Zoological Studies **60:**49 (2021)
doi:10.6620/ZS.2021.60-49

Zoological Studies

Open Access

Reproduction and Early Juvenile Growth of the Giant Clams *Tridacna noae* and *Tridacna maxima* in Taiwan

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Received 29 June 2020 / Accepted 31 May 2021 / Published 7 September 2021
Communicated by Benny K.K. Chan

The reproductive cycle, larval development, and juvenile growth of *Tridacna noae* and *Tridacna maxima* were investigated to improve their hatchery production for conservation and commercial use in Taiwan. To study the giant clam reproductive cycle, hypodermic extraction of oocytes from *T. noae* and *T. maxima* was conducted in Dongsha and Kenting, Taiwan, from March 2013 to May 2014; ripe eggs were observed in both species from March to August. There was no apparent correlation between reproduction and water temperature. Artificial fertilization was successfully conducted in *T. maxima*, *T. noae*, and a hybrid of *T. maxima*♀/*T. noae*♂. The diameter of fertilized eggs was 99.0 ± 8.1, 113.8 ± 18.5, and 116.3 ± 6.2 μm, respectively. On day 6, the hybrid died. *Tridacna maxima* grew more than *T. noae* in both 1- and 10-month-old juveniles. The juvenile growth of *T. noae* was compared between the artificial breeding sites of Penghu and Linbian, which represent the main ornamental aquatic animal culture areas of Taiwan. Starting with individuals with a shell length of 15.9 ± 2.5 mm (265 days old), the juveniles observed in Linbian were significantly larger than those in Penghu, *i.e.*, 26.6 ± 3.6 mm on day 307 vs. 18.6 ± 2.9 mm on day 321 ($p < 0.001$). This might be linked to the differences in local water temperatures between the two locations, *i.e.*, 20.3–27.0°C vs. 16.9–23.9°C, respectively. These results can be used to further research ways to maximize spat production and minimize giant clam operational costs.

Key words: Reproductive cycle, Hybrid, Larval development, Temperature, Dongsha, Kenting.

BACKGROUND

Giant clams are a small group of cardiid bivalves (Subfamily: Tridacninae) with a total of 12 living species (MolluscaBase 2020). Due to long-term exploitation pressures, giant clams are vulnerable and listed in the IUCN (International Union for Conservation of Nature) Appendix II of CITES. Recently, a comprehensive review of the taxonomy, distribution, abundance, and conservation status of giant clams was published by Neo et al. (2017). Based on the worldwide Reef Check survey, the lowest and highest density of giant clams were 0.06 individual/100 m^2 (AMPO-AM05, the Philippines in 2007) and 583.75 individual/100 m^2 (Matira, French Polynesia in 2006), respectively. In general, high densities were found in protected areas or sites with low human-related disturbance.

Six giant clam species have been recorded

Citation: Su PW, Zhang GL, Chen B, Soong K, Liu LL. 2021. Reproduction and early juvenile growth of the giant clams *Tridacna noae* and *Tridacna maxima* in Taiwan. Zool Stud **60:**49. doi:10.6620/ZS.2021.60-49.

in Taiwan (Shao et al. 2008): *Hippopus hippopus*, *Tridacna crocea*, *Tridacna derasa*, *Tridacna gigas*, *Tridacna maxima*, and *Tridacna squamosa*. However, a cryptic species related to *T. maxima* has also been recognized based on morphological and molecular evidence (Tang 2005; Kubo and Iwai 2007a; Huelsken et al. 2013). In addition, the validity of *Tridacna noae* (Röding, 1798) was confirmed with the designation of a neotype and taxonomic description by Su et al. (2014a). A more recent island-wide survey found only five giant clam species (Su et al. 2014b): *H. hippopus*, *T. crocea*, *T. squamosa*, *T. maxima*, and *T. noae*. As in other areas of the world, the density of giant clams in Taiwan is low, ranging from 0.25 to 4.75 individuals/100 m^2 (Reef Check from 2008 to 2010) (Neo et al. 2017). The most common species are *T. maxima* and *T. noae*, which have maximum densities of 1.2 and 1.8 individual/100 m^2, respectively, in Dongsha Atoll National Park (Neo et al. 2018).

Due to the continuous decline in wild populations of giant clams, artificial propagation has been conducted on several species around the world for restoration and commercial use since the 1970s, including *T. gigas*, *T. squamosa*, *T. derasa*, *T. crocea*, *T. maxima*, *T. noae*, and *Hippopus hippopus* (*e.g.*, Lucas 1994; Gomez and Mingoa-Licuanan 2006; Inoue and Kubo 2008; Mies et al. 2017; Southgate et al. 2017; Braley et al. 2018; Militz et al. 2017 2019). Inter-specific hybridization between *T. noae* and *T. maxima* was also tested for aquarium markets (Militz et al. 2019). In Taiwan, *T. maxima* was successfully propogated for the first time at the Marine Life Propagation Station, Penghu (Zhang 2006). Subsequently, *T. noae* was also produced at this station and at the Penghu Marine Biology Research Center, Fisheries Research Institute (Hsieh 2019).

However, producing enough giant clams through aquaculture to fulfill Taiwan's conservation and aquarium market needs remains a challenge, especially without knowing the reproductive biology of the target species. Hence, the reproductive cycles of the relatively abundant giant clams *T. noae* and *T. maxima* were investigated in Dongsha Atoll National Park and Kenting National Park. Artificial breeding and larval development of *T. noae*, *T. maxima*, and a hybrid of the two species were studied in Penghu. In addition, the early juvenile growth of the two giant clams was compared in Penghu and Linbian, which contain the main ornamental aquatic animal culture areas of Taiwan (Chen et al. 2012).

MATERIALS AND METHODS

Field study on the reproductive cycles of *T. noae* and *T. maxima*

The reproductive cycles of *T. noae* and *T. maxima* were monitored in coral reefs of Dongsha and Kenting (Fig. 1). The Dongsha atoll is located in the northern South China Sea. The water temperature ranges from

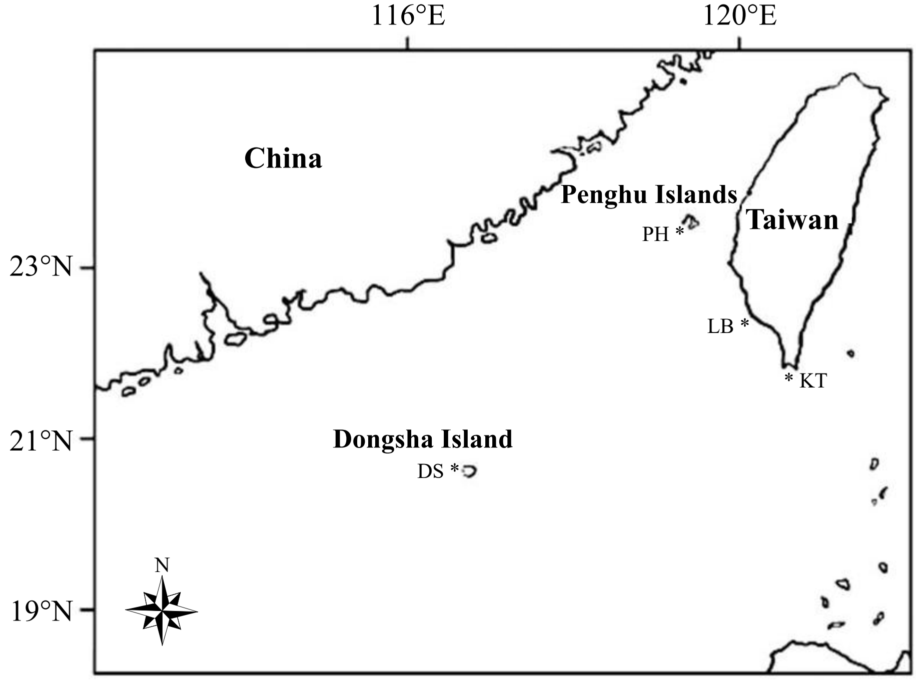

Fig. 1. Experimental sites for *Tridacna noae* and *Tridacna maxima*. DS: Dongsha; KT: Kenting; LB: Linbian; PH: Penghu.

22.3 to 34.5°C, with an average of 27.5°C in 2010–2020 (Central Weather Bureau, Taiwan). In Kenting, water temperature is 14.9 to 35.8°C, with an average of 26.3°C in 2007–2020.

Tridacna noae and *T. maxima* had low population densities, so their reproductive cycles were examined by gonad needle biopsy assay *in situ* as described by Braley (1984). A 20-cm hypodermic needle with a plastic plunger was used to extract gonadal tissue from clams (> 15 cm) on a bi-monthly basis from March 2013 to May 2014. The removed tissue was preserved in 5% buffered-formalin seawater and examined using a light microscope. At least 20 eggs were measured per individual, and gonadal development was classified into four stages based on the egg diameter: stage 0, no recognizable eggs in the sample; stage 1, egg diameter of 40–70 μm; stage 2, egg diameter of 70–90 μm; and stage 3, egg diameter > 90 μm.

Artificial breeding and larval development of *T. noae*, *T. maxima*, and their hybrid

Artificial breeding and larval development experiments were conducted on *T. noae*, *T. maxima*, and a hybrid of the two species in Penghu (Fig. 1). Penghu Islands are coral reefs located in the west of Taiwan. Its water temperature ranges from 12.1 to 32.5°C, with an average of 23.5°C in 2001–2020 (Central Weather Bureau, Taiwan). For the artificial breeding experiment, *T. noae* and *T. maxima* adults (> 21 cm) were collected from Dongsha Island and transported to the Marine Life Propagation Station in Penghu from March to April 2013.

Fifteen adults of each species were maintained in outdoor 15-ton concrete ponds with running seawater at a temperature of 20–31°C and salinity of 32–34‰. Spawning induction was conducted in June 2013. Briefly, each clam was transferred to a 1-ton tank, then oyster gonad extract was added as a stimulant (Chen 2007). The extract was made from gonad tissue collected from 10 oysters (*Crassostrea angulata*) with a shell length of 8–12 cm. The gonad tissue was removed from the oyster and gently rubbed by hand in 500 ml of seawater, then filtered through 100 and 50 μm nylon mesh to remove debris. Subsequently, the oyster gonad extract was made to a final volume of 3 L. Giant clams were induced to spawn by 1 liter of extract in each 1-ton clam tank. When clams released gametes, the eggs and sperm were separately collected from each tank with a hand-operated siphon pump and transferred to another 10-liter container.

Artificial fertilization was conducted in *T. noae*, *T. maxima*, and their hybrid within 30 min of the clams releasing gametes at a density of 25 egg/ml. Then, allosperm containing seawater was added at a volumetric ratio of 1:200 (Heslinga et al. 1990). Twenty minutes later, the egg-containing seawater was filtered through a 50 μm nylon mesh to avoid polyspermy. Subsequently, the fertilized eggs from different species were separately maintained in indoor 15-ton concrete ponds at a density of 20,000 egg/ton. During the culturing period, gentle aeration was provided with no water change. The water temperature and salinity were 27.8 ± 0.3°C (mean ± SD) and 33 ± 1‰.

On day 3, veligers were fed with *Isochrysis galbana* and *Chaetoserus muelleri* every other day at a concentration of 5×10^4 cells/ml until the larvae settled. The symbiotic zooxanthellae were prepared by the mantle from an adult clam. The mantle was cut and ground in a blender with 1-liter seawater. Then, it was filtered through a 25 μm nylon mesh to remove debris. A concentration of 50 cell/ml zooxanthellae was added in the veligers' culture pond at a density of 10 larvae/ml on day 4 (Fitt et al. 1984). The larvae settled to the bottom, and metamorphosis started on day 6. The developments of *T. noae*, *T. maxima*, and the hybrid were observed by collecting larvae with a hand-operated siphon pump and filtered through a 50 μm nylon mesh. Then, the larvae were transferred to a petri dish and examined under a dissecting microscope.

On day 12, the settled larvae were collected from the pond bottom by a hand-operated siphon pump and placed in outdoor 15-ton concrete ponds with coarse coral sand at the bottom at a density of 150 clam/m^2 and running seawater system.

Early juvenile growth of *T. noae* and *T. maxima*

For the growth comparison experiments, 7-month-old clam juveniles were shipped from Penghu to a culture farm in Linbian (Fig. 1) by plane in January 2014. Linbian is located in Taiwan's main ornamental aquatic animal culture area (Chen et al. 2012). Its water temperature is 23.2 to 32.2°C, with an average of 27.2°C in 2011 (Central Weather Bureau, Taiwan).

The growth of *T. noae* in the Penghu and Linbian sites was evaluated with an outdoor running seawater system at a density of 150 clam/m^2. For the growth comparison between *T. noae* and *T. maxima*, the experiments were conducted with three replicates in Linbian with running seawater at a density of 100 clam/m^2.

During the experimental periods, seawater temperatures were recorded with temperature loggers (TG-4100) every 1 h. Juvenile size was measured over time; before this, clams were carefully dislodged from the coral sand by cutting their byssal threads. They were then patted dry with a paper towel before being weighed, and their shell lengths measured using an electronic caliper.

The larval development and juvenile growth results were compared using *t*-test, analysis of variance (ANOVA) test, and Fisher's least significant difference post hoc test.

RESULTS

The reproductive cycle of *T. noae* and *T. maxima*

The egg developmental stages of *T. noae* and *T. maxima* were monitored from March 2013 to May 2014 in Dongsha and Kenting. The population density was low at both sites, *i.e.*, 1 to 2 individuals/100 m^2. The number of clams found from bi-monthly samplings varied from 1 to 9 individuals (Figs. 2 and 3). Clam size was smaller in Kenting than in Dongsha, *i.e.*, 17–25 cm vs. 23–29 cm for *T. noae*, respectively, and 17–28 cm vs. 22–32 cm for *T. maxima*. Egg diameters in Dongsha and Kenting reached 123 and 134 µm for *T. noae*, respectively, and 123 and 131 µm for *T. maxima*. Although the sample size was small, it is still notable that *T. noae* and *T. maxima* gonads were ripe from March to August. Water temperatures were 21.3 ± 1.1°C (January) to 30.2 ± 1.0°C (July) and 23.7 ± 0.5°C (January) to 28.4 ± 0.5°C (August) in Dongsha and Kenting, respectively; there was no correlation between reproduction and water temperature.

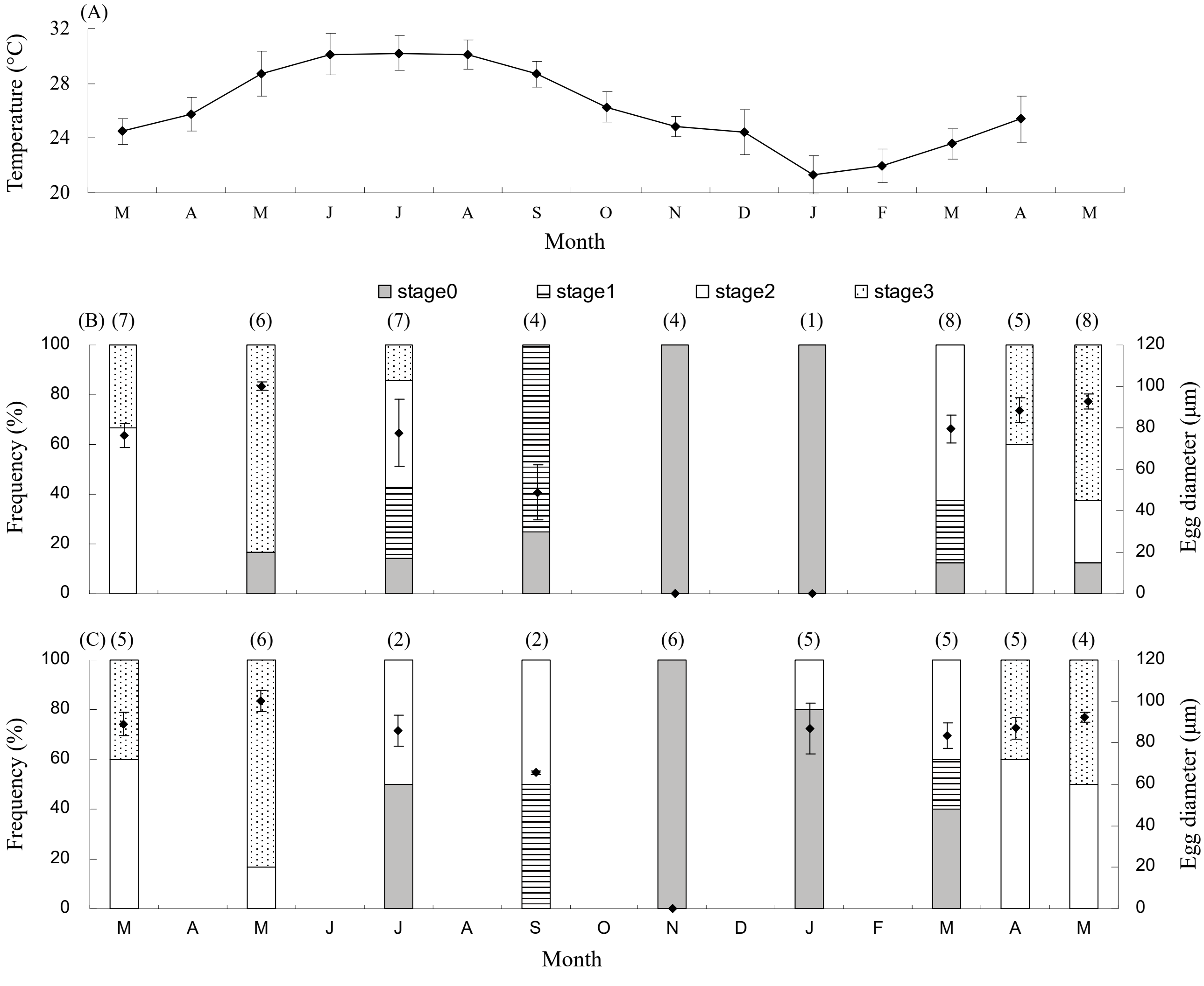

Fig. 2. Monthly water temperature (A), stages of egg development, and egg diameter of *Tridacna noae* (B) and *Tridacna maxima* (C) in Dongsha from March 2013 to May 2014. Water temperature: mean ± SD; (n): numbers of biopsy sample; ◆: mean egg diameter ± SD.

Artificial breeding and larval development of *T. noae*, *T. maxima*, and their hybrid

Artificial fertilization was successfully conducted in both species and the hybrid with sperm from *T. noae* and eggs from *T. maxima*. The major developmental stages to 10-month-old juveniles of the two species are shown in figures 4 and 5. The mantle with oval patches was clearly observed in 2-month-old *T. noae*. The diameters of fertilized eggs of *T. noae*, *T. maxima*, and the hybrid were 99.0 ± 8.1, 113.8 ± 18.5, and 116.3 ± 6.2 µm, respectively (Table 1). On day 2, water temperature was 27.8 ± 0.3°C and the shell lengths of the trochophore were 147.8 ± 5.7, 142.8 ± 4, and 151.9 ± 6.7 µm, respectively. However, the hybrid died on day 6. Larval settlement and metamorphosis were examined on day 10. On day 15, juveniles of *T. noae* and *T. maxima* had shell lengths of 200.2 ± 9.4 and 198.1 ± 14.7 µm, respectively. On day 28, juvenile *T. maxima* were significantly larger than those of *T. noae* ($p < 0.001$).

Early juvenile growth of *T. noae* and *T. maxima*

The growth of juvenile *T. noae* was determined in Penghu and Linbian. On day 265, juveniles with a shell length of 15.9 ± 2.5 mm were sent to Linbian. After 40 days, significantly larger juveniles were observed in Linbian than in Penghu—*i.e.*, 26.6 ± 3.6 mm (307-day-old) vs. 18.6 ± 2.9 mm (321-day-old) ($p < 0.001$)—and water temperature was 20.3–27.0 vs. 16.9–23.9°C, respectively (Fig. 6 and Table 2). The growth comparison of *T. noae* between sites showed a slow rate (0.7 mm/month) at 17–18°C in Penghu. In contrast, it grew from 29.5 ± 4.7 to 34.6 ± 5.0 mm within one month (1.7 mm/month) at 29°C in Linbian.

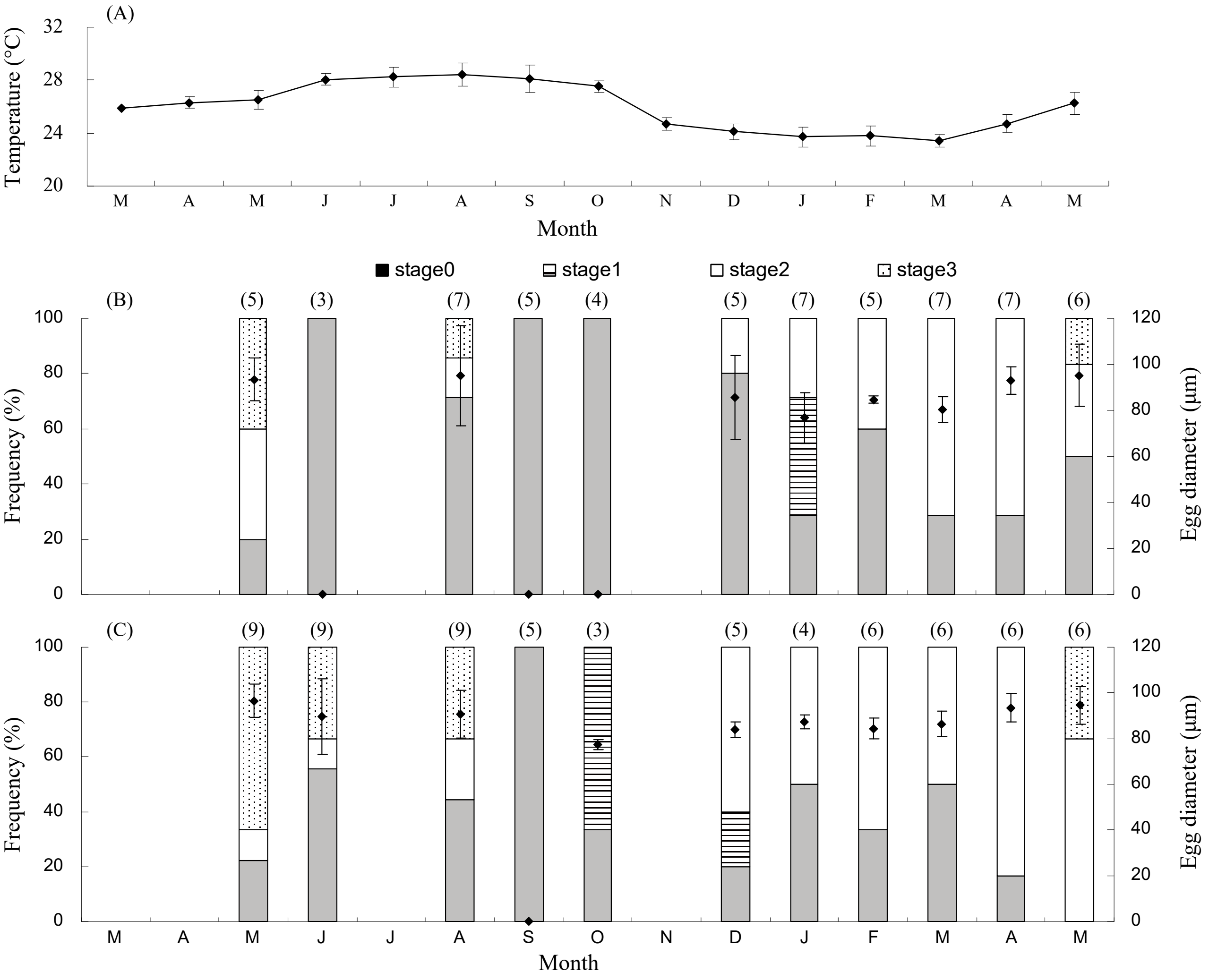

Fig. 3. Monthly water temperature (A), stages of egg development, and egg diameter of *Tridacna noae* (B) and *Tridacna maxima* (C) in Kenting from March 2013 to May 2014. Water temperature: mean ± SD; (n): numbers of biopsy sample; ◆: mean egg diameter ± SD.

There was also a difference in the growth equations as positive correlations between the shell length and body weight of *T. noae* in Penghu ($y = 0.08x - 0.87$; $R^2 = 0.87$, $p < 0.01$, $n = 125$) and Linbian ($y = 0.20x - 2.97$; $R^2 = 0.89$, $n = 98$) at the end of the experiments.

The growth of juvenile *T. noae* and *T. maxima* was compared at a water temperature range of 26.0–31.4°C in Linbian (Fig. 7). Within two months, shell lengths

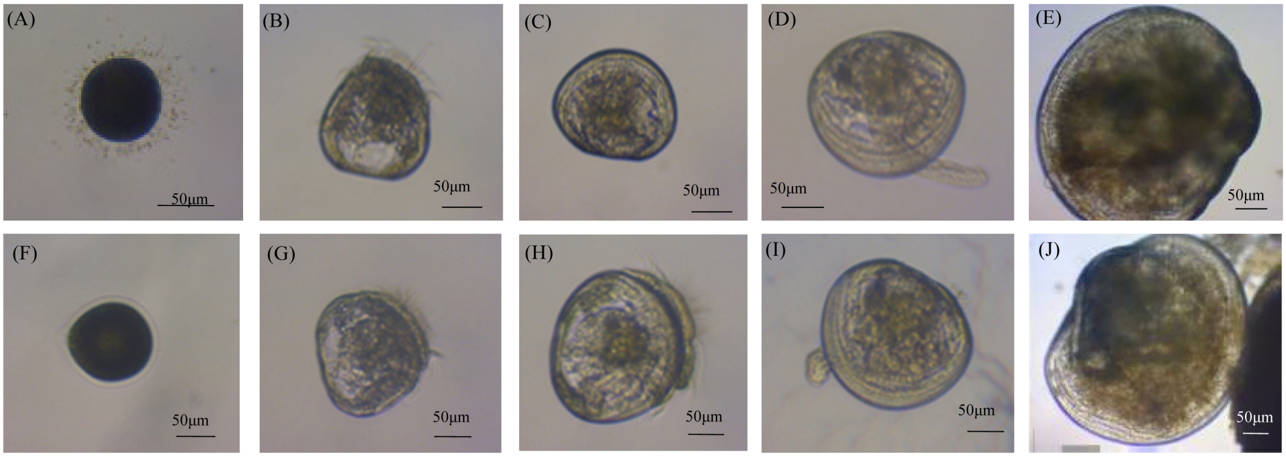

Fig. 4. Larval developmental stages of *Tridacna noae* (A–E) and *Tridacna maxima* (F–J). (A, F) Day 0 fertilized egg; (B, G) Day 2 trochophore; (C, H) Day 6 straight-hinge veliger; (D, I) Day 12 pediveliger; (E, J) Day 28 juvenile.

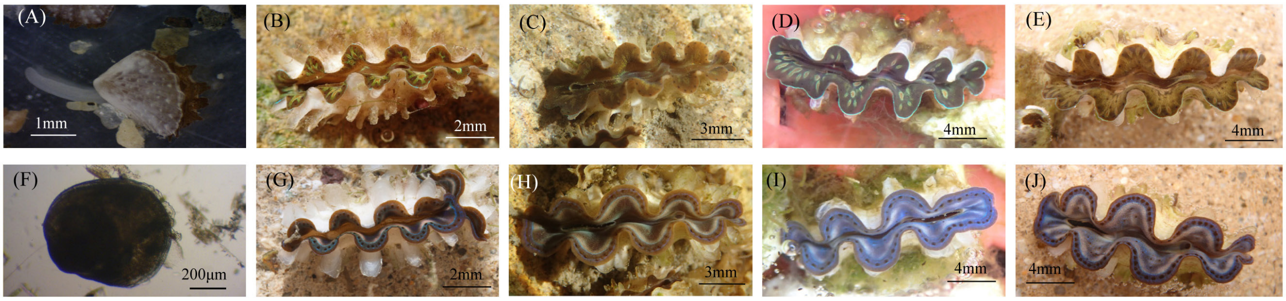

Fig. 5. Growth of juvenile *Tridacna noae* (A–E) and *Tridacna maxima* (F–J). (A, F) Month 2 Juvenile; (B, G) Month 4 juvenile; (C, H) Month 7 juvenile; (D, I) Month 9 juvenile; (E, J) Month 10 juvenile.

Table 1. Larval development of *Tridacna noae*, *Tridacna maxima*, and hybrids (mean ± SD). $^{a, b}$: significantly differ ($p < 0.05$); -: dead; (N): sample size

		T. noae		*T. maxima*		*T. maxima* ♀/*T. noae* ♂	
Stage	Day	Diameter (μm)	N	Diameter (μm)	N	Diameter (μm)	N
Fertilized egg	0	99.0 ± 8.1 ^{a}	14	113.8 ± 18.5 ^{b}	30	163.3 ± 9.9 ^{a}	15
	Day	Length (μm)	N	Length (μm)	N	Length (μm)	N
Trochophore	2	147.8 ± 5.7 ^{a}	12	142.8 ± 4.0 ^{a}	11	151.9 ± 6.7 ^{a}	14
Straight-hinge veliger	4	166.6 ± 5.9 ^{a}	48	163.3 ± 9.9 ^{a}	11	-	
D-veliger	6	181.5 ± 6.7 ^{a}	43	181.5 ± 9.2 ^{a}	30		
Pediveliger	8	192.9 ± 5.6 ^{a}	31	192.1 ± 5.0 ^{a}	36	-	
Settled larva	10	193.6 ± 5.0 ^{a}	59	194.5 ± 4.3 ^{a}	31	-	
Juvenile	15	200.2 ± 9.4 ^{a}	61	198.1 ± 14.7 ^{a}	46	-	
Juvenile	28	302.9 ± 3 3.8 ^{a}	29	376.6 ± 30.9 ^{b}	25	-	

of 10-month-old *T. noae* and *T. maxima* increased from 19.3 ± 2.8 to 30.6 ± 4.1 mm and 20.2 ± 3.1 to 33.9 ± 4.3 mm, respectively. *Tridacna maxima* grew significantly faster than *T. noae*.

DISCUSSION

The annual reproductive cycle of *Tridacna noae* was reported for the first time and compared with that of *T. maxima* in the field. The largest monitored clams were 29 and 32 cm, which fall within size ranges of other studies, *i.e.*, 26–36 cm and 27–38 cm, respectively (Kubo and Iwai 2007a b; Kishimoto et al. 2009; Borsa et al. 2015; Militz et al. 2015; Neo et al. 2018).

In Dongsha and Kenting, *T. noae* and *T. maxima* spawning peaked from March to August based on the measurement of egg diameter. It is reported that the peak spawning period of *T. maxima* varies in different geographical locations. In the subtropics, *T. maxima*

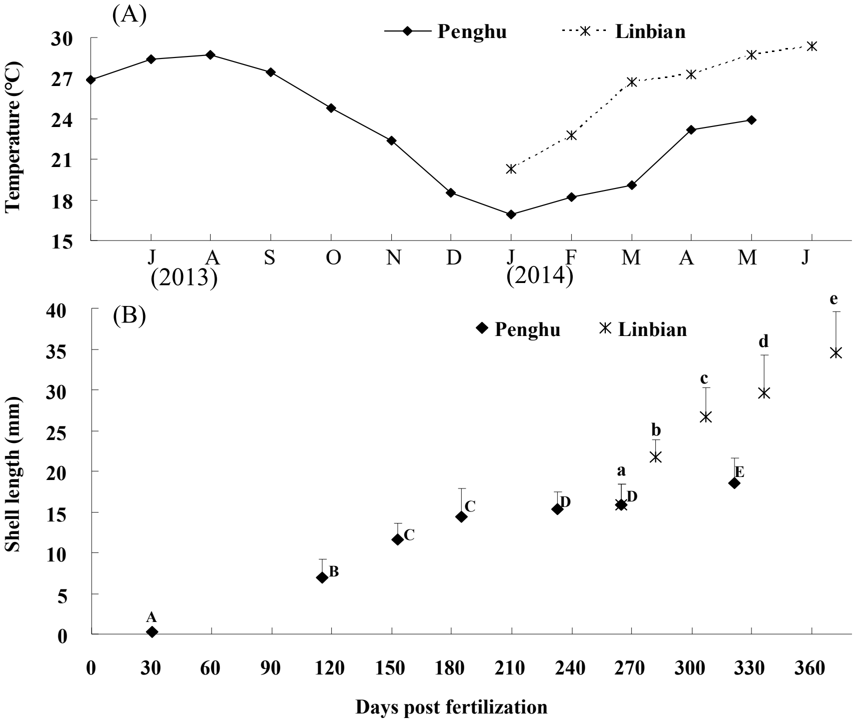

Fig. 6. Mean water temperature (A) and shell length of *Tridacna noae* juveniles (B) fertilized on June 6, 2013. Shell length: mean ± SD; A, B, C, D, E: significant difference in the cultural period of *T. noae* in Penghu; a, b, c, d, e: significant difference in the cultural period of *T. noae* in Linbian (1-way ANOVA, $p < 0.05$).

Table 2. The growth of *Tridacna noae* cultured in Penghu and Linbian. N: sample size

Site	Day of post fertilization	Sample size (N)	Shell length(mm) ± SD
Penghu	30	29	0.30 ± 0.03
	115	60	7.0 ± 2.2
	153	50	11.6 ± 2.0
	185	62	14.4 ± 3.4
	233	44	15.4 ± 2.0
	265	46	15.9 ± 2.5
	321	79	18.6 ± 2.9
Linbian	265	46	15.9 ± 2.5
	282	22	21.7 ± 2.1
	307	81	26.6 ± 3.6
	336	57	29.5 ± 4.7
	372	86	34.6 ± 5.0

spawning peaks from March to June on Ishigaki Island, Japan (Kubo and Inoue 2008) and June to September in the northern Gulf of Aquba (Roa-Quiaoit 2005). In the tropics, it is April to July in Rengis Island, Malaysia (Tan and Yasin 2000); November to March in Anae Island, Guam (Jameson 1976); and June to July in the lagoon of Tatakoto, Tahiti (Van Wynsberge et al. 2017). Jameson (1976) suggested that *T. maxima* inhabiting relatively constant temperature environments may have different spawning stimuli, depending on their local conditions. Indeed, in Tahiti, *T. maxima* spawning peaked during high oceanic water inflow and decreased under lagoon water temperatures (Van Wynsberge et al. 2017).

Moreover, a relatively long period of egg development was observed in *T. noae* and *T. maxima* in this study (Figs. 2 and 3). By comparison, the brooding oyster *Ostrea* (= *Striostrea*) *circumpicta* from the subtropical coast of Jeju Island (Korea) also exhibited a similar pattern (Lim et al. 2019). Lim and colleagues found that fully mature eggs 100 μm in diameter began to appear in March and remained until July. They suggested that the long period of oocyte maturation in *O. circumpicta* was probably linked to the relatively larger size of the oocytes and the low level of available food in the water column in southern Jeju Island.

Recent studies have cultured *T. noae* for up to 30 days and investigated aspects of its feeding ecology and early embryo and juvenile developments (Southgate et al. 2016 2017; Braley et al. 2018; Militz et al. 2017 2019). The 15-day-old *T. maxima* juvenile's growth was significantly faster than those of *T. noae* (Militz et al. 2017). Similarly, in this study, 28-day *T. maxima* juvenile showed a significantly more rapid growth rate than *T. noae* at 27.8 ± 0.3°C (Table 1). *Tridacna maxima* also grew faster than *T. noae* in 10-month-old juveniles cultured in Linbian at water temperatures of 26.0–31.4°C (Fig. 7). In addition, *T. noae* grew slower in Penghu than in Linbian, *i.e.*, 0.7 vs. 1.7 mm/month, respectively (Fig. 6 and Table 1). The difference in local water temperature might plays an important role—*i.e.*, 17–18°C in Penghu and 29°C in Linbian. Our results provide a base for further research on maximizing spat production and minimizing giant clam operational costs.

This study tested the production of a hybrid of *T. noae* and *T. maxima* for the aquarium market. The only successfully fertilized hybrid was the cross of *T. maxima* ♀/*T. noae* ♂. However, the hybrid died on day 6. Our results showed that there is reproductive isolation between *T. maxima* and *T. noae*. In contrast, with both the adult clam broodstock from Papua New Guinea, maternal and paternal crosses of *T. maxima* and *T. noae* could fertilize and yield 30-day-old juveniles

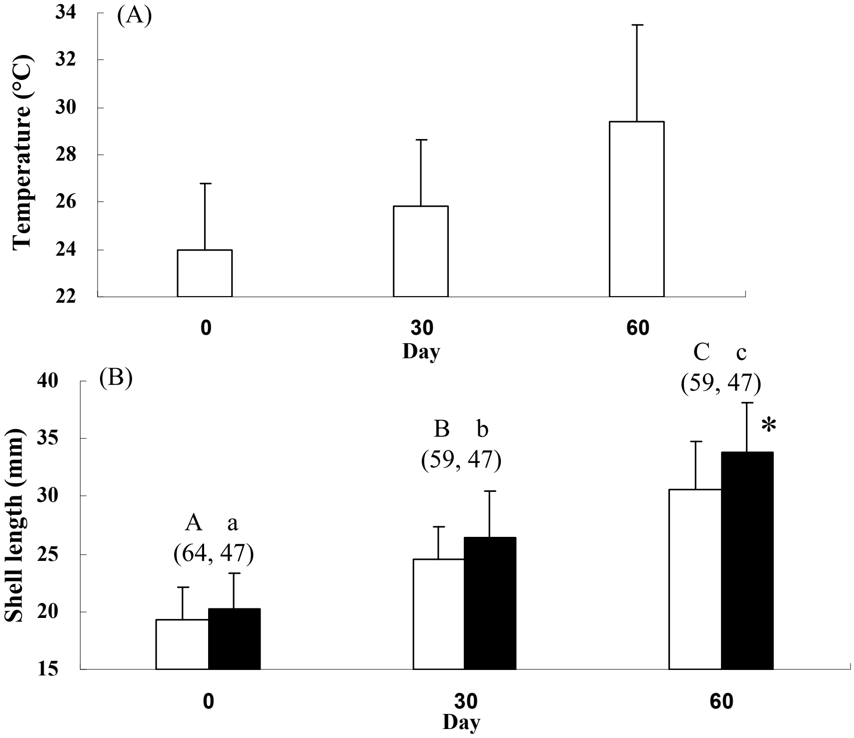

Fig. 7. Mean water temperature and shell length of *Tridacna noae* and *Tridacna maxima* cultured in Linbian. Water temperature and shell length: mean ± SD; □: *T. noae*; ■: *T. maxima*; (n): numbers of the sample; *: significant difference between *T. noae* and *T. maxima* (*t*-test, $p < 0.05$); A, B, C: significant difference in the cultural period of *T. noae*; a, b, c: significant difference in the cultural period of *T. maxima* (1-way ANOVA, $p < 0.005$).

(Militz et al. 2017 2019). Militz et al. (2019) found that the fertilization rate for the *T. noae*♀/*T. maxima*♂ cross (89.3 ± 1.8%) was higher than that of the *T. maxima*♀/*T. noae*♂ cross (40.7 ± 3.2%). Following mass mortality in the first five days of culturing, 30-day-old *T. maxima*♀/T. *noae*♂ hybrid (0.50 ± 0.14%) was superior to the *T. noae*♀/*T. maxima*♂ hybrid (0.08 ± 0.02%). However, we still do not know if hybrid juveniles can successfully develop into reproductively viable adults and produce offspring.

It is suggested that the geographical source location of broodstock may influence the survivorship of giant clam hybrids (Militz et al. 2017 2019). In the present study, the broodstocks of *T. noae* and *T. maxima* were sympatrically distributed in Dongsha. Both clam species exhibited a similar reproductive cycle, with ripe gonads from March to August (Figs. 2 and 3). Although there is a chance that hybridization occurred in the field, in our case, the two species were incapable of producing living larvae. The success of hybridization between *T. noae* and *T. maxima* may be related to differential genetic variations among the two species' populations. Based on the results of mitochondrial *COI* gene sequences, low levels of genetic diversity were found in the populations of *T. noae* and *T. maxima* in Dongsha (Neo et al. 2018). In the haplotype network analyses of *T. noae* in the Indo-West Pacific (Keyse et al. 2018), our population belonged to clade 1 (Dongsha), and that of Militz et al. (2017 2019) was in clade 2 (Papua New Guinea). For *T. maxima*, their population was in clade 4 (the Solomon Islands group, the Great Barrier Reef, the Torres Strait, and Western New Guinea), and ours was in clade 2 (the Indonesian group). There are still knowledge gaps around interspecific hybridization between *T. noae* and *T. maxima* that further research can fill.

CONCLUSIONS

The present study monitored the reproductive cycle of *Tridacna noae* for the first time and compared *Tridacna maxima* in the field. The reproductive season of both species was March to August. Artificial fertilization was successfully conducted in *T. maxima*, *T. noae*, and a hybrid of *T. maxima*♀/*T. noae*♂. However, the hybrid died on day 6. *Tridacna maxima* grew faster than *T. noae* in both 1- and 10-month-old juveniles. Significantly larger juveniles were observed in Linbian than Penghu, and this might be linked to the differences in cultural temperatures, *i.e.*, 20.3–27.0°C vs. 16.9–23.9°C, respectively. Once the reproductive biologies of *T. maxima* and *T. noae* are better understood, research can be pursued to determine effective hatchery production techniques to fulfill the conservation and aquarium market needs for these species in Taiwan.

Acknowledgments: The authors would like to express their appreciation to Ms. Meng-Ying Kuo, Hui-Ru Zhen, Mrs. Chiu-Yeh Liao, and Dong-Chi Hsu for their field work. The authors also thank Mr. Noah Last of Third Draft Editing for his English editing. The facility was generously supported by the Marine National Park Headquarters, R.O.C. and the Marine Life Propagation Station, Penghu County. Permission to collect clam tissue was granted by the Kenting National Park Headquarters and the Marine National Park Headquarters, R.O.C. This study was supported by the National Science Council, Republic of China (NSC101-2119-M110-004-MY5) to K.Y. Soong and the Ministry of Science and Technology, Taiwan (MOST 105-2311-B-110-001) to L.L. Liu. This paper is based on a thesis submitted by P.W. Su in partial fulfillment of the Master's degree requirements at National Sun Yat-sen University.

Authors' contributions: P.W.S. and P.N.C. conducted experiments. K.S. guided the data analyzes and provided advice. G.L.Z. and L.L.L. supervised the research and led the manuscript writing, with contributions from all authors.

Competing interests: The authors declare no competing interests.

Availability of data and materials: The experimental data and materials are available upon request from L.L.L.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

REFERENCES

Borsa P, Fauvelot C, Tiavouane J, Grulois D, Wabnitz C, Abdon Naguit MR, Andrefouet S. 2015b. Distribution of Noah's giant clam, *Tridacna noae*. Mar Biodivers **45:**339–344. doi:10.1007/s12526-014-0265-9.

Braley RD. 1984. Reproduction in the giant clams *Tridacna gigas* and on the north-central Great Barrier Reef, Australia, and Papua New guinea *T. derasa*. Coral Reefs **3:**221–227. doi:10.1007/BF00288258.

Braley RD, Militz TA, Southgate PC. 2018. Comparison of three hatchery culture methods for the giant clam *Tridacna noae*. Aquaculture **495:**881–887. doi:10.1016/j.aquaculture.2018.05.044.

Chen JY, Chuang CT, Kung SH. 2012. Development of Ornamental

Fish Industry in Taiwan. J Lan Yang Inst Technol **11:**167–179. (in Chinese)

Chen YC. 2007. The studies on the breeding and hatching of the *Tridacna maxima*. National Taiwan Ocean University, thesis, 56 pp. (in Chinese)

Fitt WK, Fisher CR, Trench RK. 1984. Larval biology of tridacnid clams. Aquaculture **39:**181–195.

Gomez ED, Mingoa-Licuanan SS. 2006. Achievements and lessons learned in restocking giant clams in the Philippines. Fish Res **80:**46–52. doi:10.1016/j.fishres.2006.03.017.

Hsieh HJ. 2019. Treasure in the sea - Perspectives of giant clam restoration. Fisheries News, the Fishery Research Institute **65:**42. (in Chinese)

Heslinga GA, Watson TC, Isamu T. 1990. Giant clam farming. Pacific Fisheries Development Foundation (NMFS/NOAA), Honolulu, Hawaii, pp. 141–179.

Huelsken T, Keyse J, Liggins L, Penny S, Treml EA, Riginos C. 2013. A novel widespread cryptic species and phylogeographic patterns within several giant clam species (Cardiidae: Tridacna) from the Indo-Pacific Ocean. PLoS ONE **8:**e80858. doi:10.1371/journal.pone.0080858.

Inoue K, Kubo H. 2008. Maintainig broodstocks and seed mass productions of gaint clams, *Tridacna maxima* and *T. noae*. Annu Rep Okinawa Fish Ocean Res Ctr **69:**120–123.

Jameson SC. 1976. Early life history of the giant clams *Tridacna crocea* Lamarck, *Tridacna maxima* (Roding), and *Hippopus hippopus* (Linnaeus). Pacific Science **30:**219–233.

Keyse J, Treml EA, Huelsken T, Barber PH, DeBoer T, Kochzius M, Nuryanto A, Gardner JPA, Liu LL, Penny S, Riginos C. 2018. Historical divergences associated with intermittent land bridges overshadow isolation by larval dispersal in codistributed species of *Tridacna* giant clams. J Biogeogr **45:**848–858. doi:10.1111/jbi.13163.

Kishimoto K, Kubo H, Inoue K. 2009. Sizes and abundances of giant clams *Tridacna maxima* and *Tridacna noae* in the Kabira MPA and the adjacent regions in the Ishigaki Island. Annu Re. Okinawa Fis. Ocean Res Ctr **70:**183–185. (in Japanese)

Kubo H, Iwai K. 2007a. On sympatric two species within *Tridacna* "*maxima*". Annu Rep Okinawa Fish Ocean Res Ctr **68:**205–210.

Kubo H, Iwai K. 2007b. Seasonal fluctuation on gonadal maturation of *Tridacan maxima*. Annu Rep Okinawa Prefectural Fish Ocean Res Ctr **68:**211–214.

Kubo H, Inoue K. 2008. Reproductive periodicity and histological study on giant clam *Tridacna maxima* in Okinawa. Annu Rep Okinawa Fish Ocean Res Ctr **69:**108–112. (in Japanese)

Lim NL, Lee HM, Jeung HD, Noseworthy RG, Jung S, Choi KS. 2019. Early larval development and annual gametogenesis of the brooding oyster *Ostrea circumpicta* (Pilsbry, 1904) in the shallow subtidal benthic ecosystem in Jeju Island, off the south coast of Korea. Zool Stud **58:**29. doi:10.6620/ZS.2019.58-29.

Lucas JS. 1994. The biology, exploitation, and mariculture of giant clams (Tridacnidae). Rev Fish Sci **2:**181–223.

Mies M, Dor P, Güth AZ, Sumida PYG. 2017. Production in giant clam aquaculture**:** trends and challenges. Rev Fish Sci Aquacult. doi:10.1080/23308249.2017.1285864.

Militz TA, Braley RD, Southgate PC. 2017. Captive hybridization of the giant clams *Tridacna maxima* (Röding, 1798) and *Tridacna noae* (Röding, 1798). J Shellfish Res **36:**585–592. doi:10.2983/035.036.0306.

Militz TA, Braley RD, Schoeman DS, Southgate PC. 2019. Larval and early juvenile culture of two giant clam (Tridacninae) hybrids. Aquaculture **500:**500–505. doi:10.1016/j.aquaculture.2018.10.050.

Militz TA, Kinch J, Southgate PC. 2015. Population demographics of *Tridacna noae* (Röding, 1798) in New Ireland, Papua New Guinea. J Shellfish Res **34:**329–336. doi:10.2983/035.034.0215.

MolluscaBase eds. 2020. MolluscaBase. Tridacninae Lamarck, 1819. Accessed through: World Register of Marine Species. Available at http://www.marinespecies.org/aphia.php?p=taxdetails&id=382150. Accessed 14 June 2020.

Neo ML, Liu LL, Huang H, Soong K. 2018. Thriving populations with low genetic diversity in giant clam species, *Tridacna maxima* and *Tridacna noae*, at Dongsha Atoll, South China Sea. Reg Stud Mar Sci **24:**278–287. doi:10.1016/j.rsma.2018.09.001.

Neo ML, Wabnitz CCC, Braley RD, Heslinga GA, Fauvelot C, van Wynsberge S, Andrefouet S, Waters C, Tan AS, Gomez ED, Costello MJ, Todd PA. 2017. Giant clams (Bivalvia: Cardiidae: Tridacninae): a comprehensive update of species and their distribution, current threats and conservation status. Oceanogr Mar Biol Annu Rev **55:**87–388.

Roa-Quiaoit HAF. 2005. Ecology and culture of giant clams (Tridacnidae) in the Jordanian sector of the Gulf of Aqaba, Red Sea. Ph.D. Thesis, Universitat Bremen.

Shao GJ, Peng JY, Wu WJ. 2008. Species diversity in Taiwan II. Species list, Council of Agriculture Executive Yuan Forestry Bureau, p. 796.

Southgate PC, Braley RD, Militz TA. 2016. Embryonic and larval development of the giant clam *Tridacna noae* (Röding, 1798) (Cardiidae: Tridacninae). J Shellfish Res **35:**777–784. doi:10.2983/035.035.0406.

Southgate PC, Braley RD, Militz TA. 2017. Ingestion and digestion of micro-algae concentrates by veliger larvae of the giant clam, *Tridacna noae*. Aquaculture **473:**443–448. doi:10.1016/j.aquaculture.2017.02.032.

Su Y, Hung JH, Kubo H, Liu LL. 2014a. *Tridacna noae* (Röding, 1798) - a valid giant clam species separated from *T. maxima* (Röding, 1798) by morphological and genetic data. Raffles Bull Zool **62:**124–135.

Su Y, Hung JH, Liu LL. 2014b. Review of giant clams (Mollusca, Bivalvia, Eulamellibranchia, Cardiidae, Tridacninae) from Taiwan. Platax **11:**33–52.

Tan SH, Yasin Z. 2000. The reproduction cycle of *Tridacna squamosa* and *Tridacna maxima* in Rengis Island (Tioman Island), Malaysia. J Shellfish Res **19:**963–966.

Tang YC. 2005. The Systematic status of *Tridacna maxima* (Bivalvia: Tridacnidae) based on morphological and molecular evidence. MS thesis, National Taiwan Ocean University, 114 pp.

Van Wynsberge S, AndreÂfoueÈt S, Gaertner-Mazouni N, Wabnitz CCC, Menoud M, Le Moullac G. 2017. Growth, survival and reproduction of the giant clam *Tridacna maxima* (RoÈding 1798, Bivalvia) in two contrasting lagoons in French Polynesia. PLoS ONE **12:**e0170565. doi:10.1371/journal.pone.0170565.

Zhang GL. 2006. The establishment of giant clam artificial propagation technique in the development of ecotourism in Penghu (1). Project report by the Marine Life Propagation Station, Penghu County. 122 pp. (in Chinese)