

Growth Pattern of the Tropical Sea Cucumber, *Holothuria scabra*, under Captivity

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Abstract

The growth of the juvenile sea cucumber, *Holothuria scabra*, was studied under captivity to elucidate the growth variation pattern and determine the best-fit growth model to estimate age- and size-specific growth rates. Individual growth was extremely variable, with some individuals below the mean initial weight and some expanding their original body length (L) and weight (W) by up to 6.4 and 156 times, respectively; during 84 days of culture starting at 127 days of age. Some of the smallest individuals showed a higher condition factor than larger individuals in the presence of ample food, indicating that lack of food may not be the only impediment to growth. Among the three growth models compared (von Bertalanffy, Gompertz and logistic), the Gompertz model was considered optimal to express *H. scabra* growth; both in L and W . The age- and size-specific daily growth rate for L and W up to 365 days of age, as estimated by the Gompertz model, had a range of two and nine orders of magnitude in L (0.035 – 0.96 mm/day) and W (3.4×10^{-7} – 3.5 g/day), respectively. Use of the Gompertz model over the linear model, which tends to overestimate growth rates, is encouraged to estimate the growth of *H. scabra* more accurately.

Discipline: Aquaculture

Additional key words: condition factor, Gompertz model, shooter, growth trajectory

Introduction

Tropical sea cucumbers have been heavily exploited for processing into bêche-de-mer or trepang (dried product) in many countries in the Pacific and Indian Ocean for export; mainly to the Chinese market (Carpenter & Niem 1998, Conand 2004, Hamel et al. 2001, Uthicke et al. 2004), resulting in severe depletion of wild stocks in many species. Accordingly, there has been growing interest in developing hatchery, aquaculture and stock enhancement techniques; particularly for sandfish, *Holothuria scabra*, one of the most valued tropical sea cucumber species (Battaglione et al. 1999, Purcell & Kirby 2006). There has also been growing interest in developing polyculture methods using sea cucumbers, which can consume nutrient from aquaculture debris and thus bio-mitigate environmental degradation (Ahlgren 1998, Slater & Carton 2007, Pitt et al. 2004, Watanabe et al. 2012a, Watanabe et al. 2013). However, there is a paucity of basic

biological and ecological information of *H. scabra* to design an effective polyculture system. For instance, although growth information is essential to estimate the appropriate stocking density of *H. scabra* in relation to the energy budget within the polyculture system, the growth curve for *H. scabra* is not established. It is reported that *H. scabra* can reach 40 cm in length and 2 kg in weight in India (James 1996), but the lifespan remains poorly understood.

The lack of a means of determining age for sea cucumbers is problematic for growth studies in the wild. Meanwhile, age characters in hard tissues, such as otolith and scale in teleost fishes, have not been found in sea cucumbers, nor has any age class separation method based on cohort analysis been established, which means age and size relationships can only be precisely determined under captivity. However, the growth rates of *H. scabra* reported in hatchery, aquaculture and sea ranching studies are fragmentary and often unrelated to age or size.

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To date, most growth data reported on *H. scabra* have been limited to the mean growth rate of a certain culture period with a certain initial size or age (Battaglione et al. 1999, Hamel et al. 2001, Agudo 2006, Duy 2010). The lack of growth trajectory information also makes it difficult to compare growth rates obtained in different studies dealing with different size ranges. One of a few – or the only – set of studies estimating the size-specific growth rate of *H. scabra* from long-term growth monitoring by Purcell & Kirby (2006) is based on the modified von Bertalanffy growth model (McNamara & Johnson 1995). Although the von Bertalanffy growth model is one of the most commonly used for fisheries science, its applicability to *H. scabra* remains unconfirmed.

The present study aimed to examine the growth pattern of juvenile *H. scabra* under captivity and determine the best-fit growth model to estimate age- and size-specific daily growth rates.

Materials and Methods

1. Culture and size measurements of juvenile *Holothuria scabra*

Juvenile *Holothuria scabra* produced at the sea cucumber hatchery of the Aquaculture Department, the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) in the Philippines were cultured in a tank with sand substrate (beach sand sieved with a 1 mm mesh) and aeration. The experimental *H. scabra* were born on the same day via group spawning of broodstock, with settlement at 14 days of age. As many similarly sized individuals as possible were collected at 127 days of age (n=150) to start the rearing, since younger *H. scabra* sometimes have very high mortality. The culture tank (1.5 t, made of fiberglass with a bottom area of 1 m² and water depth of 30 cm) was placed in an outdoor experimental area with roofing at SEAFDEC/AQD and neither temperature or light controlled. The water temperature, salinity, dissolved oxygen (DO) and pH were monitored daily. Eighty percent of the tank water (10- and 1 µm-filtered, UV-sterilized) was changed every 2 days in the morning, and *H. scabra* were fed with ample benthic diatom, *Navicula ramossissima* cultured at SEAFDEC/AQD (7L, approximate density 6.2×10^5 cells/mL) supplemented with ample ground commercial shrimp feed (Tateh feed, Philippines, 1 g, starting after 1 month of culture) on the same day in the evening.

Body size measurements were conducted at the beginning of the culture and every 2 weeks for every *H. scabra*, during which mortality was also recorded. *H. scabra* were anesthetized with 2% menthol-ethanol solution (Watanabe et al. 2012b) and blotted dry with paper towels before measuring body length (*L*) and breadth (*B*) to the nearest 0.01 mm with a digital caliper and body weight (*W*) to the nearest

0.01 g with a microbalance. After each measurement, the tank was washed and the sand replaced with washed sand to remove naturally occurring insect larvae.

2. Growth and condition factor analyses

The mean growth rates (*MG*) in *L* (mm/day) and *W* (mg/day) were obtained as $MG = \frac{L_t - L_0}{t}$, where *L_t* is the size at sampling on culture day (*t*) and *L₀* is the mean initial size (same for *W*). The condition factor (*K*, i.e. plumpness) was obtained as $K = \frac{W}{V}$ (g/mm³ × 10⁴), where *V* is body volume:

$$V = \frac{4}{3}\pi \left(\frac{L}{2}\right) \left(\frac{B}{2}\right)^2, \text{ assuming a spheroid body shape.}$$

The mean size at age was fitted to three growth models commonly used for fisheries science:

1) von Bertalanffy growth model: $L_t = L_\infty (1 - e^{-k(t-t_0)})$

$$\text{and } W_t = W_\infty (1 - e^{-K(t-t_0)})^{2.8}$$

2) Gompertz growth model: $L_t = L_\infty e^{-Ce^{-bt}}$ (same for *W*)

3) Logistic growth model: $L_t = \frac{L_\infty}{1 + e^{-b(t-t_c)}}$ (same for *W*),

where *k*, *t₀*, *b*, *C* and *c* are model parameters determined by the least squares method using the Microsoft Excel solver function. An exponent of 2.8 was employed for the von Bertalanffy model instead of the usual 3; based on the relationship obtained for *L* and *W* in this study (see results). For settlement size (not measured in this study), average sizes reported at SEAFDEC/AQD sea cucumber hatchery (14 days of age, 0.8 mm, 0.16 mg) were used. For the default values of asymptotes (i.e. *L_∞* and *W_∞*) for Excel solver calculations, the largest *L* and *W* reported from India (400 mm and 2000 g, James 1996) were employed. The growth models were fitted to total *H. scabra*, high-growth class (i.e. 50% fastest growing individuals for *L* and *W*) and low-growth class (50% slowest growing).

The fit of the growth models was determined from *r*² (coefficient of determination), AIC (Akaike's information criterion), as well as comparing the model predicted sizes at 365 days of age obtained in this study with the previously reported values (191, 205 and 243 mm; 150, 182 and 292 g; Agudo 2006, Purcell & Simutoga 2008, Hamel et al. 2001). The *L* and *W* relationships obtained in this study were used to estimate *W* from *L* and vice versa for those data with only *W* or *L*.

The age- and size-specific daily growth rates (*DG*) were calculated from the estimated size at age (*t*, day) from the best-fitted model from above: $DG = L_t - L_{t-1}$, and plotted against age, *L* and *W* up to 365 days of age. For correlation analyses, *p* values less than 0.01 were considered statistically significant.

Results

1. Mortality, culturing density and mean growth rate

Rearing water conditions were relatively stable throughout the culture period with water temperature ranging from

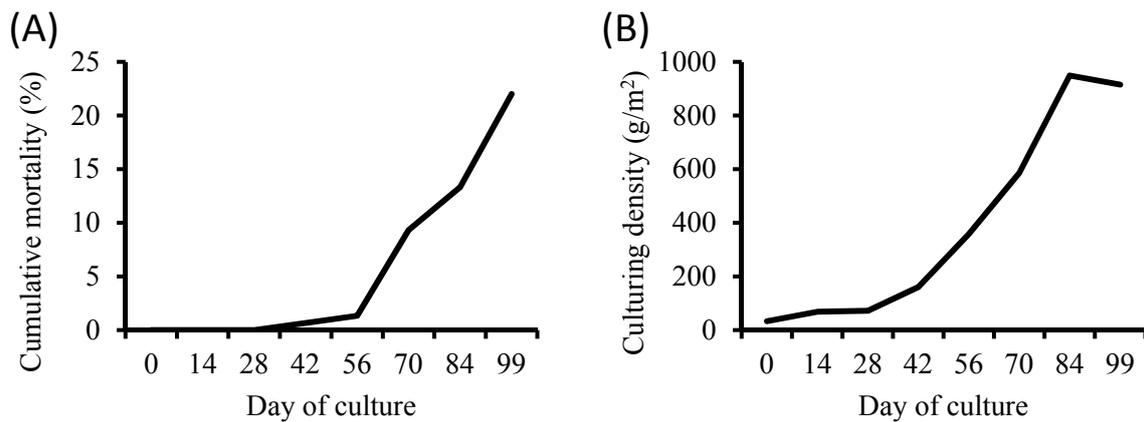


Fig. 1. Cumulative mortality (A) and culturing density (B) of *H. scabra* cultured under captivity

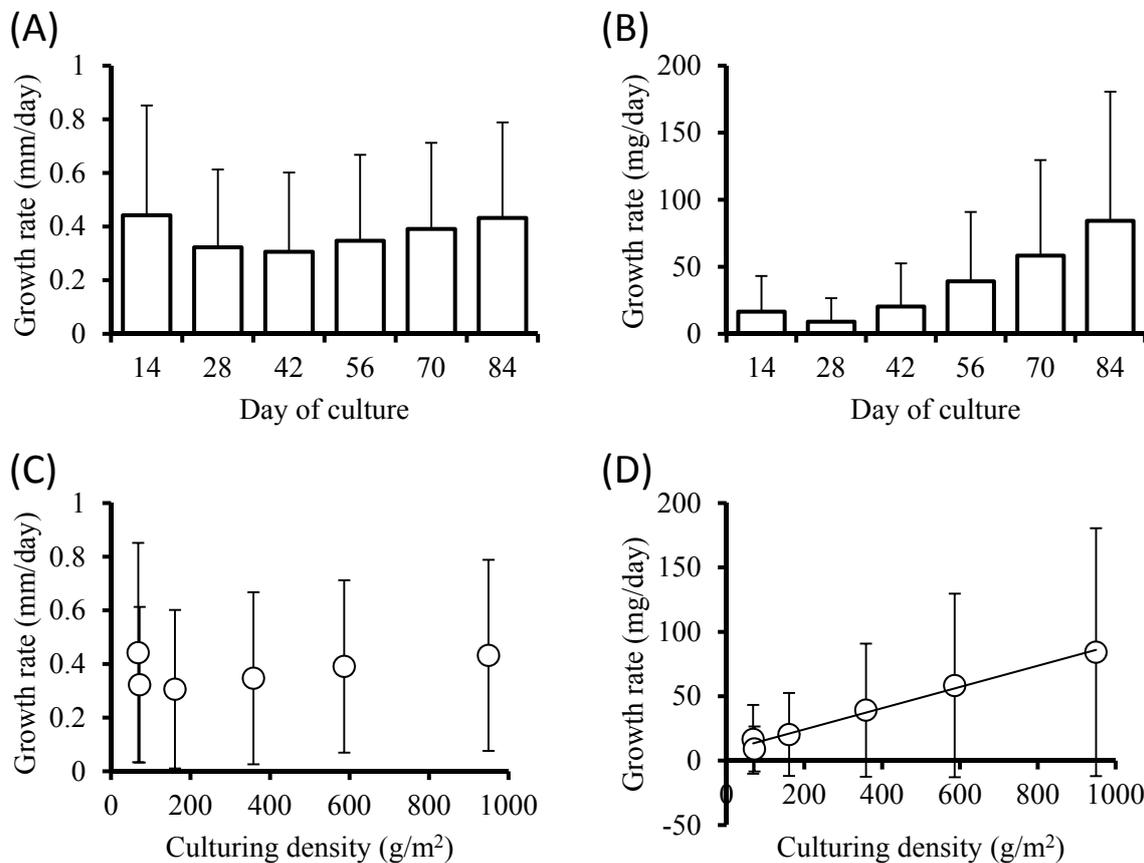


Fig. 2. The mean growth rates of *H. scabra* in body length (A) and weight (B), and relationship between culturing density and growth rate in body length (C) and weight (D)

Error bars indicate standard deviation.

24.0 – 27.2°C (with mean of $26.3 \pm 0.81^\circ\text{C}$, \pm SD), salinity (PSU) ranging from 34.0 – 35.1 (35.0 ± 0.31), DO ranging from 3.6 – 6.6 mg/L (4.6 ± 0.62 mg/L) and pH ranging from 7.2 – 7.6 (7.4 ± 0.097).

The mortality of juvenile *H. scabra* was observed to begin on the 42nd day of culture (DOC, 0.7% mortality, 169 days of age) and gradually rose until the cumulative mortality had reached 22.0% on DOC 99 (226 days of age),

whereupon the data collection was terminated (Fig. 1A). The causes of mortality were not evident; no disease symptoms, such as skin lesions due to stress and handling (Agudo 2006), were observed. The culturing density (biomass / area) of *H. scabra* increased exponentially over time from the stocking (33.4 g/m²) until DOC 84 (949.5 g/m²) due to the increase in *W* (Fig. 1B). However, on DOC 99, a decrease in density was seen (915.2 g/m²) due to increased cumulative mortality,

which is the reason data up until DOC 84 (211 days of age) were used to analyze the growth pattern.

The relationship between L (mm) and W (g) on DOC 84 effectively fitted the power function: $W = 7.4 \times 10^{-5} \times L^{2.8}$ ($r^2=0.99$, $n=127$, $p<0.0001$).

The mean growth rate (MG) from the first day (15.3 ± 3.16 mm; 0.22 ± 0.11 g) to the respective day of culture in L ranged from 0.30 ± 0.30 mm/day to 0.44 ± 0.41 mm/day (Fig. 2A). MG in W decreased from 16.6 ± 26.7 mg/day (DOC 14) to 9.13 ± 17.5 mg/day (DOC 28) and gradually increased thereafter to 84.3 ± 0.84 mg/day (Fig. 2B). There was a significant positive correlation between MG in W and culturing density (Fig. 2D, $r^2=0.99$, $n=6$, $p<0.001$, $Density = 0.082 \times W + 7.9$) but not between MG in L and the density (Fig. 2C, $r^2=0.22$, $n=6$, $p=0.35$).

2. Growth variation and condition factor

The growth of individual juvenile *H. scabra* varied considerably. Although ample feed was provided, considerable fractions of *H. scabra* had smaller L (18.5%) and W (23.1%) than the initial mean values on DOC 84, while the frequency distribution pattern of L on DOC 84 had bimodal distribution, divided around 28 mm (Fig. 3A). The frequency distribution of W was not clearly bimodal and heavily skewed to the lower end (<2.3 g, Fig. 3B), while on DOC 84, 86.9% of *H. scabra* had a higher condition factor (K) than the initial value (8.0 ± 1.7 g/mm³ × 10⁴), while some smaller individuals, typically those less than around 20 mm, had higher K values than larger ones, despite stunted growth (Fig. 3C).

3. Growth model fitting

Among the three growth models compared in this study, while r^2 was significant for all three for both L and W in all growth classes ($p<0.01$), the Gompertz model showed the smallest AIC value (i.e. indicating best fit), except for W in the low-growth class where the Gompertz and logistic model had the same AIC (Table 1). AIC for the von Bertalanffy model constantly had larger values than the other two models, particularly for L . The data fit to the Gompertz and logistic models were mutually comparable with minimal AIC difference. However, comparison of previously reported L and W for *H. scabra* at 365 days of age (○ in Fig. 4) with extrapolated values from the models indicated that the logistic model tended to greatly overestimate W (Fig. 4 lower). The Von Bertalanffy model predicted W at 365 days of age effectively for the high-growth class but underestimated the same for total class. Meanwhile, both the Gompertz and logistic models predicted L at 365 days of age effectively for total and high-growth classes, but the von Bertalanffy model greatly underestimated L (Fig. 4 upper), while the low-growth class predicted a smaller L and W than previously reported values, except for overestimation of W by the logistic model.

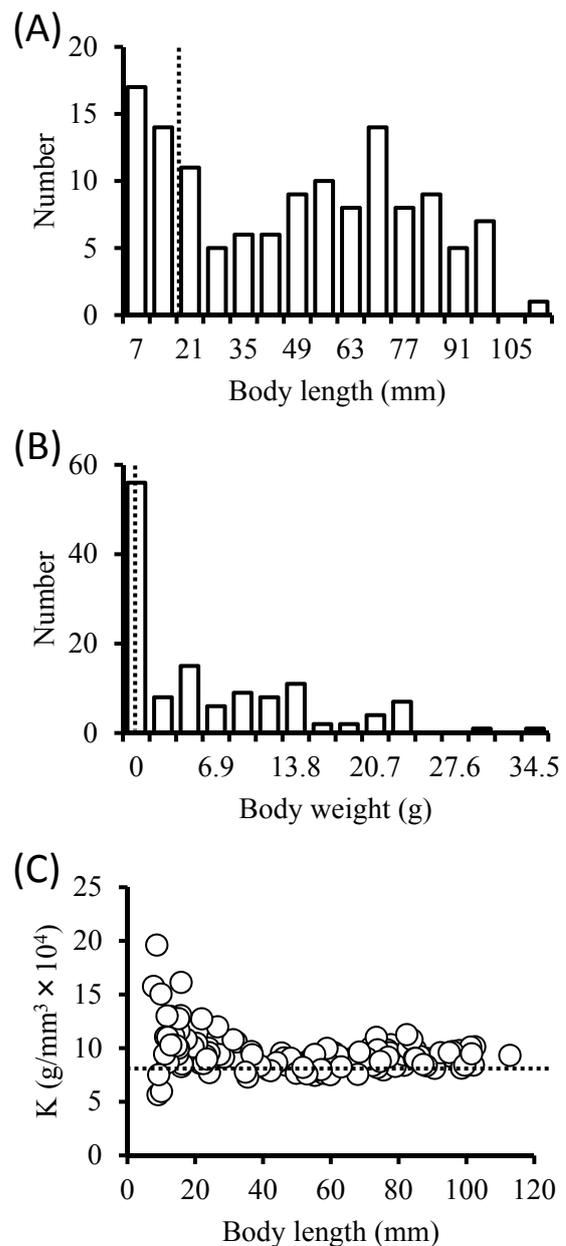


Fig. 3. Body length (A) and weight (B) frequency distribution, and relationship between condition factor and body length (C) of *H. scabra* cultured under captivity for 84 days (211 days of age)

Dashed lines indicate the mean body length, weight and condition factor (K) at the beginning of culture (127 days of age).

The Gompertz model was thus employed to derive age- and size-specific daily growth rates (DG) for up to 365 days of age (Fig. 5). Gompertz models for total growth class: $L_t = 545.6 e^{-6.57e^{-0.0018t}}$, $W_t = 1961.4 e^{-22.5e^{-0.0066t}}$

DG ranges and MG estimated for three growth classes are summarized in Table 2. Due to the non-linear growth pattern, DGs in L for the total *H. scabra* covered two orders of magnitude (0.035 – 0.96 mm/day), while DGs in W covered

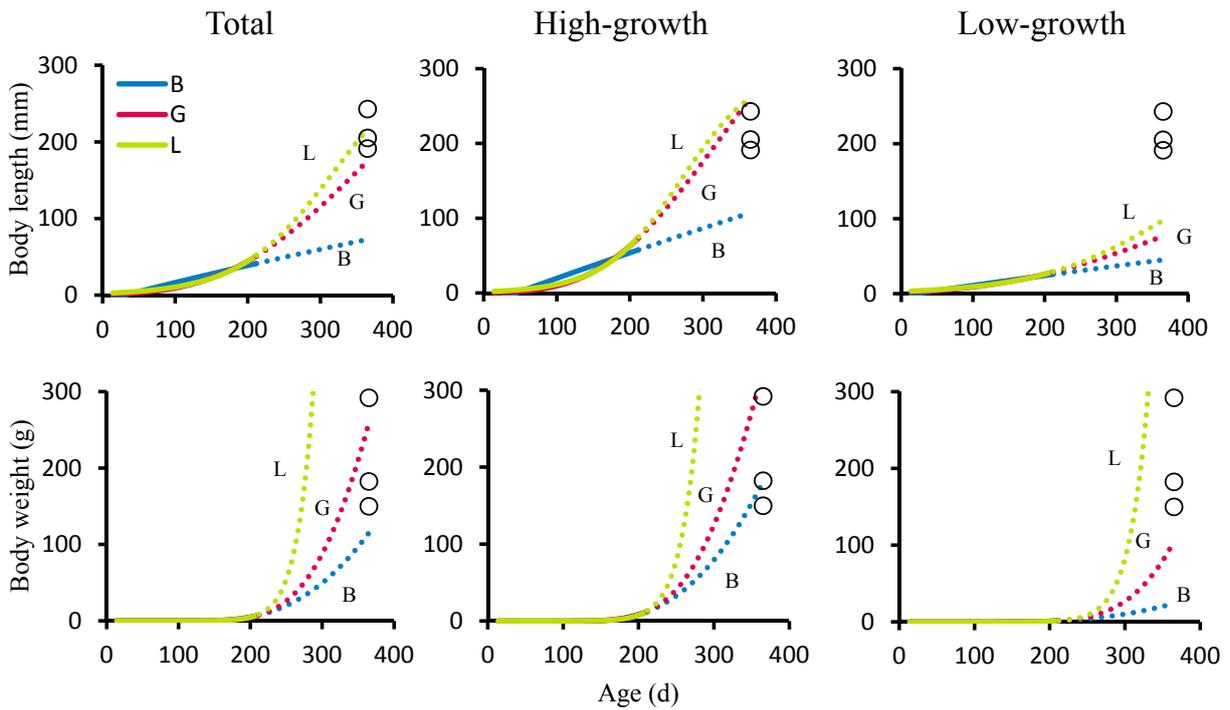


Fig. 4. Body length and weight growth trajectories estimated for cultured *H. scabra*
 B, G and L indicate the von Bertalanffy, Gompertz and logistic growth models, respectively. Dashed lines are extrapolated estimations from 212 to 365 days of age. ○'s are the body weight reported for 1-year-old *H. scabra* and body length estimated from the body weight by the equation obtained in this study. Data fitting was performed on total, high-growth class (upper 50%) and low-growth class (lower 50%).

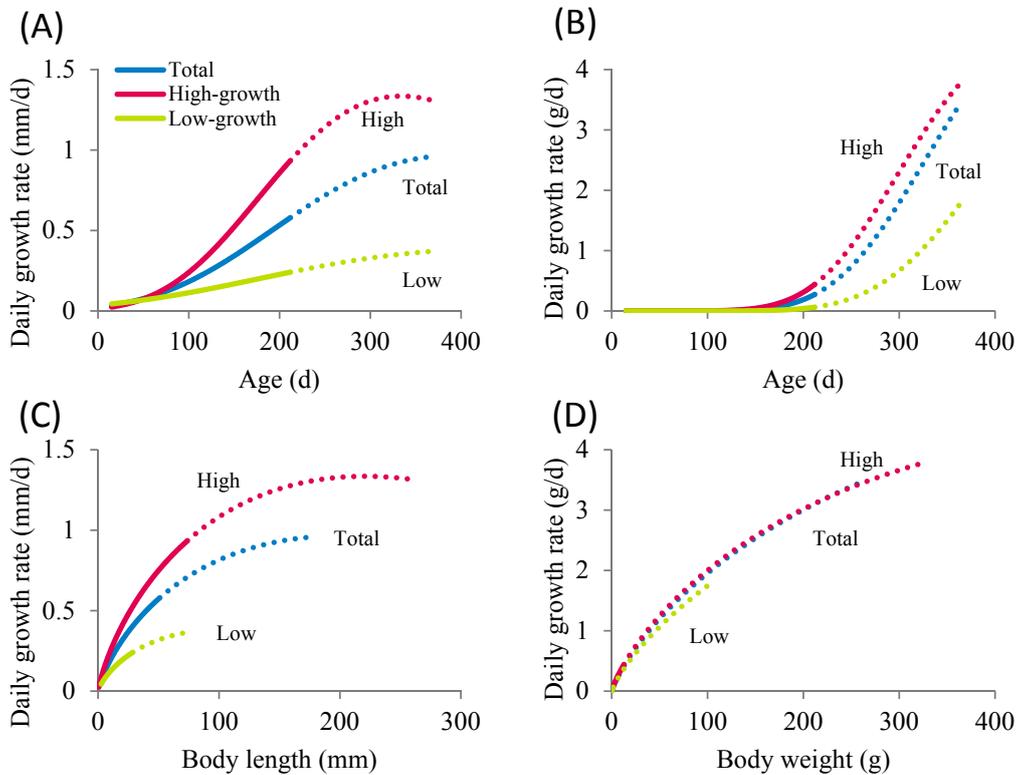


Fig. 5. Age-specific (A and B) and size-specific (C and D) daily growth rate of cultured *H. scabra* estimated by the Gompertz growth model
 Dashed lines are extrapolated estimations from 212 to 365 days of age.

Table 1. Fitting of *H. scabra* growth data from 127 to 211 days of age under captivity to three growth models

			von Bertalanffy	Gompertz	Logistic
AIC	Total	L	122.6	26.7	27.7
		W	81.3	10.5	14.3
	High-growth	L	131.0	26.5	27.2
		W	82.5	11.7	17.4
	Low-growth	L	106.6	32.6	34.1
		W	80.6	10.3	10.3
r^2	Total	L	0.85	0.99	0.99
		W	1.00	1.00	0.98
	High-growth	L	0.81	1.00	1.00
		W	1.00	1.00	0.99
	Low-growth	L	0.93	0.96	0.95
		W	0.94	0.97	0.98
p	Total	L	1.0×10^{-3}	4.0×10^{-8}	6.2×10^{-8}
		W	3.9×10^{-8}	2.2×10^{-9}	2.3×10^{-6}
	High-growth	L	2.3×10^{-3}	3.5×10^{-9}	4.8×10^{-9}
		W	1.2×10^{-8}	3.3×10^{-9}	9.5×10^{-7}
	Low-growth	L	9.3×10^{-5}	1.9×10^{-5}	3.5×10^{-5}
		W	5.4×10^{-5}	9.2×10^{-6}	3.9×10^{-6}

Data fitting was performed on total *H. scabra*, high-growth class (upper 50%) and low-growth class (lower 50%). AIC: Akaike's information criterion (i.e. smaller AIC indicates better data fitting), r^2 : coefficient of determination, p: p-value for r^2 . *L* and *W* denote body length and body weight, respectively.

Table 2. Daily and mean growth rate of *H. scabra* from settlement at 14 to 365 days of age estimated by the Gompertz growth model

	Body length growth (mm/day)		Body weight growth (g/day)	
	Daily growth rate	Mean growth rate	Daily growth rate	Mean growth rate
Total	0.035 - 0.96	0.50	3.4×10^{-7} - 3.5	0.74
High-growth	0.025 - 1.30	0.74	4.5×10^{-7} - 3.8	0.93
Low-growth	0.044 - 0.37	0.21	6.7×10^{-8} - 1.8	0.30

nine orders of magnitude (3.4×10^{-7} – 3.5 g/day) depending on the size and age.

Discussion

This study attempted to examine the growth pattern of juvenile *Holothuria scabra* cultured under captivity and determine a suitable growth model to provide a basis for hatchery, aquaculture and fishery management.

Causes of the mortality of *H. scabra* at higher culture density were not identified in this study. Battaglene et al. (1999) observed higher mortality and slower growth at high culture density in juvenile *H. scabra*, and suggested that food availability may be the limiting factor. Similarly, Slater & Carton (2007) reported that the growth of *Australostichopus mollis* was density-dependent and constrained by food limitation. However, since ample feed was supplied to *H. scabra* in this study, density effects other than food availability may have caused mortality and reduced growth at high density.

Battaglene et al. (1999) observed slow growth at a culture density exceeding 225 g/m², but the mean growth

rate continued to increase above 940 g/m² in this study (Fig. 1), possibly due to the presence of ample food. However, the individual growth of *H. scabra* varied significantly, with some individuals below the mean initial weight and some showing up to 6.4- and 156-fold increases in body length (*L*) and weight (*W*), respectively (Fig. 3). Some of the smallest individuals showed a higher condition factor (i.e. plumper) than larger individuals on DOC 84, indicating that lack of food is not the only impediment to growth.

Large growth variation is a common characteristic for sea cucumbers under captivity (Battaglene et al. 1999, James 1996, Ramofafia et al. 1997), but the causes of the variation are unclear. In this study, since juvenile *H. scabra* were obtained from group spawning of multiple broodstocks, the potential for congenital growth effects cannot be excluded. However, according to Battaglene et al. (1999), stunted juvenile *H. scabra* were due to being held at higher densities, and since they resumed growth when reared at lower densities, slow growth may not be a congenital trait. Shooters (i.e. exceptionally fast- or slow-growing individuals) are a widely known phenomenon in cultured aquatic organisms,

and considered primarily attributable to the advantage of larger individuals in feeding competitions (Nakamura & Kasahara 1955, Yamaguchi 1962, Magnuson 1962, Umino et al. 1994), although it is unknown whether larger body size is a competitive advantage in the detrital feeding of sedentary *H. scabra*. There is a need to further elucidate the causes of growth variation to improve the production efficiency of *H. scabra*.

Most growth data of *H. scabra* to date are limited to certain culture periods, and only the mean growth rates over the entire culture period are reported (Battaglione et al. 1999, Hamel et al. 2001, Agudo 2006, Duy 2010). Purcell & Kirby (2006) estimated the size-specific growth rate of *H. scabra* based on the modified von Bertalanffy growth model (McNamara & Johnson 1995), but did not confirm its applicability to *H. scabra*. Although the von Bertalanffy model is the most common for fisheries science, it showed the poorest fit to *H. scabra* growth in *L* and *W*, and its extrapolation up to 365 days of age greatly underestimated *L* compared to previously reported values (Fig. 4). Conversely, the Gompertz and logistic models, s-shaped curves often used for seaweed and shellfish growth (Akamine 2007), fitted *H. scabra* growth in *L* and *W* very precisely (Gompertz showed slightly better fit) for all three growth classes studied, and seemed to produce comparably reliable size estimates within an interpolated age range up to 211 days of age. However, since the logistic model overestimated *W* excessively at 365 days, even in the low-growth class, the Gompertz model was considered the best to express *H. scabra* growth. Further studies under different culturing conditions, particularly in the wild, should be carried out for confirmation.

Since the constants for the Gompertz model (L_{∞} , C and k) are supposed to have different values depending on the environment, constants must be determined for each environment. While it is possible to precisely determine relationships between age and size under artificial culture, it is difficult to obtain size-at-age data for wild *H. scabra* populations. Sea cucumbers lack age characters, such as otoliths in teleost fishes, and it is also difficult to determine age from cohort analysis. Shelley (1985) estimated the growth rate of wild adult *H. scabra* at 0.5 cm/month (14 g/month) based on monthly size/frequency distributions in Papua New Guinea but stated that the monthly modal progression was not clear enough to encourage further analysis. Although release-recapture of *H. scabra* with spicule staining technique is useful for studying size-at-age data, the stain can last only about 1 year (Purcell et al. 2006, Purcell & Simutoga 2008). Shrinkage (Uthicke et al. 2004) can also complicate matters. *H. scabra* attain initial maturity at 183 g (Conand 1990, some *H. scabra* spawned at 50 g of weight at SEFDEC/AQD), and gametogenesis uses stored nutrients in the body wall; resulting in body weight loss during reproduction (Morgan 2000), which also results in body length loss for *H. scabra*. Poor

nutritional condition also causes body shrinkage (Watanabe et al. 2012b) although it should be avoided under aquaculture conditions. Since *H. scabra* did not attain maturity in this study, the effects of maturation on growth curve fitting must be further examined.

The extremely high individual growth variation in *H. scabra* means growth estimation is inevitably rough and approximate at best. Nevertheless, since age- and size-specific daily growth rates based on the Gompertz model showed a range of two and nine orders of magnitude for *L* and *W* growth, respectively in one year growth (Fig. 5), using the Gompertz model should considerably facilitate growth rate prediction as compared with linear regression or the mean growth rate. For example, the Gompertz model may be particularly useful to estimate time course size-specific change in nutrient requirements and the metabolic excretion rate of *H. scabra* to design an effective polyculture method with a fed species whose excess feed and feces provide nutrients to *H. scabra* and an inorganic extractive species which is fertilized by ammonia excretion by *H. scabra*.

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