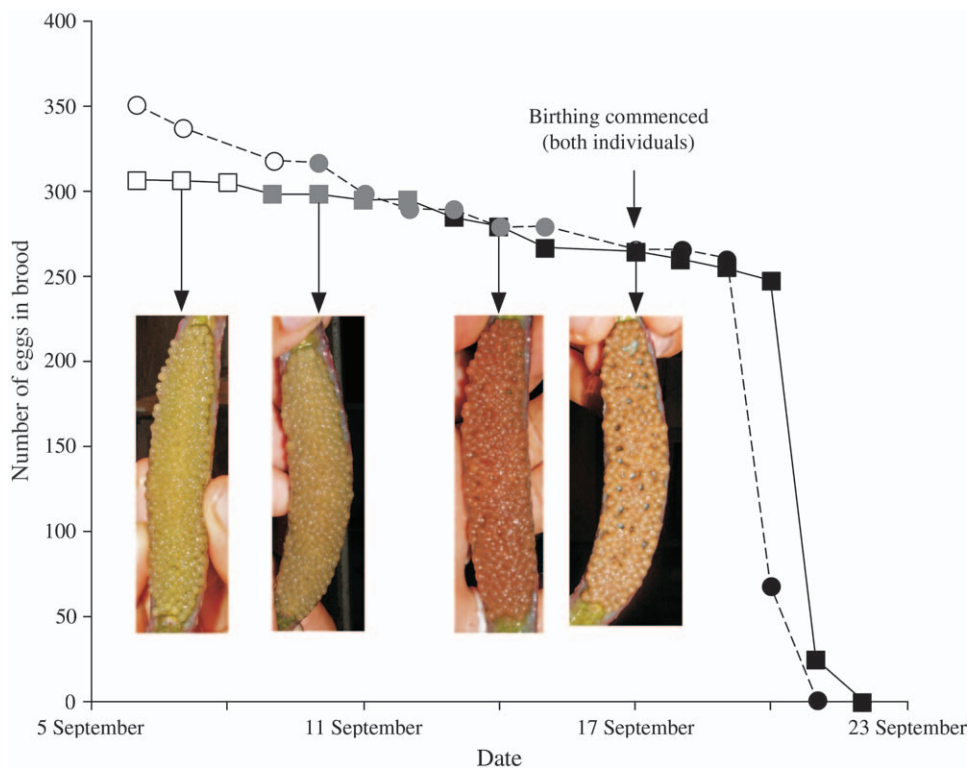


FIG. 2. Time course for two ovigerous male *Syngnathoides biaculeatus* captured on 6 September 2007 (----, male 1; —, male 2) with egg stages: clear (○, □) white (●, ■) and brown (●, ■) stages. Inset photographs show broods from male 2 at indicated time points.

## SEASONAL CHANGES IN ABUNDANCE AND SEX RATIO

At the sites where the abundance of *S. biaculeatus* was monitored, no significant changes in abundance of males, females, juveniles or all individuals over the 11 month study period were found (GLM, d.f. = 7,24,  $P > 0.05$  for all cases) (Fig. 1). Mean  $\pm$  S.D. abundance across all monitoring sites was  $20.0 \pm 9.7$  individuals  $1000\text{ m}^{-2}$  (range 5–58). CPUE at north-east Bunamotu and mainland north-east Loloata remained approximately constant while CPUE increased slightly over time at Taurama and north-west Motupore showed a marked increase in 1 month: June 2007 (Fig. 1).

Sex ratios for all sites combined were not significantly different from 1:1 for all months except August 2007, where there were significantly more females than males (all months except August 2007: Yates corrected  $\chi^2$ , d.f. = 1,  $P > 0.05$ ; August 2007, d.f. = 1,  $P < 0.001$ ).

## REPRODUCTION

The smallest brooding male found in the study was 148 mm  $L_T$ , and the largest was 278 mm  $L_T$ . Newly laid batches of eggs were initially clear, became

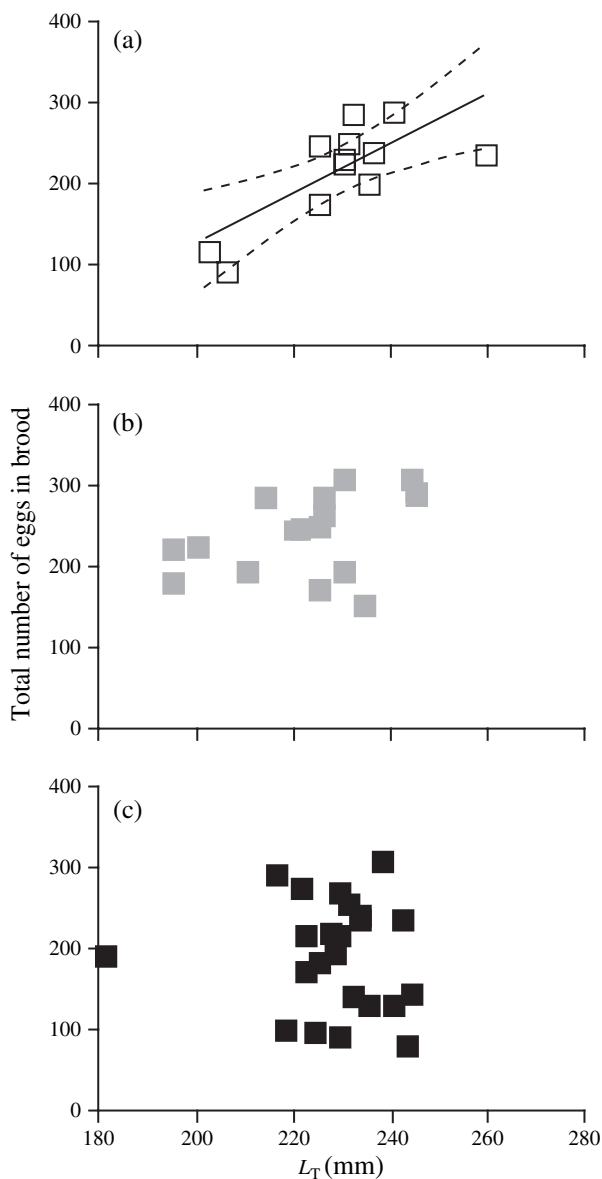


FIG. 3. Relationship between egg numbers and total length ( $L_T$ ) for identified egg stages of *Syngnathoides biaculeatus*: (a) clear ( $y = 3.063x - 484$ ,  $r^2 = 0.551$ , d.f. = 1,10,  $P < 0.01$ ), (b) white and (c) brown stages. (a) The 95% CL are given (---).

opaque white and finally brown with overgrowths of algae (Fig. 2). For the two newly ovigerous males that were maintained in aquaria, brood size gradually decreased over time as some eggs were lost: 72 eggs from a clutch of 351 for male 1 and 40 eggs from a clutch of 306 for male 2. Birth commenced 11 days after both individuals were caught, and the young were released over 2–3 days (Fig. 2). Brood size was significantly, positively related to  $L_T$  only for

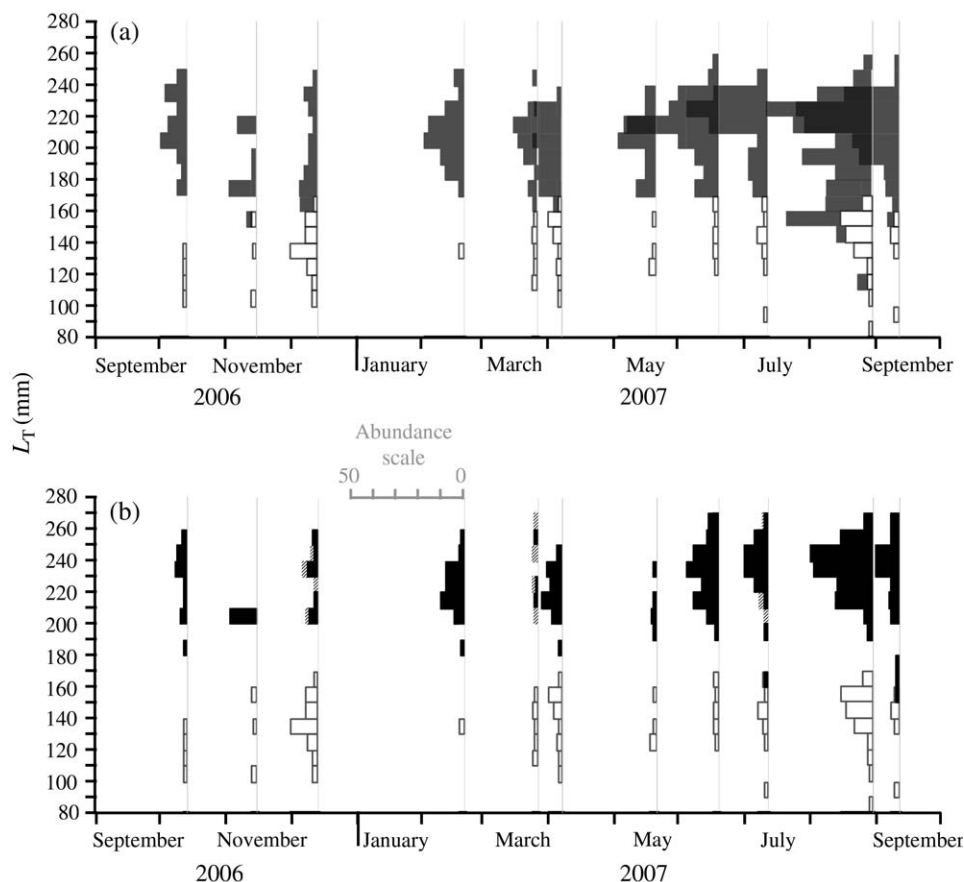


FIG. 4. Total length ( $L_T$ )-frequency of *Syngnathoides biaculeatus* sampled in Bootless Bay: (a) females (□) and juveniles (□) and (b) ovigerous males (■), non-pregnant males (▨) and juveniles (□).

the clear egg stage, while there were no significant relationships for the white and the brown egg stages (Fig. 3). The largest brood size observed was 351 (the initial count of clear eggs from male 1), and the mean  $\pm$  s.d. brood size for newly laid (clear) eggs was  $238 \pm 57$ .

Ovigerous males were found in all months. The frequency of reproduction remained reasonably constant over the entire sampling period ( $69.8 \pm 4.0\%$  where sample size  $\geq 10$ ) indicating there was no peak reproductive season. There was no relationship between the male size and the probability that he was pregnant (Wald statistic, d.f. = 1,  $P > 0.05$ ).

## GROWTH

$L_T$ -frequency plots showed no evidence of identifiable cohorts, consistent with the lack of seasonal reproduction (Fig. 4). Mean  $L_T$  of juveniles and males did not differ significantly throughout the study (GLM, d.f. = 10,76,  $P > 0.05$  for juveniles; d.f. = 10,210,  $P > 0.05$  for males). Females did show a significant



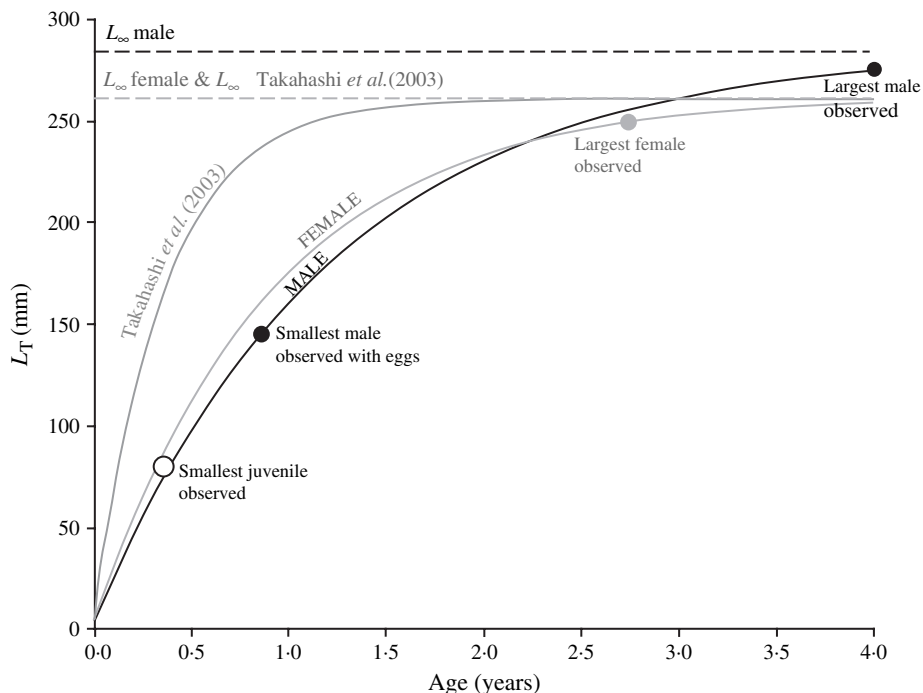


FIG. 5. Estimated von Bertalanffy growth curves for female (---○) and male (—●) *Syngnathoides biaculeatus* and comparison with growth estimate provided by Takahashi *et al.* (2003).

change in  $L_T$  over the course of the study (GLM, d.f. = 10,277,  $P < 0.001$ ) with individuals from November 2006 and September 2007 smaller than in other months. Powell–Weatherall plots provided estimates of  $L_\infty = 261$  mm for females and 285 mm for males. The growth coefficient  $K$  was estimated as  $1.10 \text{ year}^{-1}$  for females and  $0.82 \text{ year}^{-1}$  for males. Using these estimates, male *S. biaculeatus* mature at c. 1 year old and live for 2.5–4.0 years while females mature at c. 0.75 years and live for 1.5–2.5 years (Fig. 5).

## DISCUSSION

This study provides estimates of some key population and life-history variables for *S. biaculeatus* in a tropical seagrass habitat. There was little change in population structure over the course of the study, reproduction occurred year-round and growth estimates indicated that the species is fast growing and short-lived. A number of these variables differed significantly from the only other population study on this species (Takahashi *et al.*, 2003). As this species is exploited for traditional medicine use in many parts of its range (Martin-Smith *et al.*, 2003) these estimates can help assess its vulnerability and guide management through for example the development of surplus production models to estimate sustainable yields.

## ABSENCE OF SEASONAL PATTERNS IN POPULATION VARIABLES

The abundance of species with short generation times often varies in response to environmental conditions (de Silva *et al.*, 2003). Stable year-round abundance and size structure of *S. biaculeatus* in the present study may have been related to year-round reproduction and hence constant recruitment. An additional factor may have been the relative stability in the extent of seagrass habitat over the course of the study as has been observed elsewhere in Papua New Guinea (Brouns, 1987). In contrast, in subtropical Moreton Bay, Australia, *S. biaculeatus* showed seasonal fluctuations in CPUE with greatest abundance in late summer, corresponding to a recruitment pulse from a summer reproductive period from October to April (Takahashi, 2000). In Moreton Bay, reproduction occurred when the water temperature ranged from 28 to 31° C while the minimum water temperature was 21° C (Takahashi *et al.*, 2003). Bootless Bay experienced a mean annual temperature of 27.5° C (range 24–32° C). This mean annual temperature is close to the threshold for reproduction in the study of Takahashi *et al.* (2003). Thus, it would not be unreasonable to suggest that year-round reproduction was linked to higher water temperatures. Indeed, year-round reproduction was found for the worm pipefish *Nerophis lumbriciformis* (Jenyns) in Portugal but was restricted to the warmer summer months further north in the U.K. (Monteiro *et al.*, 2001).

Many syngnathid populations, particularly seahorses, display equal sex ratios for most of the year (Franzoi *et al.*, 1993; Foster & Vincent, 2004). A number of pipefish species, however, display considerable sex biases, usually male dominated, at certain times of year (Bayer, 1980; Steffe *et al.*, 1989; Vincent *et al.*, 1995; Watanabe & Watanabe, 2001). In these instances, the biased sex ratio coincides with reproductive activity and it has been suggested that migration to and from reproductive areas is responsible (Steffe *et al.*, 1989; Vincent *et al.*, 1995). For example, male *N. lumbriciformis* were observed, after mating, to leave the mating grounds while females remained longer (Monteiro *et al.*, 2006). As males with eggs were observed in all months, it does not appear likely that the biased sex ratio observed in August 2007 was due to reproductive migration. This was probably a sampling artifact.

## REPRODUCTION

Syngnathid clutch size is primarily dependant on body size and pouch structure (Lourie *et al.*, 1999). A small male syngnathid, such as *Hippocampus zosterae* Jordan & Gilbert, can have a brood of as few as five, whereas the large male *Hippocampus ingens* Girard can carry up to 2000 young (Lourie *et al.*, 2004). In this study, the initial clutch size of male *S. biaculeatus* was found to have a significant positive relationship to  $L_T$  suggesting that the abdominal brooding area enlarges with increasing body size. The loss of the size–egg number relationship during gestation, however, indicates that egg loss is not proportional to egg numbers and so is essentially stochastic, probably related to predation, parasitism or mechanical dislodgement. The

estimated brood size for both newly laid eggs (mean 238 and maximum 351) and those at the brown stage (mean 198 and maximum 313) were considerably higher (*c.* 1.5 $\times$ ) than those of Takahashi *et al.* (2003). This difference is probably due to the 20–25 mm greater size that male *S. biaculeatus* attained in Bootless Bay compared with Moreton Bay. The regression for brood size at the clear stage would indicate that this size difference represents a clutch size difference of 50–75 eggs consistent with the actual value of 53.

It is suggested that female *S. biaculeatus* produce approximately the same quantity of mature eggs as brooded by the male (Takahashi *et al.*, 2003). Male *S. biaculeatus* have only been observed carrying eggs of an equal developmental stage, indicating that they most likely receive eggs from only one female, *i.e.* within brood monogamy. Gestation in *S. biaculeatus* was relatively rapid in this study (minimum 16 days) and was similar to laboratory observations of Dhanya *et al.* (2005). Hatching of all eggs over a short period of time provided supporting evidence that all the eggs in one brood were laid at the same time. Pipefishes show a wide range of reproductive systems from monogamy to polyandry and polygynandry (Gronell, 1984; Jones & Avise, 1997; Watanabe *et al.*, 1997; Jones *et al.*, 2001). Genetic investigations would be needed to confirm within brood monogamy in *S. biaculeatus*, and whether monogamy extended across broods.

## GROWTH RATES

Growth rates of *S. biaculeatus* ( $K = 0.8\text{--}1.1 \text{ year}^{-1}$ ) in Bootless Bay were estimated to be much lower than *S. biaculeatus* in subtropical eastern Australia ( $K = 2.8 \text{ year}^{-1}$ ; Takahashi *et al.*, 2003) while  $L_{\infty}$  was comparable. The growth rate estimates from Bootless Bay are consistent with those of other pipefish and seahorse species, lying within the 95% CL of a linear regression of  $K$  and  $L_{\infty}$  for the syngnathids (Froese & Pauly, 2007). Without clear growth modes, however, the length-frequency analysis may not be particularly robust (Brey & Pauly, 1986).

These growth rate estimates imply that *S. biaculeatus* in Bootless Bay mature at *c.* 10 months of age and live between 2 and 4 years. In contrast, the estimates of Takahashi *et al.* (2003) imply that individuals mature in *c.* 3 months and live for <1.5 years. Counts of increments in the asterici of laboratory-raised juveniles *S. biaculeatus* from Moreton Bay supported the hypothesis of daily ring deposition (Takahashi, 2000). Wild-caught individuals had up to 100 rings although this was considered an underestimate due to difficulty reading older otoliths (Takahashi, 2000). Direct validation of growth rates and longevity using marked individuals would be one method of resolving this discrepancy between growth and longevity estimates.

## CONSERVATION IMPLICATIONS

It is clear that *S. biaculeatus* is a fast growing, short-lived fish species, despite the differences in some individual and population variables between the present study and the earlier work (Takahashi, 2000; Takahashi *et al.*, 2003). Population density was a similar order of magnitude to that of many of seahorse species (Foster & Vincent, 2004) and considerably smaller than

that of many temperate, seagrass-inhabiting pipefish (Ferrell *et al.*, 1993; Edgar & Shaw, 1995; Hindell *et al.*, 2000). Fecundity is low compared with similar-sized fish species but is probably offset by the lack of a free-swimming larval phase and potentially higher survival of juveniles. These life history and population characteristics suggest that *S. biaculeatus* should be relatively resilient to exploitation although the absolute numbers which can be harvested will be relatively small.

*Syngnathoides biaculeatus* has considerable potential as a flagship species to raise awareness of threats facing tropical seagrass habitat. The distribution of *S. biaculeatus* across a wide area of the Indo-Pacific (Dawson, 1985) suggests that it could be effective in mobilizing widespread support for seagrass conservation. As it appears to be found only in seagrass habitats (Kuitert, 2000; Takahashi *et al.*, 2003; this study), loss of seagrass habitat would lead to declines in populations of *S. biaculeatus*. Seagrasses and their associated ecosystems are considered to be in crisis with calls for a global conservation effort including educating the policymakers and the general public (Orth *et al.*, 2006).

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