

# **The Ecology and Culture of Giant Clams (Tridacnidae) in the Jordanian sector of the Gulf of Aqaba, Red Sea**

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## SUMMARY

Giant clams (Tridacnidae) are the largest bivalved molluscs. Two out of the eight extant species of giant clams range across the IndoPacific into the Red Sea, namely, *Tridacna squamosa* Lamarck, 1819 and *T. maxima* Roding, 1798. These two species extend into the northern basin of the Red Sea, the Gulf of Aqaba, marking the northwestern limit of their geographical distribution. The continental climate in the desert-enclosed Gulf creates harsher conditions for giant clams than those encountered in the tropical IndoPacific range. The present study highlights the ecology, distribution, reproduction and molecular genetics of giant clams in the northern Gulf of Aqaba and provides a modified culture protocol for their commercial production taking into account the unique Red Sea conditions.

At the northern tip of the Gulf in Jordan, giant clams are scarce (0.3 - 0.5 ind. per 100 m<sup>-2</sup>) and patchy compared to 4- to 7-fold higher values in the Red Sea and an order of magnitude higher numbers in the Indo-Pacific. This distribution pattern suggests that the latitudinal range of giant clams is governed by the increasingly continental climate in the Red Sea, particularly the low winter temperatures.

Along the Jordanian coast, giant clam stocks plummeted 12-fold over the past three decades. Two-third higher abundances in restricted (1.04 ± 0.4 ind. 100 m<sup>-2</sup>) compared to open-access areas, suggest that overfishing of the natural stocks and habitat loss related to sweeping rates of urban development over four decades have caused the demise of giant clams in the area, in addition to extreme events such as oil spills and extreme low tides in the area. In the wider Red Sea, the impact of human activities is also evident, e.g., higher clam abundances in unfished and protected areas in Yemen; lower abundances in areas with coastal development and shipping pressure in Djibouti. Due to the increasing coastal development from tourism, shipping, and industry taking their toll on coral reefs and their resources, there is urgent need for trans-boundary management.

The pronounced seasonality in temperature and nutrients has a differential effect on giant clams, as a function of depth: *T. maxima* (adults and juveniles) are 80-times more common on the (<3 m) reef flat than on the deeper fore-reef, whereas *T. squamosa* (mostly adults) are three times more common on the deeper fore-reef than on the reef flat (mostly juveniles). Considering the age-dependency of nutrition in giant clams (where adults are functional phototrophs and juveniles are to a larger extent heterotrophs), young specimens appear to supply their demand for nutrients by taking up plankton advected to the reef in the surface layers. Older clams, by contrast, take advantage of the rich supplies of nutrients for photosynthesis. *T. maxima* combines high supplies of both, plankton-derived nutrients and light to fuel its photosynthesis.

Growth performance of natural stocks in Jordan showed to be comparable to the Indo-Pacific, *T. squamosa* ( $\phi'$ = 3.9), *T. maxima* ( $\phi'$ = 4.2) and cultured juvenile *T. squamosa* ( $\phi'$ = 4.2), suggesting favorable growth conditions in this subtropical marginal environment.

However, the reproductive season was reduced compared to the tropics and limited to the summer months. There is a distinct reproductive seasonality of giant clams in Jordan with species-specific spawnings: *T. squamosa* spawned between June and November featuring a bi-modal spawning distribution. *T. maxima*, by contrast, showed a shorter unimodal spawning season (Jun-Sep). Regression of the ovarian follicles was protracted over winter and maturation commenced in late winter, culminating in spring. Likewise, in the landbased culture of giant clams, the combined heat- and serotonin-induced spawnings and larval to juvenile land-based rearing, showed that reproductive success is highest in summer (Jun-Sep), with protracted larval development and heavy mortality in winter. It is postulated that the ovarian maturation from winter to spring is fuelled by nutrients and phytoplankton abounding during the mixing period in winter to spring, whereas development of the lecithotrophic larvae and recruitment success are mainly governed by the better growth conditions during the warm summer period.

Modifications of existing clam culture techniques to adapt to the Red Sea conditions have been successful but the seasonalities of various controlling factors limit the success in rearing clam juveniles throughout the year. Present levels of production in the pilot culture are still experimental.

Strong separate independent lines of evidence are provided for the existence of a new species of giant clam in the northern Gulf of Aqaba, Red Sea. Molecular mitochondrial rDNA analysis, morphological taxonomic classification, and reproductive periodicity distinguish the new species, *Tridacna* nov. sp. from its sympatric congeners *T. squamosa* and *T. maxima*, as well as other reported tridacnids from the wider Indo-Pacific region. The molecular analysis and amended taxonomic classification are consistent in placing *Tridacna* nov. sp. in the subgenus *Chametrachea*. This is strongly supported by the similar morphological and behavioural characteristics of *Tridacna* nov. sp with the two members of the subgenus, *T. maxima* and *T. squamosa*, which happened to be the only two extant species in the Red Sea. Furthermore, *Tridacna* nov. sp showed species-specific reproductive seasonality with a shorter summer spawning (May-July), smaller ova diameter ( $75 \pm 2 \mu\text{m}$ ) and earlier ovarian maturation (Aug) as compared to the other two species.

The possibility of *Tridacna* nov. sp. in the Red Sea as relic population at the northernmost limit of its geographic distribution is suggested based on the following arguments: *Tridacna* nov. sp. seems to be geographically restricted at the marginal tip of the Gulf of Aqaba, suggesting a cold-water tolerance relative to its wide-ranged congeners, *T. maxima* and *T. squamosa*, and shows close

resemblance with the Red Sea fossil species *Tridacna aegyptiaca* (Chenu, 1845), which may have been wide-spread during the glacial period.

## ZUSAMMENFASSUNG

Riesenschnecken (Tridacnidae) sind als größte schalentragende Mollusken im Indopazifik weit verbreitet. Zwei der acht rezenten Arten kommen im Roten Meer vor — *Tridacna squamosa* Lamarck, 1819 and *T. maxima* Roding, 1798. Das nördliche Nebenbecken des Roten Meeres, der Golf von Aqaba, ist dabei die nordwestliche Verbreitungsgrenze beider Arten. Das extreme Kontinentalklima des wüstengesäumten Golfs bietet hier wesentlich rauere Lebensbedingungen als im Verbreitungsschwerpunkt der Muscheln im Tropengürtel des Indopazifik. Die vorliegende Arbeit untersucht die Ökologie, Verbreitung, Reproduktion und Molekulargenetik der Riesenschnecken im nördlichen Golf von Aqaba und liefert ein an die Bedingungen des Roten Meeres angepasstes Verfahren zur kommerziellen Produktion (Riesenschnecken-Marikultur).

An der Nordspitze des Golfs in Jordanien kommen Riesenschnecken nur noch fleckhaft und insgesamt weitaus seltener vor (0.3 – 0.5 Individuen pro 100 m<sup>2</sup>) als im Roten Meer oder Indopazifik, wo die Dichten vier- bis siebenfach bzw. zehnmal höher liegen. Das gefundene Verbreitungsmuster lässt darauf schließen, dass das zunehmend kontinentalere Klima mit kalten Wintertemperaturen die Häufigkeit der Riesenschnecken in ihrem geographischen Verbreitungsgebiet bestimmen.

Entlang der jordanischen Küste sind die Riesenschneckenbestände in den letzten drei Jahrzehnten auf ein Zwölftel zurück gegangen. Dieses weist — ebenso wie die im Vergleich zu frei zugänglichen Gebieten zu zwei Dritteln höheren Dichten von Riesenschnecken in geschützten Gebieten (1.04 ± 0.4 Individuen pro 100 m<sup>2</sup>)— auf eine Überfischung des natürlichen Bestandes und den Verlust von Lebensraum durch Küstenentwicklung hin, die neben Extremereignissen wie Ölunfällen und Extremtiden zum Niedergang der Population geführt haben können. Diese Vermutung wird durch die vergleichende Analyse der Bestände im übrigen Roten Meer bestätigt: so finden sich im Jemen höhere Dichten von Riesenschnecken in unbefischten und geschützten als in zugänglichen Gebieten und in Djibouti geringere Abundanzen in Gebieten, die Belastungen durch Küstenentwicklung und Schiffsverkehr ausgesetzt sind. Da die Küstenentwicklung entlang des Roten Meeres durch Tourismus, Schiffsverkehr und Industrie — und damit der Druck auf die Korallenriffe und seine nutzbaren Ressourcen — weiterhin zunimmt, ist ein grenzüberschreitendes Management dringend geboten.

Die ausgeprägte Saisonalität im nördlichen Golf von Aqaba mit warmen nährstoffarmen Sommern und kalten nährstoffreichen Wintern wirkt sich je nach Wassertiefe unterschiedlich auf die Riesenschneckenpopulationen aus: *T. maxima* (adulte und juvenile Muscheln) kommen 80-mal häufiger auf dem Rifftop (< 3 m) vor, als in tieferen Bereichen der Saumriffe und kommen damit

ganzjährig in den Genuss sehr hoher Lichtintensitäten – allerdings auf Kosten einer knapperen Nährstoffversorgung und ausgeprägter Temperaturextrema. *T. squamosa* hingegen sind dreimal häufiger im tieferen Saumriff zu finden (überwiegend adulte Tiere) als auf dem Riffdach, wo Juvenile überwiegen. Berücksichtigt man die altersabhängig unterschiedlich mixotrophe Ernährungsweise von Riesenmuscheln — Adulte sind funktional photoautotroph, Juvenile in stärkerem Maße heterotroph — so ist zu vermuten, dass junge Exemplare ihren Nährstoffbedarf vorwiegend aus dem Plankton decken, das dem Korallenriff an der Oberfläche stets zugeführt wird. Ältere *T. squamosa* hingegen nutzen das erhöhte Nährstoffangebot in den tieferen Riffbereichen für die Photosynthese. Adulte *T. maxima* decken ihren Nährstoffbedarf vermutlich aus dem aus dem erhöhten Plankton-, ihren Energiebedarf aus dem erhöhten Lichtangebot an der Oberfläche.

Das Wachstum sowohl der Wildbestände als auch der gehälterten Juvenilen in Jordanien war vergleichbar mit dem im Indopazifik, so dass davon ausgegangen werden kann, dass insgesamt ausreichende Wachstumsbedingungen in dieser subtropischen Randregion vorherrschen. Allerdings war die Reproduktionsperiode gegenüber den Tropen stark verkürzt und auf die Sommermonate begrenzt.

Es gibt eine ausgeprägte Saisonalität in der Gonadenentwicklung mit artspezifischen Laichzeiten: *T. squamosa* laicht zwischen Juni bis November und zeigt eine zwei-gipflige Verteilung in der Reproduktionsintensität. Im Gegensatz dazu zeigt *T. maxima* eine kürzere gleichförmige Laichsaison mit eingipfliger Verteilung (Juni bis September). Die Rückbildung der Ovarien-Follikel erstreckt sich über den Winter. Die neuerliche Follikelreifung beginnt im Spätwinter und ist bis zum Frühling abgeschlossen.

Reproduktionsexperimente mittels Hitze- und Serotonin- induzierter Spontanlaichungen und anschließender Larven- und Jungmuschelhaltung zeigten, dass der Reproduktionserfolg während des Sommers (Juni bis September) am größten ist. Zwar konnte ein Ablaihen auch während der Wintermonate induziert werden, aber die Larvenentwicklung war stark verzögert und die Mortalität zur Metamorphose vollständig. Aus diesen Ergebnissen lässt sich ableiten, dass die Reifung der Ovarien im Winter und Frühjahr durch die hohen Nährstoff- und Chlorophyllkonzentrationen begünstigt wird, während die Entwicklung der lecitotrophen Larven und letztlich der Reproduktionserfolg durch die Wachstumsbedingungen während der warmen Sommermonate entschieden wird.

Die Anpassung der bereits vorhanden Riesenmuschel-Hälterungstechniken an die Bedingungen des Roten Meeres waren erfolgreich, allerdings stellt die Saisonalität Herausforderungen an die Anlagentechnik, da während der Wintermonate adäquate Bedingungen für die Produktion von

Muscheln einzuhalten sind. Zur Zeit hat die Produktion von Riesenschnecken in der Kulturanlage der Aqaba Marine Science Station noch Pilot-Charakter.

Die molekulargenetischen Ergebnisse lieferten zwingende Hinweise für die Existenz einer neuen Riesenschnecke-Art *Tridacna* nov. sp., die möglicherweise als glaziale Reliktform auf den nördlichen Golf von Aqaba begrenzt ist. Die molekulare Analyse der mitochondrialen rDNA stimmt dabei mit unabhängigen Analysen der morphologisch-taxonomischen und reproduktionsbiologischen Merkmale überein und grenzt die neue Art eindeutig von ihren sympatrischen Artverwandten *T. squamosa* und *T. maxima* sowie anderen Tridacniden aus dem weiteren indopazifischen Raum ab. Die übereinstimmenden Ergebnisse ordnen *Tridacna* nov. sp. gemeinsam mit *T. maxima* und *T. squamosa* in die Untergattung *Chametrachea* überein. Von diesen sympatrischen Rote Meer - Verwandten unterscheidet sie sich durch eine besondere Reproduktionsbiologie. Sie ist gekennzeichnet durch eine frühere Reifung der Ovarien (August), ein deutlich schmalerer Durchmesser der Oozyten ( $75 \pm 2 \mu\text{m}$ ) und eine vorgezogene und kürzere Laichphase während des Frühjahrs und Frühsommers (Mai bis Juli). Vermutlich sind die Larven weniger lecitotroph und stärker an das im Frühsommer noch reichliche Planktonangebot angewiesen. *Tridacna* nov. sp. ist wahrscheinlich eine glaziale Reliktform. Hierfür spricht erstens ihr mutmaßlich auf den äußersten Nordzipfel des Roten Meer Grabensystems begrenztes Vorkommen, zweitens die Kaltwassertoleranz relativ zu den weiter verbreiteten Artverwandten *T. maxima* und *T. squamosa*, und drittens die Ähnlichkeit zur fossilen Art *Tridacna aegyptiaca* (Chenu, 1845), die vermutlich während der Eiszeit im Roten Meer verbreitet war.

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## PREFACE

This dissertation is on the giant clams in the northern Gulf of Aqaba, marking their northwestern limit of their distribution, funded by the German Federal Ministry of Education and Research (BMBF) Grant No. 03F0356A-B. The culture of the Red Sea giant clam species have been attempted from August 2002 to September 2004 at the Marine Science Station in Aqaba, Jordan. Research work has been conducted simultaneously in this period and the following are the main research themes:

*Distribution, abundance, size and growth of giant clam (Tridacnidae) in the northern Gulf Of Aqaba, Red Sea* (Chapter 2). This aspect of the research covered the assessment of the indigenous population of giant clams in the northern Gulf of Aqaba and compared with those in the wider Red Sea using secondary data on clam abundance.

*Reproductive periodicity of giant clams (Tridacnidae) in the northern Gulf of Aqaba, Red Sea* (Chapter 3). The reproductive profiles, from the field gonad biopsy and from the land-based spawning attempts, were compared to determine reproductive patterns of giant clams in the northwestern limit of their geographical distribution.

*Morphological, reproductive and molecular genetic evidence for a new species of giant clam (tridacnidae) in the northern Red Sea* (Chapter 4). This study used three separate tools (i.e., molecular mitochondrial 16s rDNA, morphological classification, and, reproductive study) to verify the taxonomic classification of some specimens of giant clams found in the northern Gulf of Aqaba.

*Giant clam culture protocol for Jordan, Gulf of Aqaba* is presented in Chapter 5. This a stepwise manual of the culture method modified from existing clam culture techniques in the tropics adapted to the conditions and climate in the subtropical margin of its geographical distribution.

The three manuscripts (chapters 2-4) presented in this thesis have been prepared for submission to peer reviewed scientific journals. The culture manual is intended for publication in a technical journal or report.

Hilly Ann Roa-Quiaoit, August 2005  
Bremen, Germany

## Chapter 1: General Introduction and Discussion



This dissertation is the first attempt so far to study in detail the ecology, biology, molecular genetics and reproduction of giant clams in the Gulf of Aqaba, Red Sea, marking the north-western limit of the distribution of the genus *Tridacna*. It adds significantly to the biodiversity of the species-poor group with the identification of a new species of restricted range and explores the potential for giant clam culture in a harsh subtropical desert climate.

Early on there has been an increasing interest on giant clams as conspicuous members of the reef ecosystem with their gigantic size and colourful mantles compared to other reef denizens. Pioneering scientific interest began very early. The unique association of giant clams with the photosynthesizing algae called zooxanthellae was explored (Yonge, 1936, 1953) and reproductive studies showed the hermaphroditism in giant clams (Stephenson, 1934; Wada, 1952), forming the baseline studies for future work on clams. There has been a surge of interest on giant clams in the 80-90s fuelled by the alarming rate of decline of the natural stocks throughout their geographical range (Lucas, 1988; Fitt, 1993). A milestone of which was the development of culture techniques for giant clams in tropical conditions (Heslinga et al, 1984; Usher & Munro, 1988; Braley, 1992; Calumpong, 1992; Bell et al., 1997).

Even then, there was not much attention directed towards giant clams at higher latitudes marking the distributional boundaries in subtropical waters. For example, in the distribution maps of key papers on the biogeography of giant clams, only one of the two Red Sea giant clam species, *T. maxima* is shown to extend all the way up to the northern tip of the Gulf of Aqaba (Rosewater 1965; Lucas, 1988; Braley, 1992). A revision of giant clam distribution and ecology at their biogeographical Red Sea margins was overdue.

This research was carried out in the framework of a bilateral project between Germany and Jordan on the sustainable aquaculture of giant clams in the Jordanian sector of the Gulf of Aqaba, to produce high-valued commodities using environmentally sound methods (BMBF Grant No. 03F0356A-B). Giant clams with their fast growth rates, high commercial value, present endangered status and mixotrophic nutrition capable of recycling nutrients (auto- and hetero-trophy) resulting in nearly 'zero waste,' were the prime targets for mariculture.

However, the mass production of giant clams has always been limited by the availability of mature clams for induced spawnings. Thus, there was a need to assess the distribution and abundance of the indigenous population in Jordan and to determine the breeding stock for aquaculture (Chapter 2). The results show a relatively scarce number of natural stocks in Jordan, which like elsewhere in its distribution, has been subjected to exploitation from harvesting and dwindling stocks from environmental pressures, mostly from deterioration or loss of reef habitat from the booming

economic development along the coast. The analysis of regional data collated from PERSGA and Reef Check surveys confirmed this same scenario for the wider Red Sea where giant clams were found in lower abundance in areas with high human impacts and environmental pressures.

Considering the temporal fluctuations of various factors affecting giant clams in this subtropical marginal sea, it necessitated a study on the natural reproductive periodicity of giant clams to help define the best period for induced spawnings and larval-early juvenile rearing for the pilot culture attempts. However, due to the scant number of broodstocks available, sacrificing the clams were not an option, so *in situ* gonad biopsy technique was applied (Braley, 1984, 1988). Although risky, the chances of killing the clams can be minimized by taking utmost care when injecting the needle past vital organs. The reproductive success of giant clams, from both the field and culture data (see Chapter 3), was found to be limited to the summer months which significantly reduces the culture potential relative to year-round spawning in tropical conspecifics (Alcazar, 1988; Trinidad-Roa, 1988; Heslinga et al., 1990). To overcome the reproductive impasse and extend induced spawning period over an entire year conditioning of the brood-stock by increasing temperature and food in land-based culture is necessary. Induced spawning and larval rearing attempts on natural unconditioned broodstock resulted in unsuccessful development and larval mortalities during the colder months. Culture results are corroborated by biopsy showing regressive ovaries over winter to spring in natural broodstock from the field.

A new re-circulating system with controlled culture and environmental conditions installed as part of the above project has recently become operational in the Marine Science Station in Aqaba. It should allow for the year-round culture of giant clams in this region, hopefully, eliminating the mortalities associated with the seasonality of environmental conditions in this desert-enclosed sea.

Over the two years of artificial induction and larval to visible juvenile land-based rearing, a culture protocol for northern Gulf of Aqaba was established, shown in detail in Chapter 5. The existing culture techniques in the tropics initially found unsuccessful were modified to adapt to the conditions of this harsh subtropical desert region.

In the course of the field work, I noticed a conspicuous variety of giant clam which did not fit into the existing identification scheme for tridacnids. Of similar size to *T. squamosa* and *T. maxima*, it differed from the former in its markedly jagged and deeply furrowed shells, reminiscent of a *T. gigas*, the largest of the genus. However, considering the limited geographical range of *T. gigas* in the Indo-Malayan area (Lucas, 1988), this was unlikely. Further, these specimens showed morphological characters shared between the two Red Sea species, so, the possibility of a hybrid of both, or, a variant of one of the species was more likely. A review of previous literatures showed

that these clams have remarkably similar feature with a fossil species in the Gulf of Suez, *T. aegyptiaca* (Chenu 1845). Considering that in a span of two decades, two giant clam species have been discovered, one of which is actually a synonym of a fossil species with limited geographic range, *T. tevoroa*, but living sympatrically with its nearest congener, *T. derasa*. This might also be the case of this new species where it exists in close proximity to the other two congeners in the Gulf of Aqaba. So, the taxonomic status of these specimens was verified using genetic analysis, which I carried out under the supervision of Dr. Kochzius (UFT, University of Bremen). Molecular analysis showed a new species of giant clam, supported by the morphological and reproductive data I collected from Jordan. This is presented in Chapter 4, where three separate lines of evidence showed the existence of a new species in the Northern Red Sea.

As shown in all chapters, the influence of temperature on giant clams over latitudinal differences is evident (e.g., distribution, abundance, reproduction) from its north-westernmost border in Jordan to the wider Red Sea and the tropical Indo-Malayan area, where the giant clams are concentrated. The unique features of the Gulf of Aqaba, in particular, the northern Gulf of Aqaba which is distinct from the southern part of the gulf and the Red Sea proper, provides very different conditions from those elsewhere in the wide Indo-Pacific range of *Tridacna*. This makes our findings all the more remarkable that the giant clams encountered at their northwestern limit are not markedly distinct e.g., in growth and size (Chapter 2) from the patterns encountered elsewhere. This suggests a marked adaptational potential to the environmental conditions at the subtropical margin of their distribution, allowing vigorous growth and large adult sizes in reasonable densities, albeit at the cost of a reduced reproductive potential and high susceptibility to anthropogenic change.

The present study shows that in spite of decades of research the Red Sea treasures are still only beginning to unfold, making it all the more important to unite the regional and international efforts to conserve this unique ecosystem and natural laboratory.

#### Literature Cited:

- Alcazar, S. 1988. Spawning and larval rearing of tridacnid clams in the Philippines. Pages 125-128 in J. Copland and J. Lucas, eds. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9, Canberra.
- Bell, J., I. Lane, M. Gervis, S. Soule & H. Tafea. 1997. Village-based farming of the giant clam, *Tridacna gigas* (L.), for the aquarium market: Initial trials in Solomon Islands. *Aquaculture Research* 28 (2):121-128.
- Braley, R. 1984. Reproduction in the giant clams *Tridacna gigas* and *T. derasa* in situ on the north-central Great Barrier Reef, Australia and Papua New Guinea. *Coral Reefs* 3:221-227.

- \_\_\_\_\_. 1988. Reproductive condition and season of the giant clams *Tridacna gigas* and *T. derasa* utilising a gonad biopsy technique. Pages 178-182 in J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- \_\_\_\_\_. 1992. *The giant clam: hatchery and nursery culture manual*. ACIAR Monograph No. 15, Canberra. 144 p.
- Calumpong, H., ed., 1992. *The giant clam: an ocean culture manual*. ACIAR Monograph No. 16, 68 p.
- Fitt, W. ed., 1993. *Biology and Mariculture of Giant Clams: a workshop held in conjunction with the 7th International Coral Reef Symposium 21-26 June 1992, Guam, USA*. ACIAR Proceedings No. 47, 154 p.
- Heslinga, G., F. Perron & O. Orak. 1984. Mass culture of giant clams (*F. Tridacnidae*) in Palau. *Aquaculture*, 39:197-215.
- \_\_\_\_\_, T. Watson and T. Isamu. 1990. *Giant clam farming*. Honolulu, Hawaii: Pacific Fisheries Development Foundation (NMF/NOAA).
- Lucas, J. 1988. Giant clam description, distribution and life history. In: Copland, J. & J. Lucas (eds) *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, pp. 21-33.
- Rosewater, J. 1965. The Family Tridacnidae in the Indo-Pacific. *Indo-Pacific Mollusca* 1(6):347-395.
- Stephenson, J. 1934. The breeding of reef animals, Part II. Invertebrates other than corals. *Scientific Report Great Barrier Reef Expedition*.
- Trinidad-Roa, M. 1988. Spawning and larval rearing of giant clams in Pangasinan, Philippines. Pages 120-124 in J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- Usher, G. and J. Munro. 1988. ICLARM Coastal Aquaculture Center: current facilities and progress. Pages 106-109 in J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- Wada. S. 1952. Protandric functional hermaphroditism in the tridacnid clams. *Oceanographic Magazine (Tokyo)* 4:25-30.
- Yonge, C. M. (1936). Mode in life, feeding, digestion and symbiosis with zooxanthellae in the Tridacnidae. *Gt. Barrier Reef Exped. 1928-29, Scient. Rept. 1*: 283-321.
- Yonge, C.M., (1953). Mantle chambers and water circulation in the Tridacnidae (Mollusca). *Proc. Zool. Soc. Lond.* 123: 551-561.

## Chapter 2: Distribution, Abundance, Size And Growth Of Giant Clam (Tridacnidae) In The Northern Gulf Of Aqaba, Red Sea



Figure 1. The two Red Sea species of giant clams, *T. maxima* (left) and *T. squamosa* (right).

## Abstract

This study compares distribution, abundance, size and growth of tridacnids in the Gulf of Aqaba with data from the Red Sea and Indo-Pacific. At the northwestern limit of distribution in Jordan, giant clams are scarce, with mean densities of *Tridacna squamosa* Lamarck, 1819 and *T. maxima* Roding, 1798 between 0.3 and 0.5 ind. 100 m<sup>-2</sup>, compared to 4 to 7-fold higher values in the Red Sea and an order of magnitude higher numbers in the Indo-Pacific. The abundance and sizes vary significantly with depth: *T. maxima* (adults and juveniles) are 80-times common on the (<3 m) reef flat ( $1.6 \pm 0.9$  ind. 100 m<sup>-2</sup>) than on the deeper (9-15 m) fore-reef, whereas *T. squamosa* (adults) are 3-times common on the deeper fore-reef ( $0.5 \pm 0.4$  ind. 100 m<sup>-2</sup>) than on the reef flat (juveniles). Two-third higher abundances in restricted ( $1.04 \pm 0.4$  ind. 100 m<sup>-2</sup>) compared to open-access areas, and, a 12-fold decline over the past four decades suggest habitat loss and overfishing of natural stocks related to sweeping rates of urban development. The growth estimates of wildstock *T. squamosa* ( $\phi' = 3.9$ ), *T. maxima* ( $\phi' = 4.2$ ) and cultured juvenile *T. squamosa* ( $\phi' = 4.2$ ) are comparable to the Indo-Pacific, suggesting favorable growth conditions in this marginal environment.

## Introduction

Giant clams belonging to the family Tridacnidae are the largest bivalves. They are commercially important for the food market (Murakoshi, 1986; Shang et al., 1994), shellcraft industry (Juinio et al., 1987) and in the live marine aquarium trade (Knop, 2004). Overexploitation of this valuable and multi-faceted resource has led to the decline of natural stocks throughout its natural Indo-Pacific range and the ecological extinction (Pandolfi et al., 2003) in some areas of the larger species (*Tridacna gigas* Linne, 1758; *Tridacna derasa* Roding, 1798) (Brown and Muskanofola, 1985; Lucas, 1988; Juinio et al., 1989). All eight described species are endangered and are included in the Convention of International Trade of Endangered Species (CITES) list (Wells et al., 1983; Hilton-Taylor, 2000). Research efforts on giant clams proliferated in the 1980s and culture techniques in the tropics for food and restocking of depleted reefs were established (Lucas, 1988; Braley, 1992; Calumpong, 1992).

Giant clams are widely distributed in the Indo-Pacific. From the southeast Pacific westwards to East Africa, its distribution extends up north to the Red Sea (Rosewater, 1965; Lucas, 1988; Braley, 1992). Two extant giant clam species are known to occur in the Red Sea, namely, *Tridacna squamosa* Lamarck, 1819 and *T. maxima* Roding, 1798. These two species exist up to the northeastern extension, the Gulf of Aqaba marking the northwestern limit of their geographical distribution. The Gulf of Aqaba, 165 km long × 15 km wide and semi-enclosed by the narrow Strait of Tiran, is characterized by a steep-sided narrow shelf with the northernmost fringing reefs in the western Indo-Pacific region.

In this paper, we study the giant clam population in the Jordanian sector of the northern Gulf of Aqaba. The Jordanian coastline, 27 km long with 13 km of discontinuous fringing reefs (UNEP/IUCN, 1988), has been greatly modified over four decades of rampant development of ports, industrial and tourism areas (Badran and Bashir, 2001), as well as extreme events such as oil spills and extreme low tides in the area (Fishelson, 1973; Loya, 1976). These factors have likely affected the indigenous stocks of giant clams, but data are thus far lacking. Here we describe the distribution, abundance and influence of several factors on the extant stocks in Jordan, and compare them with available data from the wider Red Sea and the tropical Indo-Pacific. Growth parameters from the natural stocks and cultured juveniles, from a pilot culture of giant clams in Jordan (Roquiait et al., 2004) are compared with published values in the Indo-Pacific.

## Methods

### *Distribution and Abundance in Jordan*

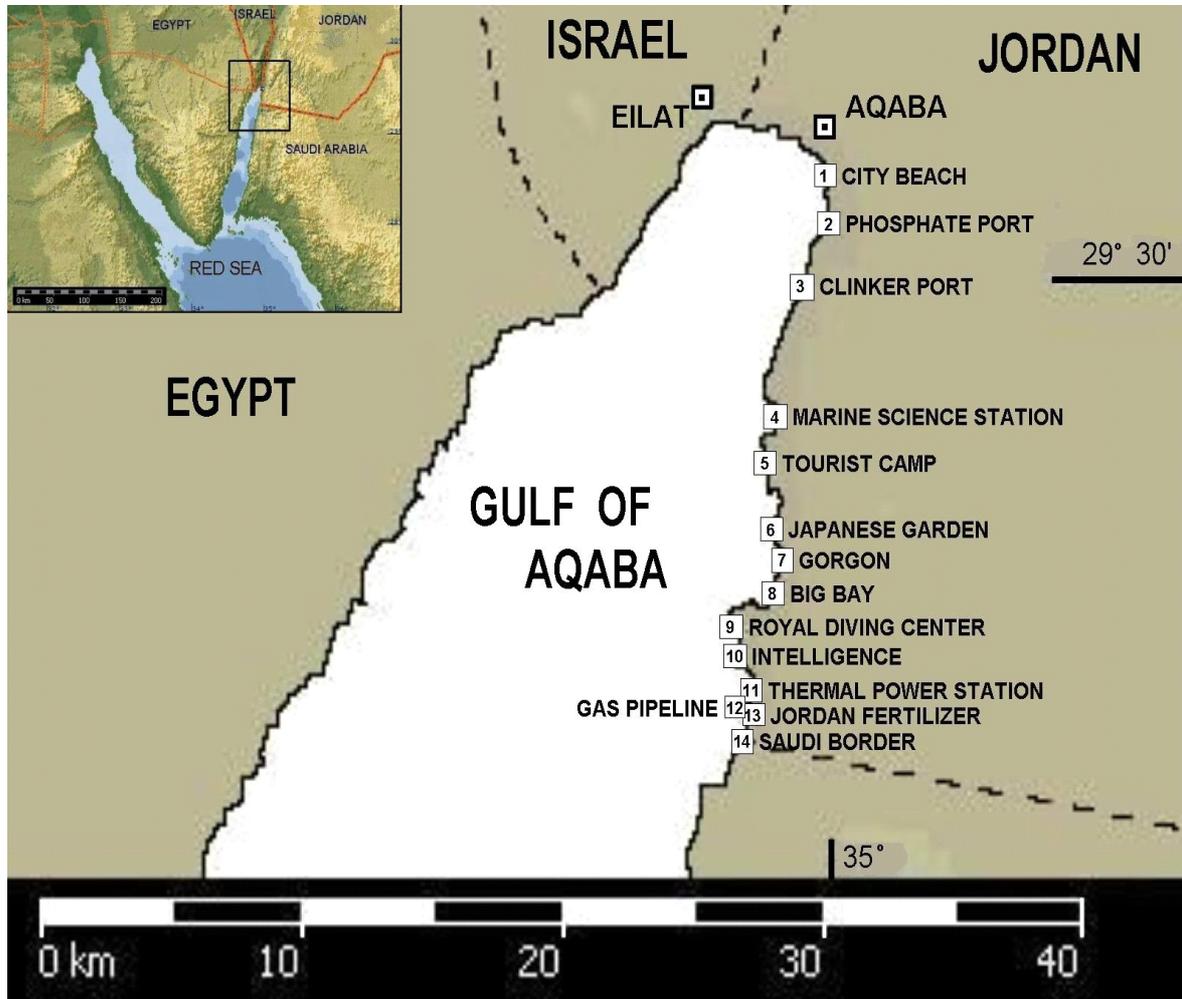


Figure 2. Survey sites in Aqaba, Jordan, showing inset map of the Gulf of Aqaba.

Surveys were conducted in Jordan (Fig. 2) at the following depths: reef flat (<3 m), shallow (3-9 m) and deeper (9-15 m) fore-reef. Shell length (SL) and corresponding depth of each clam were recorded. Triplicate belt transects (5 m width) were surveyed at 6 and 12 m depths, by either swimming along pre-determined distances thus covering a total area of 250 m<sup>2</sup> (50 m, English et al., 1994), or, by swimming for pre-determined periods of time at a constant speed (Pilcher and Alsuhaibany, 2000). This latter method was developed in response to the scarcity of natural stocks in order to increase the number of clams per transect. Inter-calibration (N = 51 surveys) showed good correspondence (see Table 1) between the two methods (T-test for dependent samples,  $p=0.79$ ), with a survey time of  $7 \pm 1.2$  minutes corresponding to an area of  $250 \pm 43$  m<sup>2</sup>. In sites

Table 1. Clam density of both species (ind. 100 m<sup>-2</sup>) as determined by belt transects (50m vs. time equivalent) as per sampling sites and depths.

Sites	Depth (m)	Belt Transect	
		pre-determined distance	pre-determined time
Big Bay	6	0.1	0.1
Royal Dive	12	0.1	0.1
Saudi Border	12	0.1	0.1
Gas Pipeline	12	0.1	0.2
JFI Port	12	0.2	0.3
MSS Reserve	6	0.3	0.3
Saudi Border	6	0.4	0.3
Royal Dive	6	0.4	0.4
Intelligence	6	0.5	0.5
Intelligence	12	0.5	0.5
Tourist Camp	12	0.6	0.8
MSS Reserve	12	0.7	0.7
Saudi Border	12	0.9	0.8
Clinker	12	0.9	1.1
MSS Reserve	12	1.5	1.4
Saudi Border	6	1.7	1.5
MSS Reserve	1	3.4	3.6

where no transects were laid, the survey time spent (sandy patches excluded) was converted to area for the calculation of clam densities.

All shell length data collected for each species were pooled and categorized according to stage of sexual maturity to estimate the population structure and depth distribution of the different life-stages. For *T. maxima*, clams were grouped into juveniles (<4 cm shell length), young adults (4-15 cm) and broodstock hermaphrodites (>15 cm) based on Manu and Sone (1995). For *T. squamosa*, clams were grouped into juveniles (<10 cm), young adults (10-20 cm) and broodstock hermaphrodites (>20 cm) based on Beckvar (1981).

The abundance data was correlated to several factors (coastal use, human activities, accessibility, fish predators and coral cover) categorized to a nominal scale (Table 2), based on site assessments and available reports, as follows: For coastal use, the conversion into heavy industries were ranked highest followed by urban-private developments (medium) and managed-marine parks (low). Human impacts were classified into urban/industrial pollutions and fishing (highest), controlled tourism (medium) and exclusive research work (low). Accessibility was classified as restricted (marine reserve and parks) and open-accessed (industrial, ports, depots and hotel areas). Health condition of the coral reef habitat was categorized based on live hard coral cover from <15% (low),

Table 2. List of factors and levels of impact affecting clam abundances.

Factors	1 Low Level	2 Medium Level	3 High Level
Human Activities	Restricted research work and scientific dives	Snorkelling, diving, swimming, tourist boats, some fishing	Fishing (hook & line, nets), gleaning, sewage & solid waste disposal, urban & industrial pollution
Coastal Zone Use	Marine reserve and managed-marine parks	Controlled tourism, hotels, dive centers, business and private coastal infrastructures, some fishing	High port/shipping activity, heavy industrial complex, oil depot, power plants, fishing
Fish Predators (Balistidae, Labridae, Lethrinidae, Tetraodontidae)	Total pooled abundance: <120 fish/ 250 m <sup>2</sup> transect	Total pooled abundance: 120 - 250 fish/ 250 m <sup>2</sup> transect	Total pooled abundance: 250 - 370 fish/ 250 m <sup>2</sup> transect
Live Hard Coral Cover	Live hard coral cover (<15%)	Live hard coral cover (15-40%)	Live hard coral cover (40-60%)

Table 3. Categories of all sampling sites under the different factors (For details on levels of impact, see Table 2).

Site No.	Site Name	Coastal Zone	Accessibility	Human activities	Coastal use	Coral cover	Fish predators
1	City Beach	Multiple-User	open-access	2	2		
2	Phosphate Port	Industrial	open-access	3	3	1	
3	Clinker	Marine Park	restricted	2	1		
4	MSS Reserve	Marine Reserve	restricted	1	1	3	2
5	Tourist Camp	Marine Park	restricted	2	1	3	3
6	Japanese Garden	Marine Park	restricted	2	1		
7	Gorgon	Marine Park	restricted	2	1		
8	Big Bay	Multiple-User	open-access	2	2		3
9	North Royal Dive	Multiple-User	open-access	2	2		
10	Intelligence	Multiple-User	open-access	2	2		
11	Thermal Plant	Industrial	open-access	3	3		1
12	Gas Pipeline	Industrial	open-access	3	3		1
13	Jordan Fertilizer Complex	Industrial	open-access	3	3		2
14	Saudi Arabia Border	Multiple-User	restricted	2	2	2	

15-40% (medium) and 40-60% (high) (Moghrabi et al., 2003). Predation by fish was correlated with the total pooled abundances of the common predators, i.e., Balistidae, Labridae, Lethrinidae and Tetraodontidae (Calumpong, 1992), using the range from <120 fish/ 250m<sup>2</sup> transect (low), 120-250 fish/ 250m<sup>2</sup> transect (medium) to 250-370 fish/ 250m<sup>2</sup> transect (high) (Khalaf and Kochzius, 2002). All surveyed sites were categorized accordingly to all factors (Table 3). Multiple correlations were done to determine the relationships between these factors and clam abundance.

#### *Red Sea secondary data collection on Tridacna spp. abundance (both species pooled)*

Abundances of giant clams between the Jordan and the southern Gulf of Aqaba (Egypt) - Red Sea populations were compared using secondary data provided by Reef Check and PERSGA (Regional Organization for the Conservation of the Environment of the Red Sea and Gulf of Aden) (Fig. 3). Similar survey methods were applied by both organizations according to the Reef Check survey method, using four 5 × 20 m long belt transects at shallow and deeper fore-reef (Hodgson et al., 2004). All data were standardized into total number of clams by depth and per site. Abundance was statistically tested for relations with physical features (shelf, water visibility), geographical location (shores, N-S gradient), ecological (live coral cover) and socio-anthropogenic (human activities). Physical features were classified into shelf width defined as narrow (<100 m) or wide (>100 m), water visibility was taken as continuous variable between 5 and 50 m. Both features were taken from topographical maps and existing reports (Pilcher and Alsuhaibany, 2000; Hassan et al., 2002; Richter and Abu-Hilal, 2004). Categorical descriptions were used for the shore location (eastern-western) and coordinates for the geographical position. The percentage coral cover was used based on the most recent coral assessment in the Red Sea in 2002 (Hassan et al., 2002). Human impacts in the Red Sea were compiled from regional status reports. Impacts due to tourism, coastal construction and development, shipping and port, collection and fishing activities (Pilcher and Alsuhaibany, 2000; Hassan et al., 2002) were normalized to low (1) and high (2) impact levels.

#### *Growth of cultured and wild stock clams in Jordan*

Due to the scarcity of indigenous stocks, a limited number of wild clams were tagged and measured at certain time intervals, 9 *T. maxima* (reef flat) and 33 *T. squamosa* (10 m depth). Growth was determined from the differences in millimeter between maximum anterior to posterior shell lengths between measurements. Cultured *T. squamosa* (n = 21) from a spawning in June 2003 were used to assess growth of the early life stages. The clams (3 - 11 months old) were reared in indoor flowing tanks with raw unfiltered seawater, illuminated 12 hours/day with artificial light source (200  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) with water temperatures maintained at 24-25 °C. This set-up ensured the availability of exogenous food sources and light for both, hetero- and autotrophic growth, respectively. After



Figure 3. Survey sites of Reef Check and PERSGA in the Red Sea (Courtesy of G. Heiss).

11 months, the clams were transferred to outdoor tanks with continuous flowing unfiltered seawater at ambient water temperature, from 20-27 °C (annual range, Aqaba, Jordan) exposed to natural sunlight with partial shading (500-1600  $\mu\text{E m}^{-2} \text{s}^{-1}$ ). Growth was monitored for each batch from 3 - 21 months.

The size increment data were transformed to von Bertalanffy (VB) growth function (Munro, 1988; Urban, 2002) and the growth parameters (growth constant,  $k$ , and asymptotic length,  $L_\infty$ ) were determined for the natural stocks and cultured juveniles. The growth performance index, phi prime ( $\phi'$ ), derived from the VB growth parameters was used (Vakily, 1992) and compared with published values in the Indo-Pacific.

The growth constant ( $k$ ) was estimated using the Munro Plot routine in the program package, FiSAT (Gayaniilo and Pauly, 1992) expressed as:

$$k = [\ln(L_\infty - L_1) - \ln(L_\infty - L_2)]/t_2 - t_1 \quad (\text{Munro, 1982}) \dots \text{Equation 1.}$$

where  $L_\infty$  was estimated from the natural stocks for each species,  $L_1$  and  $L_2$  were the shell lengths at specified time intervals  $t_2 - t_1$ (months).

The growth performance index ( $\phi'$ ) was calculated as:

$$\phi' = \log_{10} k + 2\log_{10} L_\infty \quad (\text{Pauly and Munro, 1984}) \dots \text{Equation 2.}$$

The mean phi prime was determined and compared for the indoor and outdoor set-ups of the cultured stocks of 10 to 11 months-old and 12 to 18 months-old clams, respectively.

#### *Data analyses*

All data were subjected to test of normality using Shapiro-Wilk  $W$ -test. Data not normally distributed were analyzed using non-parametric statistics. Comparison of means was determined using Student  $t$ -test and non-parametric Kolmogorov-Smirnov test. Nonparametric Spearman Rank Order (and Gamma) Correlations tests were done on abundance and length data per species with several factors (i.e., depth, zone, etc.). All statistical tests were run using the program Statistica 6.0 Package at a significance level of 0.05.

## Results

### *Population abundance in Jordan*

The mean abundance of *T. maxima* ( $0.5 \pm 0.3$  ind.  $100 \text{ m}^{-2}$ ) was higher compared to *T. squamosa* ( $0.3 \pm 0.2$  ind.  $100 \text{ m}^{-2}$ ). Clam abundance (Table 4) was significantly correlated only with depth. In *T. maxima*, abundance was inversely correlated with depth ( $r = -0.84$ ,  $p = 0.05$ ): the bulk of the population was found on the reef flat ( $1.64 \pm 0.9$  ind.  $100 \text{ m}^{-2}$ ), with only few ( $0.35 \pm 0.4$  ind.  $100 \text{ m}^{-2}$ ) and scattered ( $0.02 \pm 0.04$  ind.  $100 \text{ m}^{-2}$ ) specimen on the shallow (3-9 m) and deeper fore-reef (9-15 m), respectively. In *T. squamosa*, on the other hand, abundance was positively albeit moderately correlated with depth ( $r = 0.39$ ,  $p = 0.05$ ). Its lowest abundances were found on the reef flat ( $0.16 \pm 0.1$  ind.  $100 \text{ m}^{-2}$ ), increasing in the shallow ( $0.25 \pm 0.2$  ind.  $100 \text{ m}^{-2}$ ) and deeper fore-reef ( $0.47 \pm 1.1$  ind.  $100 \text{ m}^{-2}$ ).

Table 4. Abundance (ind.  $100 \text{ m}^{-2}$ ) of *T. squamosa* and *T. maxima* in the reef flat (<3 m), shallow (3-9 m) and deeper (9-15 m) fore-reef.

Site No.	<i>T. squamosa</i>				<i>T. maxima</i>			
	deeper	shallow	flat	mean	deeper	shallow	flat	mean
1			0.2	0.2			0.8	0.8
2	0.0	0.00		0.0	0.0	0.0		0.0
3	0.9	0.00	0.2	0.4	0.0	0.4	0.6	0.3
4	0.8	0.20	0.4	0.5	0.1	0.0	2.0	0.7
5	0.4	0.13	0.0	0.2	0.1	0.2	1.3	0.5
6	1.5	0.29	0.2	0.7	0.0	0.0	2.9	1.0
7	0.3	0.49		0.4	0.1	0.8		0.4
8	0.0	0.80		0.4	0.0	0.9		0.5
9	0.1	0.13	0.0	0.1	0.0	0.0	1.1	0.4
10	0.5	0.53		0.5	0.0	0.0		0.0
11	0.6	0.00		0.3	0.0	1.0		0.5
12	0.1	0.12		0.1	0.0	1.0		0.5
13	0.3	0.16		0.2	0.0	0.2		0.1
14	0.5	0.33	0.1	0.3	0.0	0.1	2.9	1.0
Mean	0.47	0.25	0.16	0.31	0.02	0.35	1.64	0.47
STD	0.43	0.24	0.13	0.19	0.04	0.42	0.95	0.32

The size distribution of both species (Figs. 4A-B) was also significantly correlated with depth. In *T. maxima* (Fig. 4), the majority of clams of different age-groups were found on the reef flat while only a few were found at deeper depths ( $r = 0.25$ ,  $p = 0.05$ ). Its population has a very high proportion of young hermaphrodites (82%) with smaller proportion of juveniles and only few older clams (Fig. 5A).

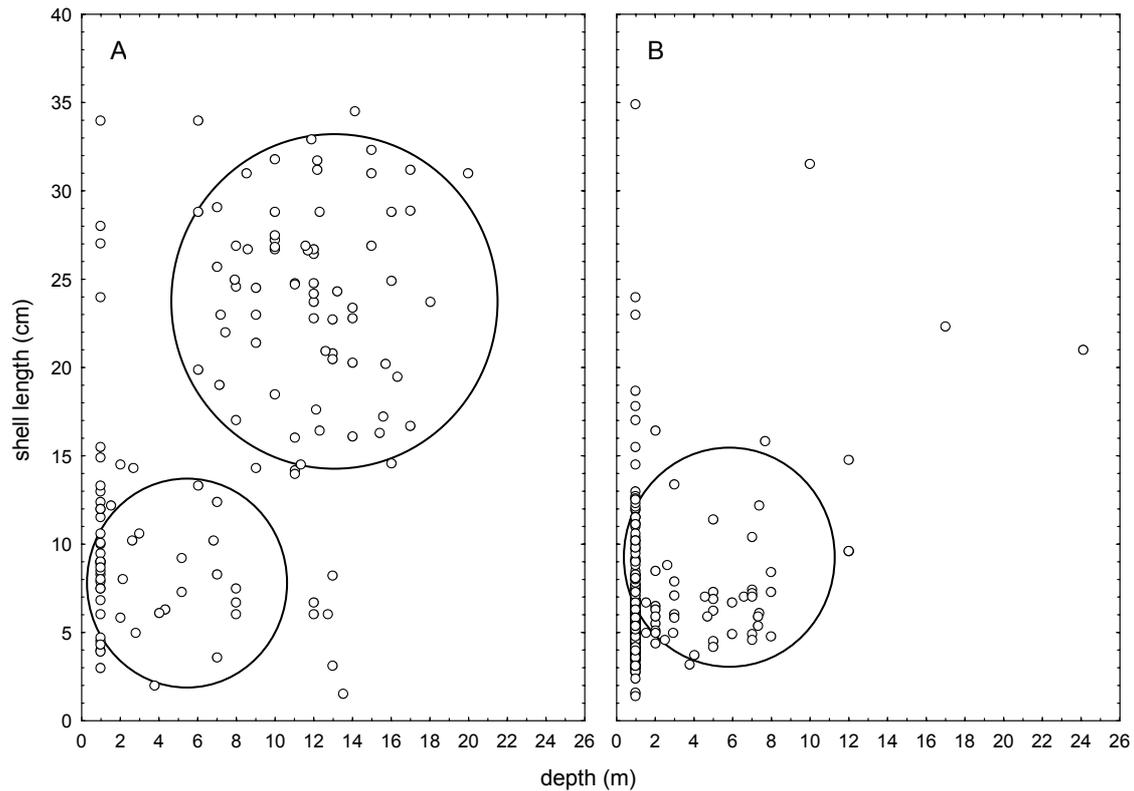


Figure 4. Size-depth distribution of *T. squamosa* (A) and *T. maxima* (B).

In *T. squamosa* (Fig. 5), size was moderately correlated with depth ( $r = 0.49$ ,  $p = 0.05$ ): juveniles occurred mostly on the reef flat, young adults were found at all depths while the majority of the larger old clams were found in the deeper fore-reef. Interestingly, *T. squamosa* featured a higher proportion of adults (43%) than juveniles and sub-adults.

We found no north-to-south gradient in either of the species along the Jordanian coast. However, in both species clam abundances appeared to vary as a function of natural and anthropogenic factors (Fig. 6). We found an inverse relationships between clam densities and both, human impact and coastal use in *T. squamosa* ( $p = 0.05$ ,  $r = -0.49$  and  $-0.31$ , respectively) and *T. maxima* ( $p > 0.05$ ,  $r = -0.12$  and  $-0.23$ , respectively). Furthermore, abundances were significantly ( $p = 0.04$ ) higher in the restricted access zones ( $1.04 \pm 0.4$  ind.  $100 \text{ m}^{-2}$ ) than in the open-access areas ( $0.56 \pm 0.3$  ind.  $100 \text{ m}^{-2}$ ), particularly when comparing the marine parks with multiple-use and industrial zones (Fig. 7). There was a significant positive relationship with coral cover only in *T. squamosa* ( $r = 0.74$ ,  $p = 0.05$ ), but no significant correlation with fish predators in both species.

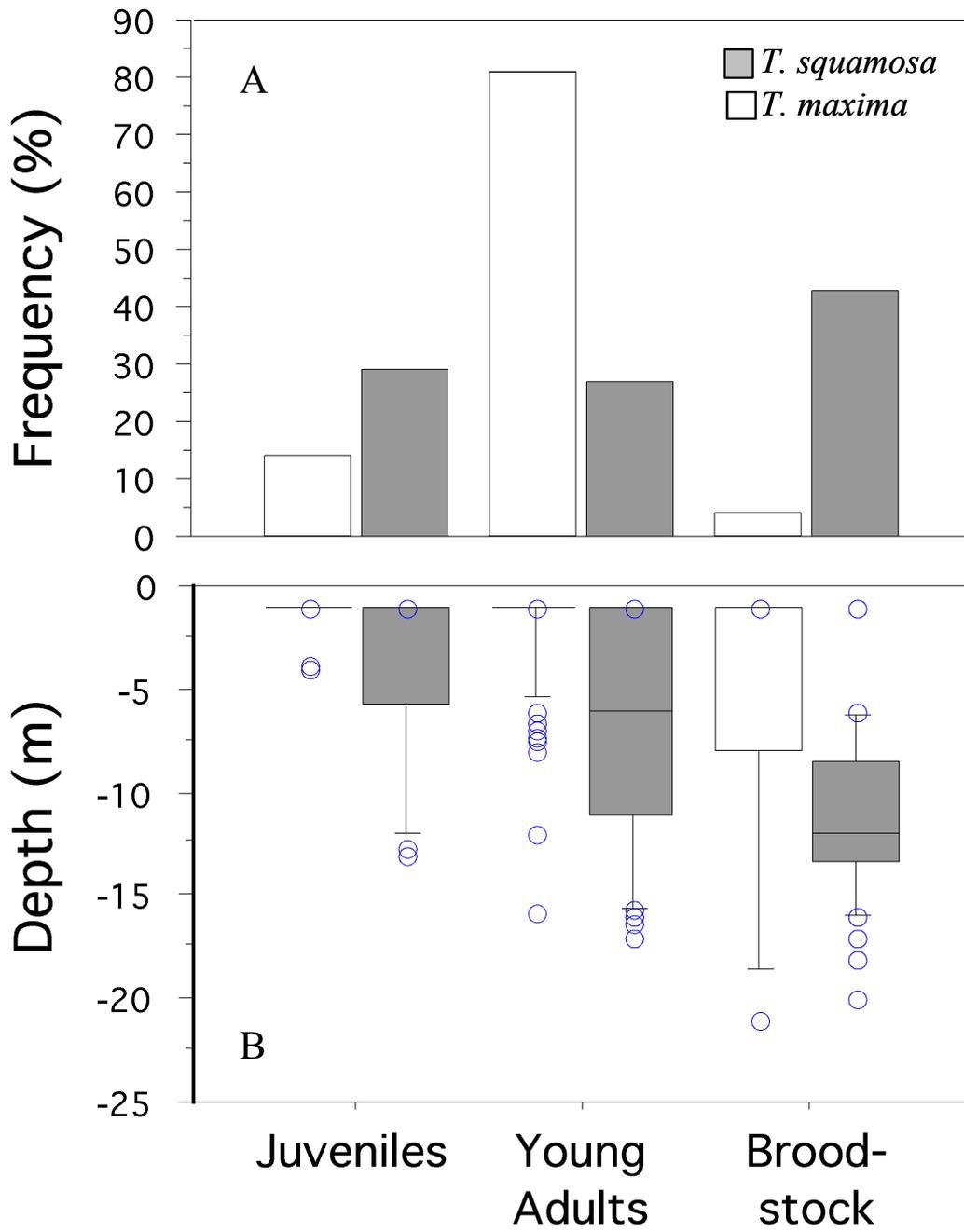


Figure 5. Percentage composition of the different age-group based on sexual maturity (A) and the distribution with depth (B) of *T. squamosa* and *T. maxima*.

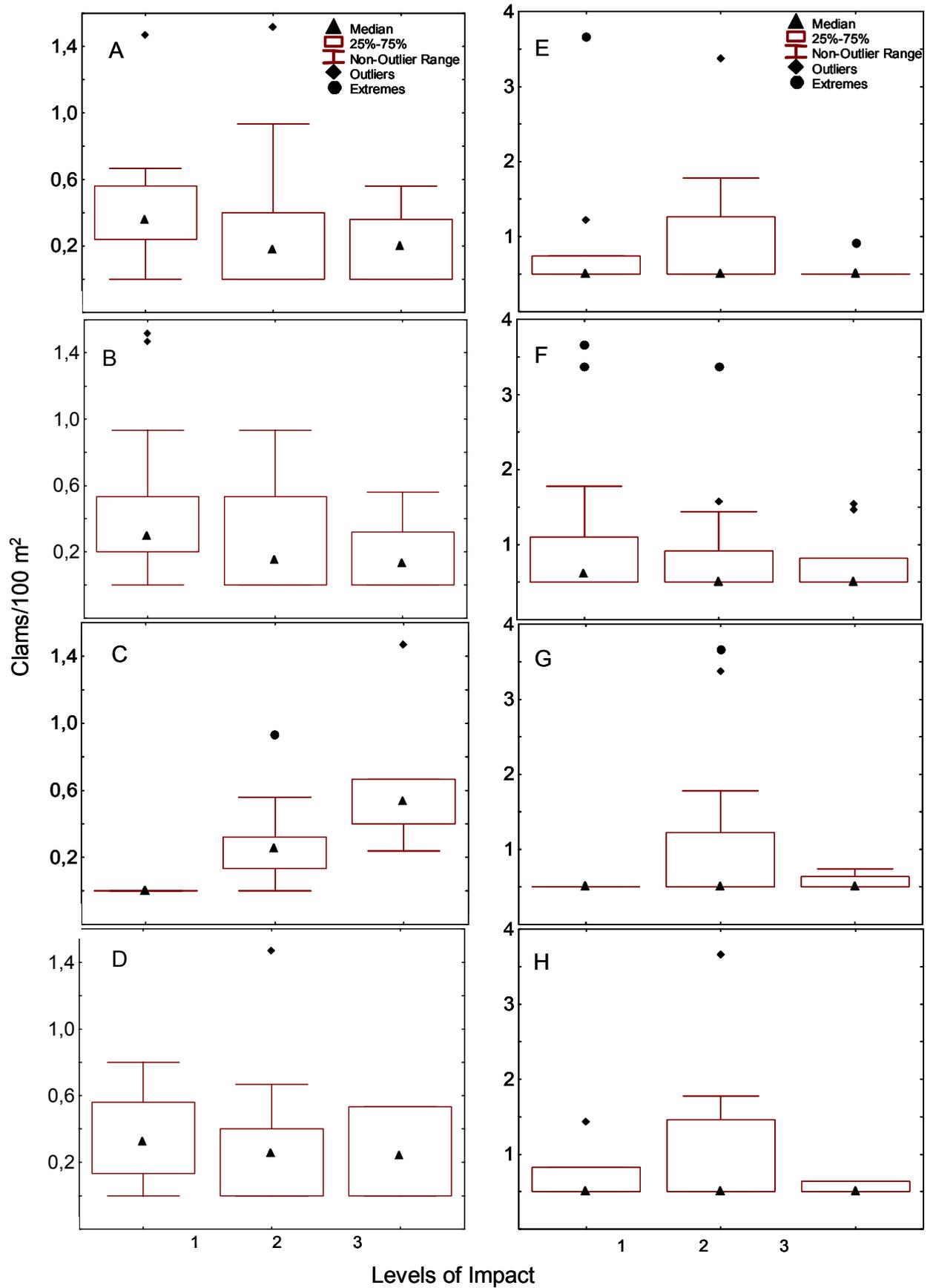


Figure 6. Abundance of *T. squamosa* (A-D) and *T. maxima* (E-H) in relation to human impact (A, E), coastal use (B, F), live coral cover (C, G) and fish predators (D, H).

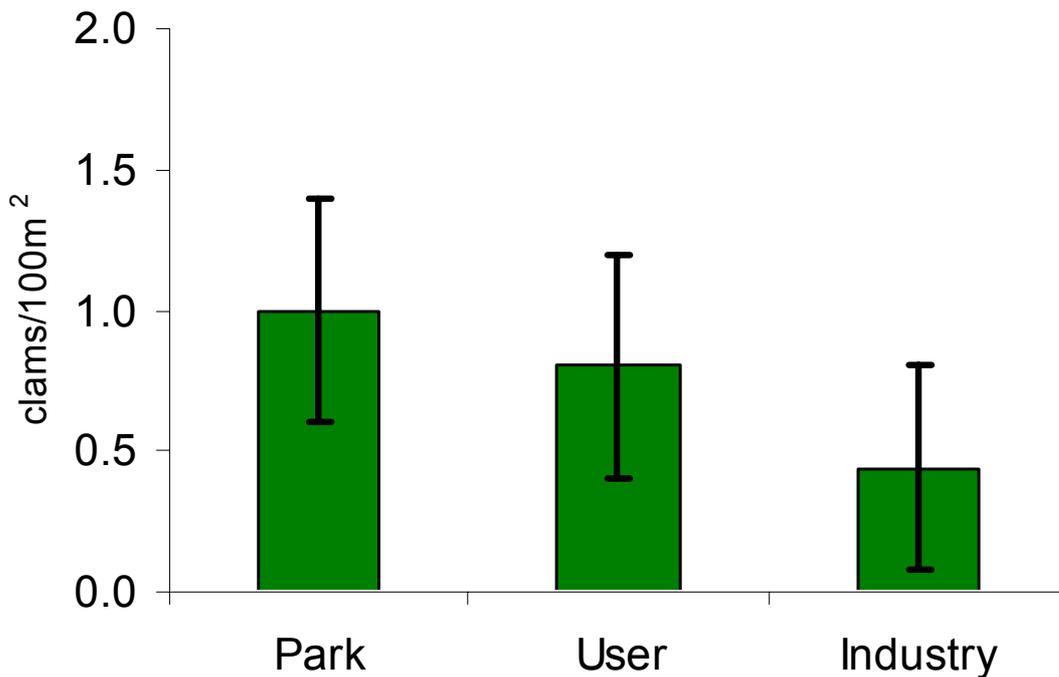


Figure 7. Mean clam abundance of both species (pooled) in different coastal zones in Jordan.

#### *Comparison to Red Sea populations*

We found a large spatial heterogeneity in clam abundances (both species, pooled data) in the Red Sea, which differed significantly ( $p = 0.05$ ) between countries, geographical areas and shore locations. Abundances increased 10-fold along the western shores from Israel southwards to the Sinai peninsula (Fig. 8), rebounding to moderate abundances along the Red Sea coast of Egypt, Sudan and Djibouti. On the eastern shores, abundances also increased from Jordan in the northern Gulf of Aqaba to Saudi Arabia in the Red Sea proper. The Yemeni coast along the Red Sea showed abundances similar to Jordan, however, the former had a higher maximum abundance (5.0 versus 3.5 ind. 100 m<sup>-2</sup>, respectively).

Egypt harbored the densest clam populations in the Red Sea reaching a maximum of ~80 ind. 100 m<sup>-2</sup> in the Sinai peninsula (see Fig. 8). This was followed by Saudi Arabia with abundances around 20 ind. 100 m<sup>-2</sup> in the northern reefs (Al-Wajh Bank), a lower peak (<10 ind.100 m<sup>-2</sup>) going south in Djibouti and Sudan and lowest values in Yemen (<5 ind.100 m<sup>-2</sup>). Abundances varied markedly along the long Saudi Arabian coastline, with higher mean values in the Central Red Sea (Al-Wajh,

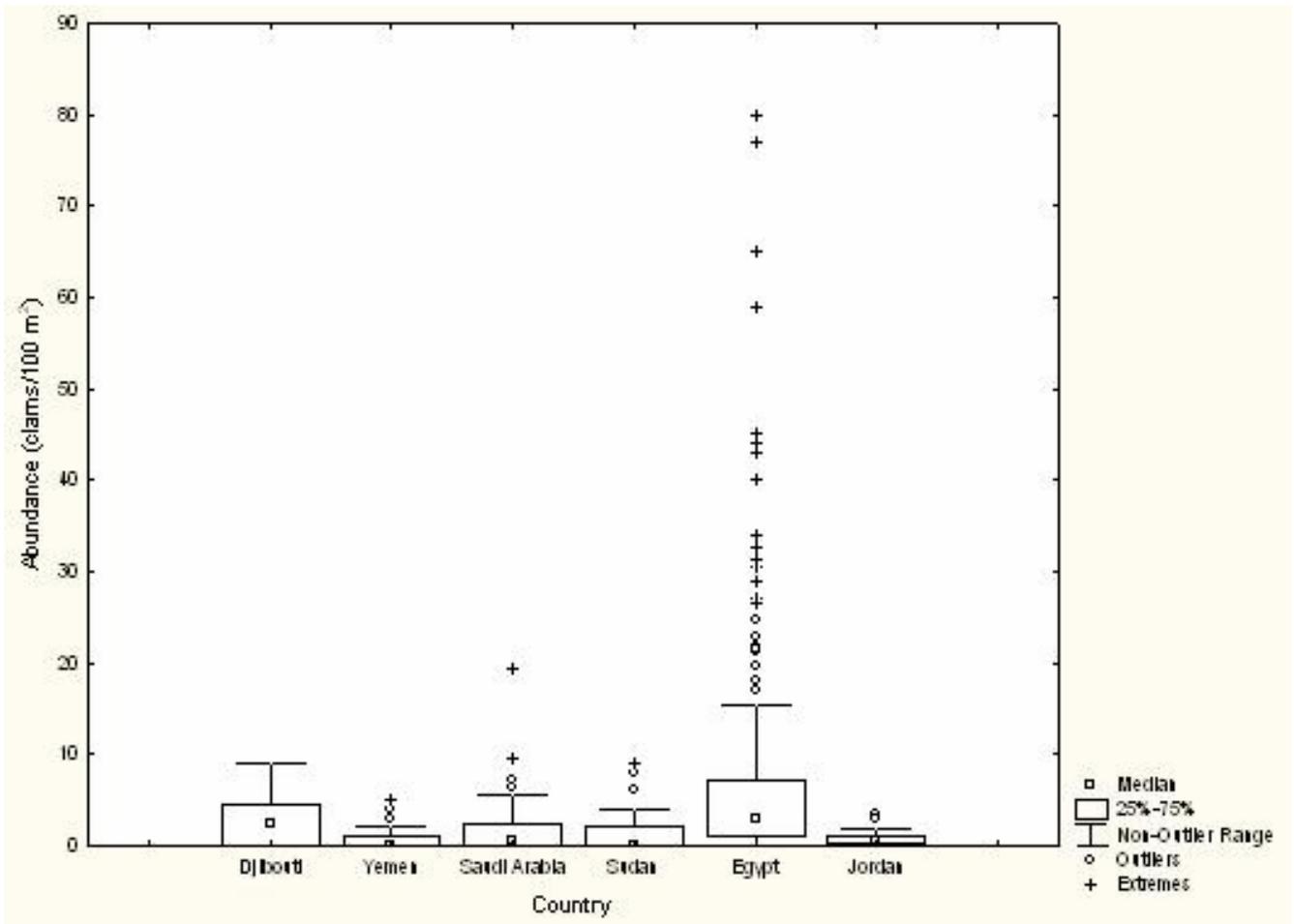


Figure 8. Clam abundance in the wider Red Sea.

$3.8 \pm 5.8$  ind.100 m<sup>-2</sup> and Jeddah,  $3.3 \pm 3.4$  ind.100 m<sup>-2</sup>) compared to Southern Red Sea (Farasan,  $0.2 \pm 0.3$  ind.100 m<sup>-2</sup>).

The influence of human impacts were significant in some countries in the Red Sea (Fig. 9) where higher abundances were found in areas of lower impacts in Yemen ( $r = 0.65$ ,  $p = 0.05$ ) and Djibouti ( $r = 0.70$ ,  $p = 0.05$ ). The visibility of the water column affected abundance significantly ( $r = 0.44$ ,  $p = 0.05$ ) as supported by high clam densities in clear than in turbid waters. Shelf width and live coral cover showed no significant correlation with clam abundance.

#### *Growth of cultured and wild stock clams in Jordan*

The estimated growth parameters for *T. squamosa* and *T. maxima* in Jordan as well as published values in the tropics were listed in Table 5. The wildstocks of *T. maxima* ( $\phi' = 4.2$ ) had significantly ( $p = 0.047$ ) higher growth performance index than *T. squamosa* ( $\phi' = 3.9$ ). However,

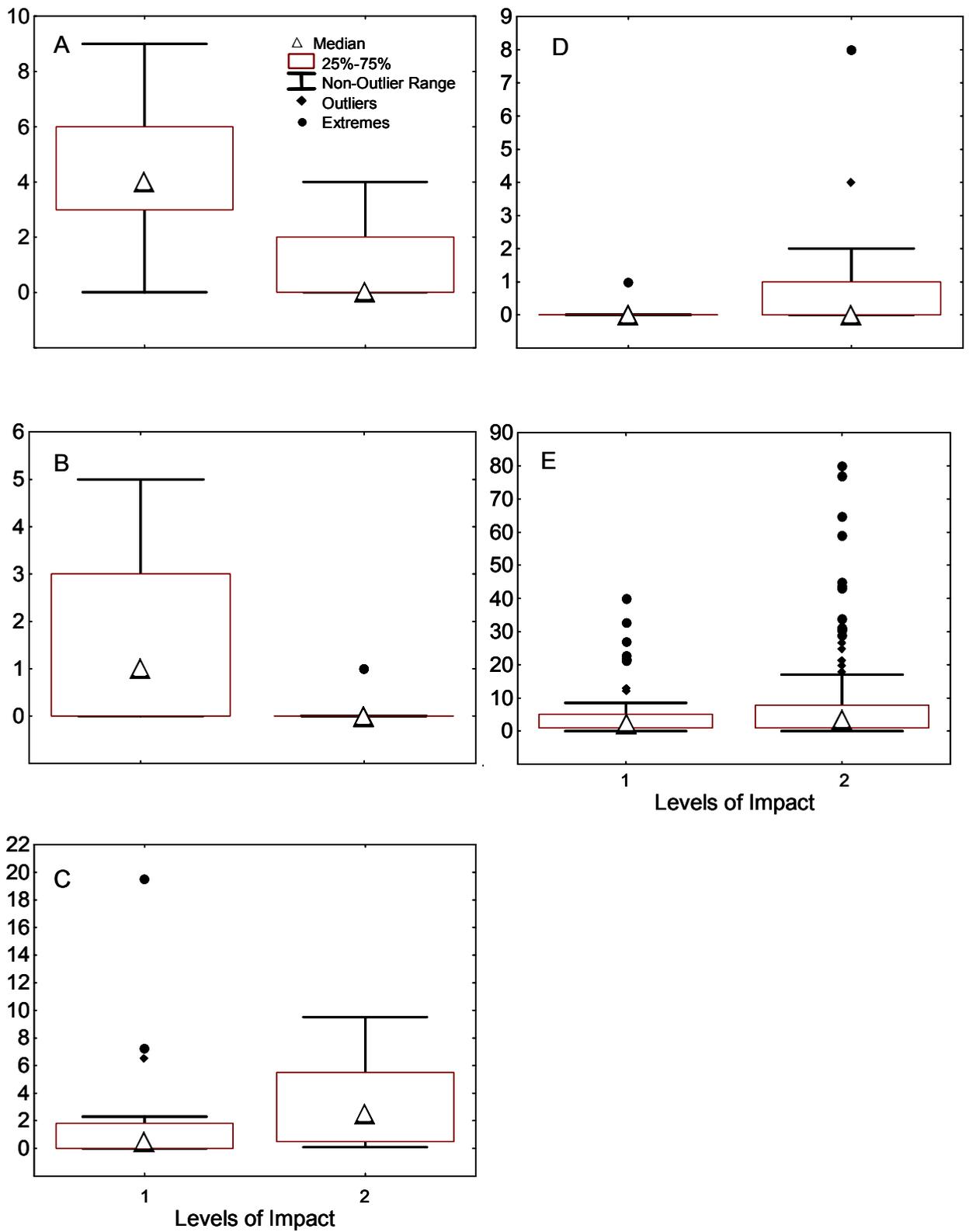


Figure 9. Abundance of both species (pooled) in relation to human impact in Djibouti (A), Yemen (B), Saudi Arabia (C), Sudan (D) and Egypt (E).

Table 5. Growth parameter estimates for *T. squamosa* and *T. maxima* from Jordan and from published values in the literature: phi-prime ( $\phi'$ ), growth constant (k) and maximum length ( $L_{\infty}$ ).

Country	Life stage	$\phi'$	K	$L_{\infty}$	References
<i>T. squamosa</i> wildstocks:					
Jordan	wildstock adults	3.9	0.06	37.0	this paper
Philippines	wildstocks	4.3	0.23	30.0	Villanoy et al 1988
Papua New Guinea	wildstocks	4.3	0.14	38.5	Munro & Heslinga 1983
Papua New Guinea	wildstocks	4.3	0.20	31.5	Vakily 1992, Munro & Gwyther 1981
Palau	wildstocks	4.2	0.09	40.0	Munro & Heslinga 1983
<i>T. squamosa</i> cultured stocks:					
Jordan	indoor juveniles	4.4	0.17	37.0	this paper
Jordan	outdoor juveniles	4.2	0.12	37.0	this paper
Jordan	mean_cultured juveniles	4.2	0.11	37.0	this paper
Thailand	fieldbased juveniles	3.6	0.02	40.0*	Adulyanukosol, 1997 (*fixed $L_{\infty}$ )
Thailand	landbased juveniles	3.3	0.01	40.0*	Adulyanukosol, 1997 (*fixed $L_{\infty}$ )
<i>T. maxima</i> wildstocks:					
Jordan	wildstock adults	4.2	0.18	29.1	this paper
Papua New Guinea	wildstocks	4.2	0.28	24.3	Vakily 1992, Munro & Gwyther 1981
Papua New Guinea	wildstocks	4.0	0.11	30.5	Munro & Heslinga 1983
Australia	wildstocks	4.0	0.27	19.5	Jameson 1978
Australia-GBR	wildstocks	3.7	0.07	27.5	Munro & Heslinga 1983
Tonga	wildstocks	3.9	0.08	30.5	Munro & Heslinga 1983
Cook Islands	wildstocks	3.6	0.10	19.7	Sims & Howard 1988
Cook Islands	wildstocks, reef flat	3.5	0.10	18.1	Sims & Howard 1988
Cook Islands	wildstocks, lagoon	3.7	0.10	23.2	Sims & Howard 1988
French Polynesia	wildstocks	3.6	0.26	12.4	Vakily 1992, Richard 1981

the growth rates for both species were not significantly different ( $p = 0.10$ ), *T. maxima* ( $0.67 \pm 0.77$  mm/mo) and *T. squamosa* ( $0.66 \pm 0.73$  mm/mo). The maximum shell lengths estimated from the asymptotic growth function ( $L_{\infty}$ ) were close to the actual maximum shell length recorded ( $L_{\max}$ ), both in *T. squamosa* ( $L_{\infty} = 37.0$  cm vs.  $L_{\max} = 36.0$  cm SL) and *T. maxima* ( $L_{\infty} = 29.1$  cm vs.  $L_{\max} = 28.4$  cm SL). For the cultured *T. squamosa* juveniles, growth performance index was  $\phi' = 4.2$  with a growth rate of  $3.0 \pm 1.27$  mm/month. The growth performance in the land-based rearing conditions was significantly ( $p = 0.001$ ) higher in the indoor setup than the outdoor setup,  $\phi' = 4.4$  vs. 4.2, respectively.

## Discussion

Our study extends the geographic range of the giant clam *Tridacna squamosa* from the Red Sea proper (Rosewater, 1965; Lucas, 1988; Braley, 1992) to the northernmost tip of the Gulf of Aqaba, confirming previous reports on the co-occurrence of *T. squamosa* and *T. maxima* in that area (Schuhmacher, 1973; Mergner and Schuhmacher, 1974).

However, present giant clam densities in Jordan are very low (1.0 ind. 100 m<sup>-2</sup>, both species pooled data, this study), an order of magnitude lower than the average densities in Jordan in the 1970's

(Mergner and Schuhmacher, 1974) and up to 40-150 times less than the values reported for *T. maxima* in south Sinai (10 - 160 ind. 100 m<sup>-2</sup>, Kilada et al., 1998) or Saudi Arabia (22 ind. 100 m<sup>-2</sup>, Bodoy, 1984). The abundance of giant clams (both species pooled) is lowest at its northwestern limit in Jordan, increases 4-fold towards the wider Red Sea (4.0 ind. 100 m<sup>-2</sup>, over-all mean for Red Sea), and seem to increase further into the Indian Ocean, western (28 ind. 100 m<sup>-2</sup>, Gomez and Alcala, 1988; Lucas, 1994) and to the eastern Pacific border where maximum abundances are found (260 ind. 100 m<sup>-2</sup>, Lucas, 1994).

The 12-fold decrease over three decades in Jordan, two-third higher abundances in restricted compared to open-access areas and the significant influence of coastal use and human impacts suggest a decline in clam abundance due to habitat loss and over-fishing. Collection of giant clams has been reported contributing to the reduction of natural stocks in Jordan (RSMPP, 2003) as elsewhere in the Red Sea-Indopacific region (Brown and Muskanofola, 1985; Juinio et al., 1987; Copland and Lucas, 1988; Lucas, 1994; Tuyen, 1994; Courtois de Vicoise and Chou, 1999; Bruckner, 2001; Ashworth et al., 2004). However, data on clam harvest in the Gulf of Aqaba are lacking. Coastal conversion has been rampant in Jordan over the last 40+ years, where 70% of the coastline were developed for ports, industries and tourism (Badran and Bashir, 2001), likely decimating giant clam stocks due to modification and loss of suitable reef habitats (Courtois de Vicoise and Chou, 1999; Epstein et al., 1999; Hassan et al., 2000; Zuschin et al., 2000). This, coupled with pollution from the cities of Aqaba, Eilat (Israel) and Taba (Egypt), may have caused considerable harm and high larval mortalities (Belda et al., 1993; Duquesne and Coll, 1995; Elfving et al., 2002; Blidberg, 2004; Blidberg and Tedengren, in press; Blidberg et al., in press), although the occurrence of small clams (marine park) indicates that recruitment is unabated, though probably at low levels.

Our data suggest that predation by fish plays a minor role in both species, in line with previous studies showing that fish predation is not a serious threat for giant clams in the field (Govan, 1992). The overall scarcity of large adult *T. maxima* broodstock is somewhat puzzling. Ruling out predation leaves us with mortality due to disease or parasitoid infection, but both field and laboratory observations indicate a higher susceptibility for infection and mortality in *T. squamosa* which is equally represented by individuals of all ages. Natural events including episodic extreme low tides have also been known to wipe out part or all of the populations on the reef flats (Fishelson, 1973; Loya, 1976) and may explain the truncated size distribution of the shallow water species *T. maxima*.

In the wider Red Sea, the impact of human activities have been found significant in Yemen where clam abundances are higher in unfished (Hassan et al., 2002) and protected areas (Sinha, 2004).

Likewise in Djibouti, abundances are lower in areas with coastal construction, dredging, land filling and shipping pressure (Hassan et al., 2002). Although, a significant relationship was not observed in the other Red Sea countries, presently, there is still an increasing demand from tourism, shipping, development and fishing (e.g., Saudi Arabia, Gladstone, 2002) putting heavy pressure on the resources and the need for trans-boundary management (Gladstone et al., 2003).

The distribution of giant clams as functional phototrophs may be also influenced significantly by water visibility (Schuhmacher, 1973) explaining the high abundances of *Tridacna* in the Northern and Central Red Sea where narrow shelves expose the reefs to clear oceanic waters (Pilcher and Abou Zaid, 2000; Richter and Abu-Hilal, 2004). Giant clams are scarcer along the Southern Red Sea and Yemen (Kamaran Island) where waters are turbid and visibility poor (Hassan et al., 2002), but abound in the offshore island of Socotra (Gulf of Aden) where waters are clearer.

Our abundance and size data from Jordan suggest different depth preferences between the two species: *T. maxima* of all ages are most abundant in the shallows (see Fig. 5). Virtually all of the juveniles and four fifths of the adult population were found on the reef top, underlining the close association of this species with reef flats (Zuschin and Piller, 1997). Only scattered specimens, including large broodstock adults were found in the deeper parts of the reef. *T. squamosa*, by contrast, showed a wider vertical range of distribution as well as a conspicuous zonation by age (Fig. 5): juveniles settled preferentially in the top 5 m, whereas older adults were found progressively deeper, median depths of occurrence extending from 6 m in young adults to 12 m in adult broodstock. Preliminary comparative data on photosynthetic performances suggest that *T. maxima* is indeed capable of exploiting higher levels of light (C. Jantzen, University of Munich, unpubl. data). Light-enhanced calcification may result in faster shell growth of *T. maxima* on reef flats, where ambient light levels are at their maximum, thus conferring a competitive advantage to this high-light specialist, particularly during the vulnerable young stages. It does not explain, however, the paucity of *T. maxima* in deeper waters.

In fact, both species, appear to fare equally well at the lower range of light intensities, as shown by similar light compensation depths (C. Jantzen, University of Munich, unpubl. data) and growth performances (Munro and Gwyther, 1981; Munro and Heslinga, 1983; Vakily, 1992), the latter of which are similar to the values we obtained for juvenile *T. squamosa* under low/moderate light levels.

Giant clams are mixotrophic, capable of growing as 'solar animals' (Braley, 1992) through the photosynthate translocated from the zooxanthellae living in their tissues, or alternatively by living on plankton and other organic material acquired by both, filter-feeding and dissolved uptake from

the surrounding water (Hawkins and Klumpp, 1995) or through digestion of the zooxanthellae (Maruyama and Heslinga, 1997).

A critical factor in the mode of nutrition appears to be the age of the animal (Fitt et al., 1993): Heterotrophy is particularly important during the early life stages, whereas zooxanthellar photosynthesis increases in importance with increasing size of the animal. Filter feeding on particles between 2 and 50 $\mu$ m is able to provide 65% of the total carbon needed both for respiration and growth in small clams (100 mg dry tissue wt), whereas large clams (10 g) acquire only 34% of their carbon from this source (Klumpp et al., 1992).

Young specimens thus appear to exploit preferentially ambient fluxes of plankton, while older clams take advantage of ambient fluxes of nutrients. In the Red Sea, the cross-shore circulation carries plankton-rich surface water into the reef (Niemann et al., 2004, Genin et al., 2005), where it is intercepted by a diverse assemblage of coral reef planktivores (Richter et al., 2001; Yahel et al., 2005). The organic material harnessed by the reef biota is remineralized in the coral reef framework (Richter et al., 2001) and sediments (Rasheed et al., 2002, Wild et al., 2004). The nutrient-enriched near-bottom waters are carried downslope and offshore (Richter et al., 2001).

We can thus assume that the first settlement and growth of young clam, particularly *T. maxima* is facilitated in shallow water by *mixotrophy* under the combined high fluxes of plankton and light. In contrast, adult broodstock may thrive better in less saturating light conditions somewhat deeper in the water column where nutrient levels are high enough to support maximum *phototrophic* growth. The scales of *T. squamosa* or “fluted” clam may support exposing the photosynthetic mantle in sub-saturating light environments, providing a competitive advantage over the less ornamented *T. maxima* in deep waters.

Our first estimates of the growth performance indices in the subtropical waters of the Red Sea in the northern Gulf of Aqaba, *T. squamosa* ( $\phi' = 3.9$ ) and *T. maxima* ( $\phi' = 4.2$ ), showed values within range for tridacnid species (Vakily, 1992). These two species are medium-sized tridacnids that have lower growth index than fast-growing species, *T. derasa* ( $\phi' = 4.4 - 4.5$ ) and *T. gigas* ( $\phi' = 4.7 - 4.9$ ) (Munro, 1988; Vakily, 1992). The general trend among widely distributed bivalves is the linear increase in growth index and size with water temperature (Vakily, 1992) due to the higher metabolic costs of shell deposition in the low water temperature in the higher latitudes (Clarke, 1983). Both Red Sea giant clam species are not completely consistent with this trend. *T. squamosa* (see Table 5) has a lower growth index but similar asymptotic size compared to those in the Indo-Pacific (Munro and Gwyther, 1981; Munro and Heslinga, 1983; Villanoy et al., 1988; Vakily, 1992). On the other hand, *T. maxima* has a similar growth index and asymptotic size to those in the

tropics, whilst, both parameters are decreasing towards the tropical southeastern Pacific border (Jameson, 1978; Munro and Gwyther, 1981; Richard, 1981; Munro and Heslinga, 1983; Sims and Howards, 1988; Vakily, 1992). The growth performance indices and growth rates in Jordan for wildstocks are similar to the tropics and the cultured juveniles are at par with tropical field-grown clams (Hardy and Hardy, 1969; Belda and Gomez, 1988; Solis et al., 1988; Klumpp and Griffiths, 1994; Bell et al., 1997). The higher maximum length increment for cultured *T. squamosa* juveniles compared to those in the tropics (4.9 mm/mo. vs 4.5 mm/mo., respectively, Lucas, 1994) shows a high potential for growth in land-based tanks in Jordan (Adulyanukosol, 1997), especially if light and water temperature are constant.

The giant clams in the northern Gulf of Aqaba are at the margin of their northwestern distribution, exposed to different and perhaps harsher conditions than in the tropics, e.g., temperature (Gomez and Mingoa-Licuanan, 1998; Blidberg et al., 2000) and salinity (Blidberg, 1999). For instance, reproductive success in Jordan is short and limited (June-August) and larval development is protracted in winter (Roa-Quiaoit et al., in prep.). In the absence of rivers, nutrients in the euphotic layer of the Gulf of Aqaba are replenished only seasonally, leading to alternate states of stratification and nutrient depletion in summer; and mixing, nutrient and chlorophyll saturation in winter (Rasheed et al., 2002). Perhaps, a bigger size and lower number in the subtropical border is a 'K-strategy,' which has been suggested for clams by Yamaguchi (1977), of compensating for the stressful conditions that inhibit growth and reproduction. *T. maxima*, in particular, appears to have higher average sizes and minimal densities in its northwestern subtropical limit; while in the tropical southeastern limit, the clams are of smaller average sizes but at maximum densities than in other areas in eastern Polynesia and the Pacific, e.g., 70 ind. m<sup>-2</sup> (Richard, 1981; Sims and Howard, 1988; Lewis et al., 1988).

The very low abundance of the two Red Sea species at the subtropical northwestern border of its distribution as compared with those elsewhere in the tropics suggests the strong influence of temperature in the distribution and abundance of these giant clams in its geographical range. However, our first estimates on the growth performance and size of the Red Sea populations showed inconsistent patterns with other bivalves which seemed to suggest the controlling influence of factors other than temperature.

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## Literature Cited

- Adulyanukosol, D. 1997. Growth of giant clams *Tridacna squamosa* Lamarck under laboratory and natural conditions. Proc. 7<sup>th</sup> Workshop of the Tropical Marine Mollusc Programme (TMMP) on Central and West Java, Indonesia, 11-22 November 1996. Part 1. Vol. 17, no. 1, pp. 269-274. Spec. Publ. Phuket Mar. Biol. Cent.
- Ashworth, J, R. Ormond and H. Sturrock. 2004. Effects of reef-top gathering and fishing on invertebrate abundance across take and no-take zones. J. Exp. Mar. Biol. Ecol. 303 (2):221-242.
- Badran, M and B. Bashir. 2001. Ecotourism, the way out of a pressing investment dilemma on the Jordanian coast of the Gulf of Aqaba. Scientific Committee on Problems of the Environment-SCOPE XIth General Assembly and Scientific Symposia, September 24-28, 2001, Bremen, Germany.
- Beckvar, N. 1981. Cultivation, spawning and growth of the giant clams *Tridacna gigas*, *Tridacna derasa* and *Tridacna squamosa* in Palau, Caroline Islands. Aquacult. 24:21-30.
- Belda, C. and E. Gomez. 1988. Growth of giant clams in Bolinao, Pangasinan. Pages 178-182 in J. Copland and J. Lucas, eds. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9, Canberra.
- \_\_\_\_\_, E. Cuff and D. Yellowlees. 1993. Modification of shell formation in the giant clam *Tridacna gigas* at elevated nutrient levels in sea water. Mar. Biol. 117:251-257.
- Bell, J., I. Lane, M. Gervis, S. Soule and H. Tafea. 1997. Village-based farming of the giant clam, *Tridacna gigas* (L.), for the aquarium market: Initial trials in Solomon Islands. Aquacult. Res. 28 (2):121-128.
- Blidberg, E. 1999. Physiological responses of the fluted giant clam, *Tridacna squamosa*, exposed to decreased irradiance and reduced salinity. Proc. 9<sup>th</sup> Workshop of the Tropical Marine Mollusc Programme (TMMP), Indonesia, 19-29 August 1998. Part 1. Vol. 19, no. 1, pp. 85-91. Spec. Publ. Phuket Mar. Biol. Cent.
- \_\_\_\_\_. 2004. Effects of copper and decreased salinity on survival rate and development of *Tridacna gigas* larvae. Mar. Environ. Res. 58:793-797.
- \_\_\_\_\_. and M. Tedengren. In press. Survival and development in giant clam larvae (*Tridacna gigas*) exposed to benzo[a]pyrene. Submitted to Marine Pollution Bulletin.
- \_\_\_\_\_. T. Elfwing, P. Plantman and M. Tedengren. 2000. Water temperature influences on physiological behaviour in three species of giant clams (Tridacnidae). Proc. 9<sup>th</sup> Int. Coral Reef Symp. 1:561-565.
- \_\_\_\_\_. M. Tedengren and T. Elfwing. In press. Effects of petroleum on bioenergetics and DNA integrity in the giant clam *Hippopus hippopus*. Submitted to Aquatic Toxicology.
- Bodoy, A. 1984. Assessment of human impact on giant clams (*Tridacna maxima*) near Jeddah, Saudi Arabia. Proc. Symp. Coral Reef Environ. Red Sea, Jeddah, Jan. 1984, pp. 472-490.

- Braley, R. 1992. The giant clam: hatchery and nursery culture manual. ACIAR Monograph No. 15, Canberra. 144 p.
- Brown, J. and M. Muskanofola. 1985. An investigation of stocks of giant clams (Family Tridacnidae) in Java and of their utilization and potential. *Aquacult. Fish. Manage.* 16(1):25-39.
- Bruckner, A. 2001. Tracking the trade in ornamental coral reef organisms: The importance of CITES and its limitations. *Aquarium Sci. Conserv.* 3(1-3):79-94.
- Calumpong, H. 1992. The giant clam: an ocean culture manual. ACIAR Monograph No. 16, Canberra. 68 p.
- Clarke, A. 1983. Life in cold water: the physiological ecology of polar marine ectotherms. *Oceanogr. Mar. Biol. Ann. Rev.* 21:341-453.
- Copland, J. and J. Lucas. 1988. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9, Canberra. 274 p.
- Courtois de Vicoise, G. and L. Chou. 1999. Future of giant clam mariculture in Singapore: Problems and potential solutions. Proceedings of an International Workshop on the Rehabilitation of Degraded Coastal Systems, 19-24 Jan. 1988, No. 20, pp. 119-122. Spec. Publ. Phuket Mar. Biol. Cent.
- Duquesne, S. and J. Coll. 1995. Metal accumulation in the clam *Tridacna crocea* under natural and experimental conditions. *Aquat. Toxicol.* 32:239-253.
- Elfwing, T, E. Blidberg and M. Tedengren. 2002. Physiological responses to copper in giant clams: a comparison of two methods in revealing effects on photosynthesis in zooxanthellae. *Mar. Environ. Res.* 54:147-155.
- English, E. C. Wilkinson and V. Baker. 1994. Survey manual for tropical marine resources. Australian Institute of Marine Science, Townsville. 390 p.
- Epstein, N, R. Bak and B. Rinkevich. 1999. Implementation of a small-scale 'no-use zone' policy in a reef ecosystem: Eilat's reef-lagoon six years later. *Coral Reefs* 188 (4):327-332.
- Fishelson, L. 1973. Ecological and biological phenomenon influencing coral-species composition on the reef tables at Eilat (Gulf of Aqaba, Red Sea). *Mar. Biol.* 19:183-196.
- Fisher, C., W. Fitt, and R. Trench. 1985. Photosynthesis and respiration in *Tridacna gigas* as a function of irradiance and size. *Biol. Bull.* 169:230-245.
- Fitt, W., T. Rees, R. Braley, J. Lucas and D. Yellowlees. 1993. Nitrogen flux in giant clams: size-dependency and relationship to zooxanthellae density and clam biomass in the uptake of dissolved inorganic nitrogen. *Mar. Biol.* 117:381-386.
- Gayanilo, S. and D. Pauly. 1992. FAO-ICLARM Fish Stock Assessment Tools (FiSAT). Rome, Italy.
- Genin, A., J. Jaffe, R. Reef, C. Richter and P. Franks. 2005. Swimming against the flow: a mechanism of zooplankton aggregation. *Science (Wash.)*. 308(5723): 860-862.
- Gladstone, W. 2002. Fisheries of the Farasan Islands (Red Sea). *Naga* 25(3-4):30-34.

- Gladstone, W., F. Krupp and M. Younis. 2003. Development and management of a network of marine protected areas in the Red Sea and Gulf of Aden region. *Ocean Coast. Manage.* 46 (8):741-761.
- Gomez, E. and A. Alcalá. 1988. Giant clams in the Philippines. Pages 51-53 in J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- \_\_\_\_\_ and S. Mingo-Licuanan. 1998. Mortalities of giant clams associated with unusually high temperatures and coral bleaching. *Reef Encounter* 24.
- Gomez, E., H.A. Roa and M.A. Juinio. 1994. Assessment of *Tridacna crocea* in an exploited Philippine reef. In: L. Chou, ed. *The Third Asian Fisheries Forum*. Asian Fisheries Society, Manila, Philippines.
- Govan, H. 1992. Predators and Predator Control. Pages 41-49 in N. Calumpong, ed. *The Giant Clam: An Ocean Culture Manual*. Australian Centre for International Agricultural Research (ACIAR) Monograph 16, Canberra.
- Hassan, M., M. Kotb and A. Al-Sofyani. 2002. Status of coral reefs in the Red Sea-Gulf of Aden. Pages 45-52 in C. Wilkinson, ed. *Status of coral reefs of the world: 2000*, Australian Institute of Marine Science, Townsville.
- Hawkins, A. and D. Klumpp. 1995. Nutrition of the giant clam *Tridacna gigas* (L.). 2. Relative contributions of filter-feeding and the ammonium-nitrogen acquired and recycled by symbiotic alga towards total nitrogen requirements for tissue growth and metabolism. *J. Exp. Mar. Biol. Ecol.* 190:263-290.
- Heslinga, G., F. Perron and O. Orak. 1984. Mass culture of giant clams (F. Tridacnidae) in Palau. *Aquacult.* 39:197-215.
- Hilton-Taylor, C. 2000. 2000 IUCN Red List of Threatened Species. Gland, Switzerland and Cambridge, UK:IUCN.
- Hodgson, G., W. Kiene, J. Mihaly, J. Liebeler, C. Shuman and L. Maun. 2004. Reef Check Instruction Manual: A guide to Reef Check coral reef monitoring. Reef Check, Institute of the Environment, University of California, Los Angeles.
- Hulings, N. 1989. A review of marine science research in the Gulf of Aqaba. *Publications of the Marine Science Station Aqaba, Jordan*, Vol. 6, 267 p.
- Jameson, S. 1978. Growth of the small giant clam *Tridacna maxima* (Roding). Contribution 00, Sea Grant Coherent Project (04 - 4 158-4) University of Guam, 7 p.
- Jantzen, C. 2005. Photosynthetic performance of giant clams (*Tridacna squamosa* and *T. maxima*) in the Gulf of Aqaba, Red Sea. Diploma Thesis, University of Munich, Munich, Germany
- Juinio, A., R. Menez and C. Villanoy. 1987. Use of giant clam resources in the Philippines. *Naga* 10:7-8.
- \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_ and E. Gomez. 1989. Status of giant clam resources of the Philippines. *J. Moll. Stud.* 55:431-440.
- Khalaf, M. and M. Kochzius. 2002. Changes in trophic community structure of shore fishes at an industrial site in the Gulf of Aqaba, Red Sea. *Mar. Ecol. Prog. Ser.* 239:287-299.

- Kilada, R., S. Zakaria and M. Farghalli. 1998. Distribution and abundance of the giant clam *Tridacna maxima* (Bivalvia: Tridacnidae) in the northern Red Sea. Bull. Nat. Inst. of Oceanogr. and Fish., A.R.E. 24:221-240.
- Klumpp, D. and C. Griffiths. 1994. Contributions of phototrophic and heterotrophic nutrition to the metabolic and growth requirements of four species of giant clam (Tridacnidae). Mar. Ecol. Prog. Ser. 115:103-115.
- \_\_\_\_\_ and J. Lucas. 1994. Nutritional ecology of the giant clams *Tridacna tevoroa* and *T. derasa* from Tonga: influence of light on filter-feeding and photosynthesis. Mar. Ecol. Prog. Ser. 107:147-156.
- \_\_\_\_\_, B. Bayne and A. Hawkins. 1992. Nutrition of the giant clam *Tridacna gigas*(L.). I. respiration and growth. J. Exp. Mar. Biol. Ecol. 155:105-122
- Knop, D. 2004. Giant Clam Buyer's Guide. Coral - The Marine Aquarium Magazine 1(3):36. Ecosystem Publications, California 92705, USA.
- Loya, 1976. Recolonisation of Red Sea coral reefs affected by natural catastrophes and man-made perturbations. Ecol. 57:278-289.
- Lucas, J. 1988. Giant clam description, distribution and life history. Pages 21-33 in J. Copland and J. Lucas, eds. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9, Canberra.
- Lucas, J. 1994. The biology, exploitation, and mariculture of giant clams (Tridacnidae). Rev. Fish. Sci. 2(3):181-223.
- Lewis, A., T. Adams and E. Ledua. 1988. Fiji's giant clam stocks – A review of their distribution, abundance, exploitation and management. Pages 66-72 in J. Copland and J. Lucas, eds. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9, Canberra.
- Manu, N. and S. Sone. 1995. Breeding season of the Tongan shellfish. 3. Elongated giant clam (kukukuku), *Tridacna maxima*. Fish. Res Bull Tonga 3:25-33.
- Maruyama, T. and G. Heslinga. 1997. Fecal discharge of zooxanthellae in the giant clam *Tridacna derasa*, with reference to their in situ growth rate. Mar. Biol. 127:473-477.
- Mergner, H. and Schuhmacher H. 1974. Morphologie, Okologie und Zonierung von Korallenriffen bei Aqaba, (Golf von Aqaba, Rotes Meer). Helgolander Wiss. Meeresunters. 26: 238-358.
- Moghrabi, S., M. Al-Zibdah, A. Abelson, N. Chadwick-Furman and Y. Loya. 2003. Coral Reef Monitoring Team. The Red Sea Marine Peace Park (RSMPP) Cooperative Research, Monitoring and Management Program Final Report, Phase 1, pp. 55-63.
- Munro, J. 1982. Estimation of the parameters of the von Bertalanffy growth equation from recapture data at variable time intervals. J. Cons. Int. Explor. Mer. 40:199-200.
- \_\_\_\_\_ 1988. Growth, mortality and potential aquaculture production of *Tridacna gigas* and *T. derasa*. Pages 218-220 in J. Copland and J. Lucas, eds. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9, Canberra.
- \_\_\_\_\_ and J. Gwyther. 1981. Growth rates and maricultural potential of tridacnid clams. Proc. 4<sup>th</sup> Int. Coral Reef Symp. 2: 633-636.

- \_\_\_\_\_ and G. Heslinga. 1983. Prospects for the commercial cultivation of giant clams (Bivalvia: Tridacnidae). *Proc. Gulf Caribb. Fish. Inst.* 35:122-134.
- Murakoshi, M. 1986. Farming of the boring clam, *Tridacna crocea* Lamarck. *Galaxea* 5: 239-254.
- Niemann, H., C. Richter, H. Jonkers and M. Badran. 2004. Red Sea gravity currents cascade near-reef phytoplankton to the twilight zone. *Mar. Ecol. Prog. Ser.* 269:91-99.
- Pandolfi, J., R. Bradbury, E. Sala, T. Hughes, K. Bjorndal, R. Cooke, D. McArdle, L. McClenachan, M. Newman, G. Paredes, R. Warner and J. Jackson. 2003. Global trajectories of the long-term decline of coral reef ecosystems. *Science Wash.* 301 (5635):955-958.
- Pauly, D. and J. Munro. 1984. Once more on the comparison of growth of fish and invertebrates. *Fishbyte* 2(1): 21.
- Pilcher, N. and M. Abou Zaid. 2000. The status of coral reefs in Egypt – 2000. Pages 178-182 *in* C. Wilkinson, ed. *Status of coral reefs of the world: 2000*. Australian Institute of Marine Science, Townsville.
- \_\_\_\_\_ and A. Alsuhaibany. 2000. Regional status of coral reefs in the Red Sea and the Gulf of Aden. Pages 35-54 *in* C. Wilkinson, ed. *Status of coral reefs of the world: 2000*. Australian Institute of Marine Science, Townsville.
- Rasheed, M., M. Badran, C. Richter and M. Huettel. 2002. Effect of reef framework and bottom sediment on nutrient enrichment in a coral reef of the Gulf of Aqaba, Red Sea. *Mar. Ecol. Prog. Ser.* 239:277-285.
- Richard, G. 1981. A first evaluation of the findings on the growth and production of lagoon and reef molluscs in French Polynesia. *Proc. 4<sup>th</sup> Int. Coral Reef Congress* 2:637-641.
- Richter, C. and M. Wunsch. 1999. Cavity-dwelling suspension feeders in coral reefs – a new link in reef trophodynamics. *Mar. Ecol. Prog. Ser.* 188:105-116.
- \_\_\_\_\_ and A. Abu-Hilal. 2004. Seas of the Arabian Region. Pages 1371-1410 *in* A. Robinson and K. Brink, eds. *The Sea*, Vol. 14, President and Fellows of Harvard College, USA.
- \_\_\_\_\_, M. Wunsch., M. Rasheed, I. Koetter and M. Badran. 2001. Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity dwelling sponges. *Nature* 413: 726-730.
- Roa-Quiaoit, H., C. Richter and M. Al-Zibdeh. 2004. Towards a sustainable aquaculture of giant clams (Tridacnidae) in the Jordanian sector of the Gulf of Aqaba. Presented at the 6<sup>th</sup> International Aquarium Congress, Monterey CA USA, Dec. 5-10, 2004.
- Roa-Quiaoit, H., M. Al-Zibdeh and C. Richter. In prep. Reproductive periodicity of giant clams (Tridacnidae) in the northern Gulf of Aqaba, Red Sea.
- Rosewater, J. 1965. The Family Tridacnidae in the Indo-Pacific. *Indo-Pacific Mollusca* 1(6):347-395.
- Schuhmacher, H. 1973. The light-dependent colonization of pillars by sessile animals and algae from the coral reef at Eilat (Red Sea). *Helgolander Wiss. Meeresunters* 24 (1-4):307-326.

- Shang, Y., L. PingSun and J. Brown. 1994. Test marketing of giant clams for food in Honolulu, Guam and Saipan and observations in Okinawa and Taiwan. Pages 69-87 in Economics of commercial giant clam mariculture.
- Sims, N. and N. Howard. 1988. Indigenous tridacnid clam populations and introduction of *Tridacna derasa* in the Cook Islands. Pages 34-40 in J. Copland and J. Lucas, eds. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9, Canberra.
- Sinha, P. 2004. Coastal resource management in Yemen – Problems and prospects. J. Indian Ocean Stud. 12 (1):103-113.
- Solis, E., J. Onate and M. Naguit. 1988. Growth of laboratory-reared giant clams under natural and laboratory conditions. Pages 201-206 in J. Copland and J. Lucas, eds. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9, Canberra.
- Tuyen, H. 1999. Checklist of marine molluscs of Con Dao National Park, Vietnam. Proc. 9<sup>th</sup> Workshop of the Tropical Marine Mollusc Programme (TMMP), Indonesia, 19-29 August 1998. Part 2. Vol. 19, no. 2, pp. 403-406. Spec. Publ. Phukrt Mar. Biol. Cent.
- UNEP/IUCN. 1988. Coral Reefs of the world. Pages 145-151 in UNEP Regional Seas Directories and Bibliographies, IUCN, Gland, Switzerland and Cambridge, UK, and UNEP, Nairobi.
- Urban, H. 2002. Modeling growth of different developmental stages in bivalves. Mar. Ecol. Prog. Ser. 238:109-114.
- Vakily, J. 1992. Determination and comparison of bivalve growth, with emphasis on Thailand and other tropical areas. ICLARM Tech. Rep. 36, 125 p.
- Villanoy, C., A. Juinio and L. Menez. 1988. Fishing mortality rates of giant clams (Family Tridacnidae) from the Sulu Archipelago and Southern Palawan, Philippines. Coral Reefs 7:1-5.
- Wells, S., R. Pyle and N. Collins. 1983. Giant clams. Pages 97-107 in The IUCN Invertebrate Red Data Book, Gland, Switzerland.
- Wild, C. M. Rasheed, U. Werner, U. Franke, R. Johnstone and M. Huettel. 2004. Degradation and mineralization of coral mucus in reef environments. Mar. Ecol. Prog. Ser. 267:159-171.
- Yamaguchi, M. 1977. Conservation and cultivation of giant clams in the tropical Pacific. Biol. Conserv. 11:13-20.
- Yahel, G., J. Sharp, D. Marie, C. Haese and A. Genin. 2005. In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon.
- Zuschin, M. and W. Piller. 1997. Molluscan hard-substrates associations in the northern Red Sea. Mar. Ecol. 18(4):361-378.
- Zuschin, M., J. Hohenegger and F. Steininger. 2000. A comparison of living and dead molluscs on coral reef associated hard substrata in the northern Red Sea- implications for the fossil record. Palaeogeogr., Palaeoclimatol., Palaeoecol. 159(1-2):167-190.

### Chapter 3: Reproductive periodicity of giant clams (Tridacnidae) in the northern Gulf of Aqaba, Red Sea



Figure 10. The *in situ* biopsy of the gonad using biopsy needle (left) and plastic syringe (right).

## Abstract

The reproduction of giant clams was studied in the Jordanian sector of the Gulf of Aqaba, Red Sea, the northwestern border of its distribution. *In situ* gonad biopsy (16 months) and pilot culture (~2 years) data on gonad maturity, egg stages and egg sizes showed a pronounced seasonality with species-specific summer spawnings in this subtropical marginal sea. *T. squamosa* Lamarck, 1819 featured an extended spawning season (Jun-Nov) with two spawning peaks, indicated by spent gonads (30%), extremely low gonad state indices (1-2) and high frequencies of regressive (80%) and spawned eggs (20%). *T. maxima* Roding, 1798, by contrast, showed a shorter unimodal spawning season (Jun-Sep) shown by low gonad state indices (1-2) and high frequencies of spent gonads (50-80%). The gonad state indices in both species peaked in June concomitant with the build-up of mature eggs in *T. squamosa* ( $r = 0.59$ ) and *T. maxima* ( $r = 0.53$ ) at the height of the growing season, with maximum ova diameters of  $94 \pm 13 \mu\text{m}$  and  $95 \pm 9 \mu\text{m}$ , respectively. Mean egg sizes of *T. squamosa* ( $89 \pm 10 \mu\text{m}$ ) and *T. maxima* ( $87 \pm 9 \mu\text{m}$ ) were smaller in the Red Sea than in the tropical Indo-Pacific. Reproductive success was highest in summer (Jun-Sep), with protracted larval development and heavy mortality in winter, suggesting that both temperature and food control the reproductive success of giant clams at high latitudes. Adaptations of existing clam culture techniques to Red Sea conditions are discussed, using combined heat- and serotonin-induced spawnings and larval to juvenile land-based rearing.

## Introduction

Giant clams are the largest of all shelled marine organisms. Their meat is a traditional food source in the South Pacific and the adductor muscles are highly prized delicacies in Japan, China, Honolulu and Guam (Murakoshi, 1986; Shang et al., 1994). The shells are used for souvenirs, jewelries, ornamental objects, tiles and construction materials (Brown and Muskanofola, 1985; Juinio et al., 1987). Live giant clams are highly in demand in the growing billion-dollar tropical marine aquarium industry in Europe and North America (Bell et al., 1997; Bruckner, 2001; Knop, 2004; Lindsay et al., 2004). The over-exploitation and habitat loss of this valuable resource has led to the severe depletion throughout its geographic range and ecological extinction (Pandolfi et al., 2003) of the larger species in some areas (Lucas, 1988; Juinio et al., 1989). The commercial trade of wild giant clams is prohibited under the Convention of International Trade of Endangered Species (CITES) (Wells et al., 1983; Hilton-Taylor, 2000).

Culture techniques on giant clams in the tropics have been developed over the past two decades (Heslinga et al., 1984; Usher and Munro, 1988; Braley, 1992; Calumpong, 1992; Bell et al., 1997). Giant clams are protandric hermaphrodites (Wada, 1952). After a brief period of maleness, the clams become functional or simultaneous hermaphrodites where the male and female acini occur in close proximity (Nash et al., 1988; Roa, 1997). The release of sperm precedes the spawning of up to hundreds of millions of eggs to avoid self-fertilization in these hermaphrodite broadcast spawners (Lucas, 1994). In spite of their high fecundity, the natural recruitment success in giant clams is low (Lucas, 1994). The fertilized eggs develop into swimming larval veligers (after 2 days), become settled pedi-veligers (7-9 days) and then undergo metamorphosis (2-3 weeks) to adapt to a phototropic mode of existence by taking up photosynthesizing algal symbionts known as zooxanthellae (Lucas, 1988).

Information on the reproduction of giant clams is still fragmentary (Lucas, 1994; Svane, 1996). Initial studies on the reproduction of giant clams were done in laboratory settings (e.g., Rosewater, 1965; Hardy and Hardy, 1969; La Barbera, 1975) with subsequent field studies in the 1980's (e.g., Braley, 1984; Shelley & Southgate, 1988; Nash et al., 1988). Reproductive patterns of giant clams at the margins of their geographic distributions have been studied at their northeastern border in Japan (e.g., Murakoshi and Kawaguti, 1986), southeastern Asian margin in Australia (e.g., Braley, 1988). In the Red Sea, spawning of *T. squamosa* and *T. maxima* appears to take place seasonally during the stratified period, during June and October (Kilada, 1994), but given the abundances reported (Kilada et al., 1998), the separation of species appears questionable (Chapter 2). Virtually nothing is known about giant clam recruitment in the Gulf of Aqaba forming the northeastern extension of the Red Sea, nor of the factors governing the reproductive activity of *Tridacna* in a

marginal sea subjected to pronounced fluctuations in temperature, nutrient and plankton conditions (Genin et al., 1995; Lindell and Post, 1995; Richter & Abu-Hilal, 2004).

In this paper, we study the reproduction of the two Red Sea species, *Tridacna squamosa* Lamarck, 1819 and *T. maxima* Roding, 1798, at their northwestern limit of distribution in the Jordanian sector of the Gulf of Aqaba (Rosewater 1965; Lucas, 1988; Braley, 1992; Chapter 2). The Gulf of Aqaba is 165 km long × 15 km wide and characterized by the northernmost fringing reefs in the western Indo-Pacific region (UNEP/IUCN 1998). We combined field and laboratory data from *in situ* gonad biopsy and pilot culture of giant clams (Roa-Quiaoit et al., 2004) and compared the reproduction patterns to published reports in the tropics and in relation to the particular environmental conditions in the northern Gulf of Aqaba (Klinker et al., 1978; Reiss and Hottinger, 1984; Hempel and Richter, 2002).

## **Methods**

### *Gonad Biopsy*

Due to the given mortality risk involved with the gonad biopsy technique (Braley, 1988), the protected status of giant clams in Jordan (RSMPP, 2003) and the overall scarcity of giant clams in Jordan (Roa-Quiaoit & Richter, submitted), only a limited number of wild adult giant clam broodstock was available for sampling in the marine reserve in front of the Aqaba Marine Science Station (MSS). *In situ* biopsy of the gonad was carried out using SCUBA between March 2003 and June 2004 (16 months) on a total of six *T. maxima* (13.8 – 25.6 cm shell length (SL) and seven *T. squamosa* (23.1 – 30.5 cm SL) in their natural fore-reef locations between 2 and 16 m depth. We used an aspiration biopsy needle with a 10 ml plastic syringe to sample the gonad tissue by injecting around 1-inch from the exhalant opening of the mantle, taking particular care not to injure the liver (Braley, 1984, 1988). Collected gonad samples were placed in 2% buffered formalin and examined in the microscope. Three parameters (egg stage, egg size and gonad state) were determined for each broodstock in each month.

The egg developmental stages used were based on Braley (1988), namely: early (developing), mature (ripe), regressive (post-spawning condition), spawned (empty egg sac) and spent (no eggs, resting) (Fig. 11). The mean frequency distribution of the different stages was determined using triplicate random counts of 30 eggs. The mean ova diameter of mature eggs was measured from 30 randomly selected eggs using a calibrated eyepiece ocular micrometer.

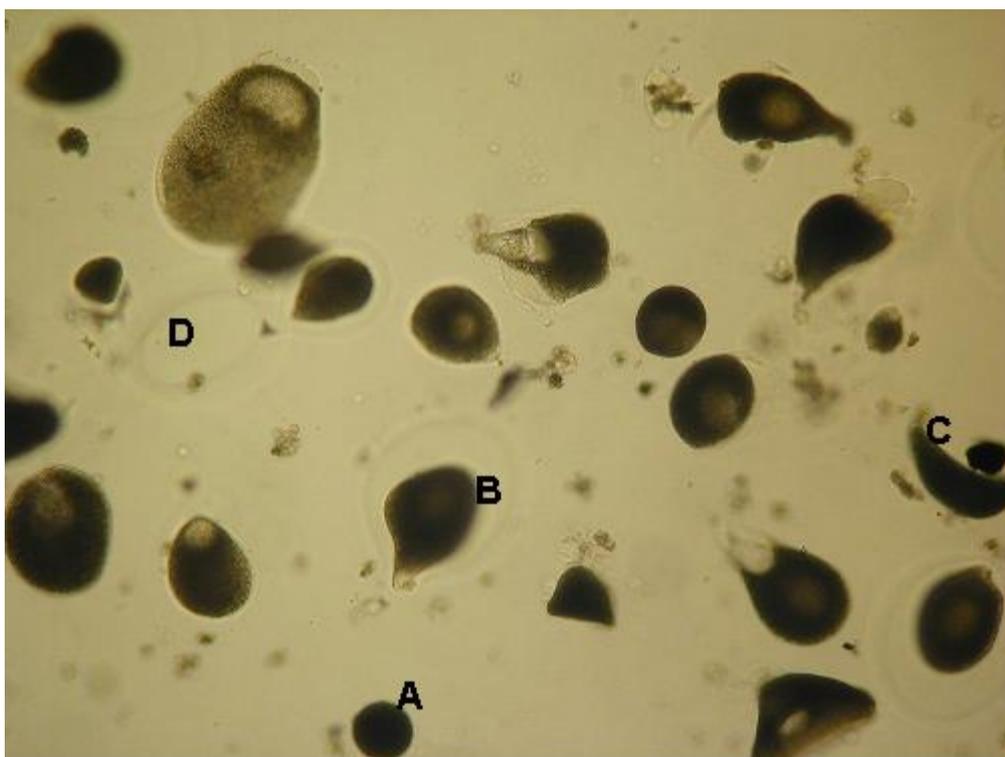


Figure 11. Different developmental stages of the egg: A) early, B) mature with egg sac, C) regressive, and, D) empty egg sac indicating spawning, magnification: 150x.

Table 6. The gonad state index based on the gonad biopsy extract *in situ*.

Nominal scale	Biopsy extract categories	<i>In situ</i> gonad biopsy extract and sampling conditions
1	Empty	clear, no visible eggs in extract and weak resistance from plunger
2	Few	few visible suspended eggs in extract and weak resistance from plunger
3	Not concentrated	loosely suspended egg extract and low resistance from plunger
4	Concentrated	many suspended and settled eggs in extract with strong resistance from plunger
5	Very concentrated	white tissue clump of concentrated eggs in extract and very strong resistance from plunger, in most cases, an abrupt stop

Since sacrificing the clams was out of the question, the state of the gonad could only be estimated semi-quantitatively by assessing the total volume of the eggs extracted and gauging the intensity of resistance of the syringe plunger during biopsy (see Table 6). We used the following categories: empty, few, not concentrated, concentrated and very concentrated gonad extracts and assigned a nominal scale between 1 to 5 (Dix and Ferguson, 1984).

#### *Data Analysis*

All data were tested for normality and homogeneity of variances using Shapiro-Wilk W-test and Kruskal-Wallis ANOVA by Rank test, respectively. Non-parametric statistical tests were applied to non-normally distributed data. For each of the two species, we tested for differences in frequencies of egg stages, egg sizes and gonad state between samplings (months) using the non-parametric Kruskal-Wallis test. Correlations between the above variables were determined using Spearman Rank Order Correlation tests. All statistical tests were run using the program Statistica 6.0 Package at a significance level of 0.05.

#### *Culture of giant clams*

Broodstocks of both species were collected and placed in the shallow (*T. maxima*) and deeper (*T. squamosa*) fore-reefs in the MSS Reserve for induced spawnings in the landbased tanks. Initial attempts to induce spawning using combination of heat stress and serotonin-injection patterned after Braley (1992), one-hour air exposure and warm water immersion, were applied but were not successful. We modified the thermal stress by reversing to immersion in cold water (24-26°C) after one-hour sun-exposure, immediately followed by serotonin-injection which was effective in inducing the release of gametes.

Initial attempts in rearing larvae were done in static water without antibiotics (Heslinga et al., 1984; Crawford et al., 1986), but the prevailing high air temperatures in Jordan (>40°C) resulted in mortalities due to heat build-up and bacterial contamination in the outdoor tanks. We applied the flow-through system (Braley, 1992) and were successful in reducing heat and contamination, but in some occasions resulted in overflow (filter clogging) and loss of larvae if the flow-through cones were not tight. We were able to rear larvae to pediveligers and post-metamorphosed clams with this flowing system using different types of flow-through drains as shown in Fig. 12. The postmetamorphosed juveniles were reared in outdoor PE and cement tanks with filtered (10-25 µm) seawater and natural light. Some mortalities were encountered in small juveniles (<1 year-old) produced from late summer-spawning in the outdoor tanks during colder months (20-23°C). But, this was alleviated by rearing the clams indoors with immersible heaters and artificial light source.

Table 7. Culture rearing protocol applied in Aqaba, Jordan from induced spawning to visible juveniles (>3-mos.).

Protocol outline on the giant clam spawning, larval and juvenile rearing for Aqaba, Jordan (as of Jan 2004).		
DAY	STAGE	ACTIVITIES
0	Broodstocks	Spawning of clam broodstocks (emersion, heat stress, serotonin)
0	Eggs	Separate egg releasing clam in individual bins with aeration. Count eggs per clam, fertilize and stock in hatching tanks (20-25 eggs/ml) Flowing filtered seawater (5uM) with 'flow-thru screen/drain (55uM)'
>3 hrs	Fertilization rate	Sampling and determine fertilization rate
Day 1 -2	Trochophores	Check for trochophores Turn off air 1hour, siphon bottom water (unfertilized eggs, etc.)
Day 2 - 4	Veligers	Check for veligers. Harvest both water column and tank bottom. If bacterial infection, separate harvested water column from bottom samples. Stock veligers in larval tanks (1-3 vel/ml) w/ flowing filtered seawater (5L/min) Start algal feeding (10000-15000 cells/ml)
Day 5-7	Veligers	Algal feeding (10000-15000 cells/ml) (daily or every two days)
Day 7-9	Veligers	Check larval stage. Harvest larvae to nursery bins (5 clams/ sq m), slow water flow (FSW) Put filter bag (coarse-fine cloth) on inlet pipe. Moderate aeration. Start daily algal feeding in outdoor tanks (10000-15000 cells/ml)
Day 9+	Pedi-veligers	Add zooxanthellae (20 - 100 cells/ml) Check up for presence of foot
Day 14	Post-metamorphic clam	Start stronger water flow, add sleeve over drain pipe, >50-60cm water depth Add zooxanthellae (20 - 100 cells/ml) (optional) Start Weekly cleaning of tanks for algal fouling (by fanning-snorkeling)
Day 30	Microscopic clam	Check-up for live clams. Sampling (lengths, presence of zoox, etc.)
Day 60-90	Visible juveniles	Check for visible clams. Harvest, count and measure. Transfer on trays and placed in raceways. Water column to 30-50cm, add biological grazers ( i.e., Trochus snails). Start of nitrogen spiking, 50uM (3x/week)

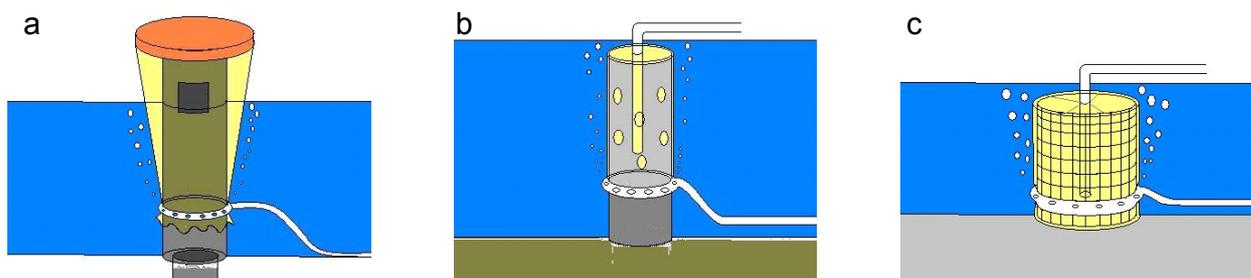


Figure 12. The different flow-through drains developed in Jordan using 50 micrometer screen covered over the drain pipe (a), suspended bored cylinders (b) and plastic mesh wires (c), each with a strong bottom air-ring to keep the larvae away from the drains.

In over 2-year cycles, we modified and improved the rearing method to fit the conditions in Jordan. We were able to produce relatively small number of juveniles of which were spawned during summer months of June-August. The resulting culture protocol applied in Jordan was outlined in Table 7. Details of the culture rearing were described in the culture protocol for Aqaba, Jordan (Roa-Quiaoit et al., in prep.).

## Results

### *Reproductive seasonality of natural stocks*

We found a distinct reproductive seasonality in both species of giant clams in Jordan. We observed natural spontaneous spawnings in the summer months (June to September), but not during the colder months. Likewise, spawned eggs and spent gonads in both species were limited to the summer period (see Figs. 2-3a). Ovarian maturation commenced in early winter and culminated in spring (Dec-May), in parallel with an increase in gonad state during this period (see Figs. 2-3b). The maximum ova diameter was observed in June for both species (see Figs. 2-3c).

*T. squamosa* showed an extended spawning season (Fig. 13a) with a major spawning in June to August followed by a minor spawning in October to November, as shown by the extremely low gonad state indices during this period. Gonad state was inversely correlated with the frequency of spent gonads ( $r = -0.31$ ), spawned ( $r = -0.25$ ) and regressive ( $r = -0.29$ ) eggs (Fig. 13b), and positively related to the frequencies of mature eggs ( $r=0.59$ ;  $p<0.05$  in all cases). The gonad state index started to increase in December and peaked in June, where eggs also reached maximum diameter ( $94 \pm 13 \mu\text{m}$ ), compared to an over-all annual mean of  $89 \pm 10 \mu\text{m}$  (Fig. 13c).

*T. maxima* (Fig. 14a) also commenced spawning in June showing partial but sustained spawnings over October and seemed to have a major release in September. Again, gonad state was inversely related to the frequency of spent gonads from June to September (Fig. 14b). This species seemed to show an early onset of maturity (October) corresponding to the simultaneous increase of both gonad state indices and mature egg frequencies ( $r = 0.53$ ) until it reached maximum values in March to May. Mature eggs of *T. maxima* reached its full size ( $95 \pm 9 \mu\text{m}$ ) also in June, with an over-all mean of  $87 \pm 9 \mu\text{m}$  (Fig. 14c).

Both species of giant clam thus showed consistent patterns of significant strong to moderate correlations between the gonad state indices and the frequencies of the egg stages in the Gulf of Aqaba.

### *Culture of giant clams*

Existing culture techniques in the tropics were applied (Heslinga et al, 1984; Usher and Munro, 1988; Braley, 1992), but were not fully successful due to the pronounced wide-ranged fluctuations ( $>15^{\circ}\text{C}$ , Richter & Abu-Hilal, 2004) of temperature in Jordan

Artificial induction of spawnings in wild broodstocks of giant clams, larval to post-metamorphic rearing, early stage and land-based nursery culture, carried out for almost two- yearly cycles, showed reproductive success only in summer (Fig. 15). During the warmer months, development was rapid at the usual developmental rate in the tropics, with larvae attaining pediveliger stage after only 7-9 days. In winter, by contrast, development was protracted and chronology was delayed, with subsequent total mortality of post-metamorphic clams. Fertilized eggs developed to rotating gastrula in 1- 4 days compared to only 3-6 hours in summer. Likewise, the larval swimming stage persisted for 2- 30 days in winter, compared to 5-7 days in summer. The induced spawnings in the colder months, although successful, resulted in poor egg quality (late maturity or regressive), extended development always leading to larval mortalities, even if reared indoor with relatively warmer waters ( $24\text{-}25^{\circ}\text{C}$ ).

Marked diel and seasonal variations in ambient temperatures, with up to  $5\text{-}10^{\circ}\text{C}$  differences between day-time maximum and night-time minimum, and  $35^{\circ}\text{C}$  between average summer maximum and average winter minimum, led us to modify the established culture protocol for tropical giant clams in the following ways: e.g., Spawning was induced by a heat stress following the change in temperature from warm air exposure and immersion in cold ambient water; A flow-through system of larval rearing compared to static or batch was successful in rearing larvae to postmetamorphosis, reducing heat and bacterial contamination; During winter months, visible juveniles ( $<1$  year old) were reared indoors with artificial light source and constant warm water temperature using immersible heaters.

## **Discussion**

We found a marked seasonality in the reproductive cycle of the two species of giant clams in the subtropical northwestern margin of their distribution. In both, *Tridacna maxima* and *T. squamosa* spawning in the northern Gulf of Aqaba is restricted to the warmer months, when sea surface temperatures are between  $24 - 27^{\circ}\text{C}$ . Species-specific reproductive variability (Newell et al., 1982; Sastry, 1987) showed an extended spawning season for *T. squamosa* (June-November) with two spawning peaks and a shorter incessant season for *T. maxima* (June-September). Regression of the ovarian follicles is protracted over winter and maturation commenced in late winter, culminating in spring.

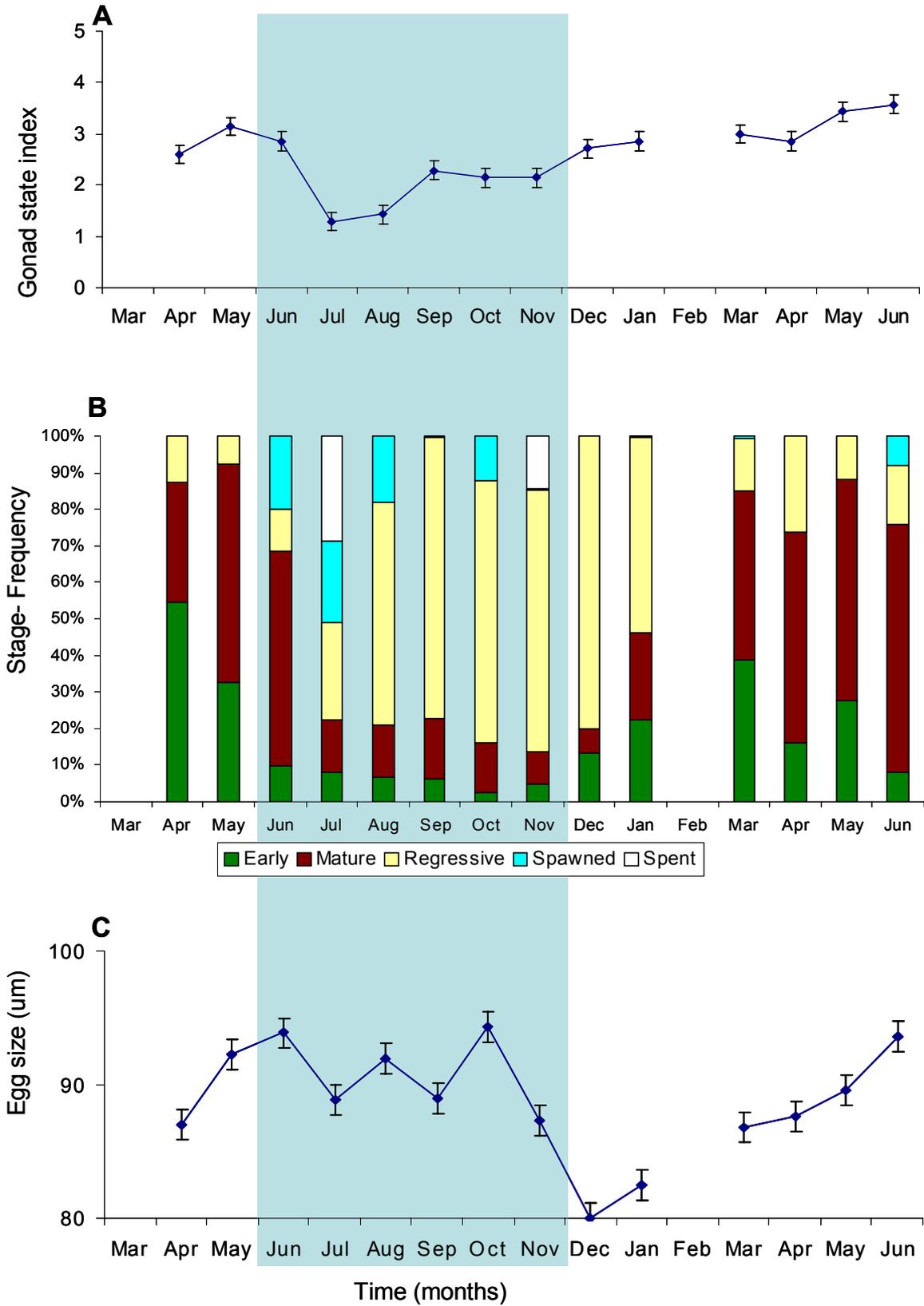


Figure 13. The gonad state index (a), frequencies of the different egg stages (b) and egg size (c) from the gonad biopsy extracts of *T. squamosa* (n=7), showing progressive maturity of eggs (Dec-May) culminating in spawning (Jun-Nov), no bar means no data.

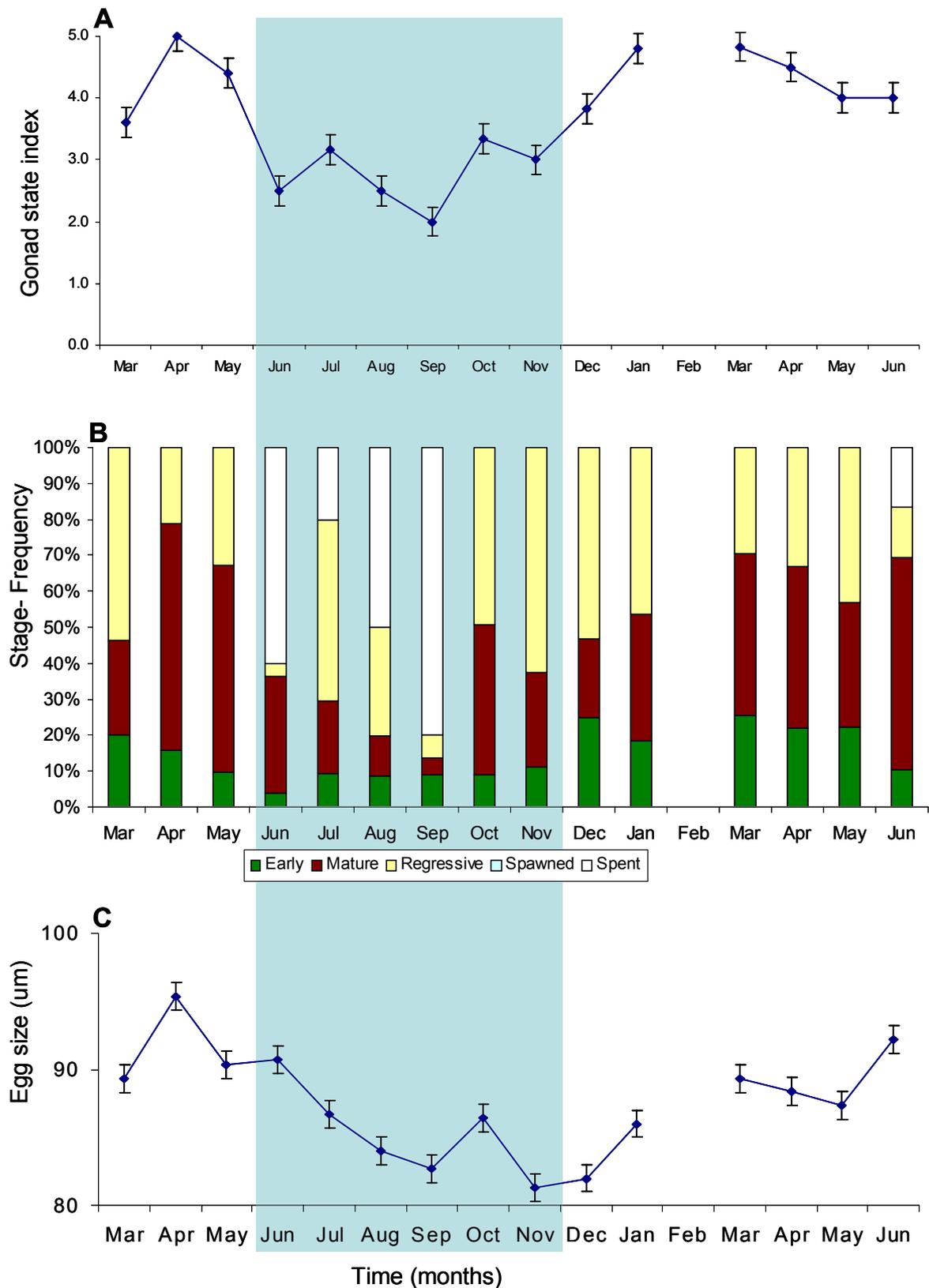


Figure 14. The gonad state index (a), frequencies of the different egg stages (b) and egg size (c) from the gonad biopsy extracts of *T. maxima* (n=6), showing progressive maturity of eggs (Oct-May) culminating in spawning (Jun-Sep), no bar means no data.

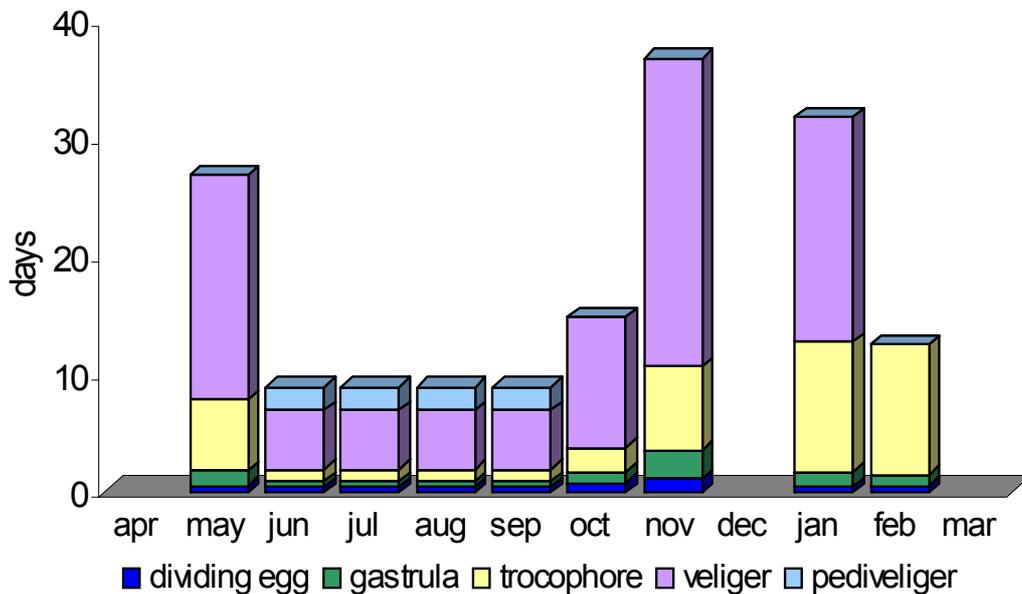


Figure 15. Larval development of giant clams over an annual cycle showing protracted larval development and mortality in the winter months (Oct-May).

The artificial induced spawnings showed consistent pattern with *in situ* gonad biopsy where there is pronounced seasonality in reproductive success in the summer months (June to September), but protracted larval development and mortality in winter. This is similar at the high latitude northeastern border of giant clam distribution in Japan where winter induced spawnings yield regressive eggs with poor survival rate in the low water temperatures (Kawaguti, 1991). In the warmer months (from May-September), early stage development progresses similar to the tropics, e.g. the Philippines, Palau or Solomon Islands (Heslinga et al, 1984; Usher and Munro, 1988; Braley, 1992).

The reproductive pattern in the northern Gulf of Aqaba suggests that both, temperature and nutrients control reproduction, compounding earlier reports (Himmelman, 1975, 1980; Newell et al., 1982; McDonald and Thompson, 1986; Hesselman et al., 1989). However, contrary to the tropics, where seasonality is generally moderate and related to changing wind and rainfall patterns (e.g. Longhurst and Pauly, 1987) and temperature hence unrelated to nutrient levels, the subtropical Gulf of Aqaba is characterized by pronounced seasonal changes in both, temperature and nutrients (Reiss and Hottinger, 1984). Alternating periods of stratification with warm and nutrient-poor waters on the one hand, and mixing with cold and nutrient rich on the other (Klinker et al., 1978) set the stage for a seasonal succession of phytoplankton (Lindell and Post, 1998), with a major

bloom in spring and a smaller bloom in fall (Genin et al., 1995). Our data show that gonad development is already in full swing at the height of the mixing period in March where temperatures are lowest, nutrient levels highest and phytoplankton in its early bloom, suggesting that the ripening of gametes is fuelled primarily by nutrients. Spawning, by contrast, and successful development of larvae to post-metamorphic juveniles is restricted to the summer months, when surface water nutrient levels are below detection levels (Klinker et al., 1978; Badran and Forster, 1998) and plankton concentrations at their annual minimum (Genin et al. 1995, Richter & Abu-Hilal, 2004).

Due to the close proximity of the reef to oceanic waters in the Gulf of Aqaba (Hulings 1989), and cross-shore circulation advecting oceanic surface water to the shore (Richter et al., 2001; Niemann et al., 2004), similar seasonalities in nutrient and plankton levels are found in the coral reefs (Rasheed et al., 2002). We can therefore postulate that the ovarian maturation from winter to spring coincides with the reef saturation of nutrients and chlorophyll during the mixing period in winter to spring. Indeed, many of the bivalve hatcheries in the world condition broodstocks through food rations of microscopic unicellular algae to hasten reproductive maturity in the tanks (Aiken, 1993; Coutteau and Sorgeloos, 1993; Laing and Lopez-Alvarado, 1994). Our data show that the winter supply of exogenous nutrients from the Gulf of Aqaba to the broodstock giant clams in the coral reef is sufficient to meet the high energy cost (Sastry, 1979) of gametogenesis, in spite of the low temperatures (21°C).

The reproductive success of giant clams in summer, in both natural and artificial spawning attempts, points to the importance of temperature in the early stage development from eggs to postmetamorphic juveniles, as contrasted by the high winter larval mortalities observed in the laboratory. The only marginal roles of nutrients and plankton in depleted reef waters in summer supports previous reports that larval clams are mainly lecithotrophic (Manahan, 1983; Heslinga et al., 1990) or only facultative planktotrophs (Southgate 1993), against competing claims that they may be predominantly planktotrophic (Gwyther & Munro, 1981; Fitt et al., 1984; Southgate et al., 1992). The short spawning period, particularly of *T. maxima* in summer, suggests that nutrients and particular food may be ultimately limiting recruitment success by controlling the build-up of energy reserves in the eggs.

The cross-shore circulation in the Red Sea carrying plankton-rich surface water into the reef (Niemann et al., 2004, Genin et al., 2005) and nutrient-enriched near-bottom waters cascading to the offshore (Richter et al., 2001) may be important in controlling the early life-stages of clams where heterotrophic filter feeding is significant compared to the larger adults which rely significantly on its zooxanthellar photosynthates (Klumpp et al., 1992; Fitt et al., 1993; Klumpp &

Griffiths, 1994, Klumpp & Lucas, 1994). Larval clams are shown to filter phytoplankton from 2 to 10 µm size and naked flagellate algae (Fitt et al., 1984; Braley, 1986; Southgate, 1990 Braley, 1992), and visible juveniles between 2 and 50µm (Klumpp et al., 1992).

We envision the following life cycle scenario for giant clams in the Gulf of Aqaba: A successful spawning in summer produces lecithotrophic larvae which undergo rapid development to metamorphosis, largely independent of the low ambient supplies of food. Postmetamorphic clams benefit from both, the prevailing warm temperatures well into September and October, and the eroding offshore thermocline enhancing the supply of food and nutrients into the reef. The early juveniles which are still to large part heterotrophic filter feeders benefit from the ample supply of plankton during the winter months, after which they gradually develop into functional phototrophs which are independent of the scarce supply of plankton during the stratified summer months, benefiting however from the regeneration of nutrients in the near-reef bottom boundary layer (Richter et al., 2001; Rasheed et al., 2002).

To account for the particular climatic conditions in the Gulf of Aqaba, we modified the clam culture methods developed in the tropical Pacific. There are different methods in hatching and larval rearing, using static waters (Heslinga et al., 1984), static with antibiotics (Crawford et al., 1986) and alternate water change (Gwyther & Munro, 1981). The use of the flow-through system in Jordan, which has been tried experimentally only in some facilities (Braley, 1992), has been successful in rearing larvae, prevented bacterial contamination and heat build-up in the outdoor tanks, and, eliminated frequent larval harvest. The modified combination of heat stress and serotonin-injection has been effective in stimulating clams to spawn.

One option to insure spawning success in artificial induction is to use clams with full mature gonads, which can be determined by doing gonad biopsy, although, the risk of mortality is high. The gonad biopsy technique has been a reliable tool in estimating the condition of the gonad (Braley, 1984, 1988) and has been significantly supported by the egg-stage frequencies and gonad state index developed in this study. Results from statistical correlations in both species showed good consistency between these two variables and seem to be a rough but reliable indicator in following the condition of the gonad.

If we integrate the gonad state indices and egg stage frequencies with the gonad developmental stages used by Nash et al. (1988), we can postulate the reproductive periodicity of giant clams based on the following: an 'empty' gonad extract may mean spent gonad; a 'few' gonad extract could be a partly spent gonad; a 'not concentrated' extract either means early developing or regressive gonad; a 'concentrated' extract means a partially full gonad of mature and/or regressive

eggs; and, a ‘very concentrated’ extract means a ripe gonad, most likely followed by spawning (see also Figs. 2-3).

In the subtropical borders of the distribution of giant clams, natural spawning are observed to occur in summer: in the northeastern border in Japan, spawning is limited in summer (Jun-Aug) for *T. crocea* (Murakoshi and Kawaguti, 1986; Murakoshi, 1987); and, in the southeast Asian border in Australia, an austral summer spawning is found for *H. hippopus* and *T. crocea* (Shelley and Southgate, 1988), and, *T. gigas* and *T. derasa* (Braley, 1984; Nash et al., 1988). In the tropical Indo-Pacific, spawning occurs throughout the year (Palau, Heslinga et al., 1990; Philippines, Trinidad-Roa, 1988; Alcazar, 1988).

The variation in the reproductive periodicities over latitudinal difference have been observed also in other groups of bivalves (Barber & Blake, 1983) and other invertebrates, e.g. echinoderms (Pearse, 1968). Results from our study showed an inverse relationship between the seasonal extent of the spawning periods in giant clams with latitude, where restricted summer spawnings at high latitudes contrast with year-round spawning in the tropics. In the smaller spatial scale in the northernmost reefs in the Red Sea alone, *T. maxima* has a shorter spawning period in the northern tip of the Gulf of Aqaba (June to September, Jordan) compared to the southern end of the Gulf in Sinai, Egypt (June to December, Kilada et al., 1998), where oceanographic conditions are quite different (Hempel and Richter, 2002; Richter and Abu-Hilal, 2004) and seasonality much reduced (Klinker et al., 1978).

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## **Literature Cited**

- Aiken, D. (ed.). 1993. Bivalve culture. World Aquaculture, Vol. 24.
- Alcazar, S. 1988. Spawning and larval rearing of tridacnid clams in the Philippines. Pages 125-128 in J. Copland and J. Lucas, eds. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9, Canberra.
- Badran, M. and P. Forster. 1998. Environmental quality of the Jordanian coastal waters of the Gulf of Aqaba, Red Sea. Aquat. Ecosyst Health Manag 1:75-89.

- Barber, B. and J. Blake. 1983. Growth and reproduction of the bay scallop, *Argopecten irradians* (Lamarck) as its southern distribution limit. *J. Exp. Mar. Biol. Ecol.* 66:247-256.
- Beckvar, N. 1981. Cultivation, spawning and growth of the giant clams *Tridacna gigas*, *Tridacna derasa* and *Tridacna squamosa* in Palau, Caroline Islands. *Aquacult.* 24:21-30.
- Belda, C. and E. Gomez. 1988. Growth of giant clams in Bolinao, Pangasinan. Pages 178-182 in J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- \_\_\_\_\_, E. Cuff and D. Yellowlees. 1993. Modification of shell formation in the giant clam *Tridacna gigas* at elevated nutrient levels in sea water. *Mar. Biol.* 117:251-257.
- Bell, J., I. Lane, M. Gervis, S. Soule and H. Tafea. 1997. Village-based farming of the giant clam, *Tridacna gigas* (L.), for the aquarium market: Initial trials in Solomon Islands. *Aquacult. Res.* 28 (2):121-128.
- Braley, R. 1984. Reproduction in the giant clams *Tridacna gigas* and *T. derasa* in situ on the north-central Great Barrier Reef, Australia and Papua New Guinea. *Coral Reefs* 3:221-227.
- \_\_\_\_\_. 1988. Reproductive condition and season of the giant clams *Tridacna gigas* and *T. derasa* utilising a gonad biopsy technique. Pages 178-182 in J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- \_\_\_\_\_. 1992. The giant clam: hatchery and nursery culture manual. ACIAR Monograph No. 15, Canberra. 144 p.
- Brown, J. and M. Muskanofola. 1985. An investigation of stocks of giant clams (Family Tridacnidae) in Java and of their utilization and potential. *Aquacult. Fish. Manage.* 16(1):25-39.
- Bruckner, A. 2001. Tracking the trade in ornamental coral reef organisms: The importance of CITES and its limitations. *Aquarium Sci. Conserv.* 3(1-3):79-94.
- Calumpong, H. 1992. The giant clam: an ocean culture manual. ACIAR Monograph No. 16, Canberra. 68 p.
- Coutteau, P. and P. Sorgeloos. 1993. Substitute diet for live algae in the intensive rearing of bivalve mollusks – a state of the art report. *World Aquaculture* 24(2): 45-42.
- Copland, J. and J. Lucas. 1988. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra. 274 p.
- Crawford, C., W. Nash & J. Lucas. 1986. Spawning induction, and larval and juvenile rearing of the giant clam, *Tridacna gigas*. *Aquaculture*, 58: 281-295.
- Dix, T. and A. Ferguson. 1984. Cycles of reproduction and condition in Tasmanian blue mussels, *Mytilus edulis planulatus*. *Aust. J. Mar. Freshw. Res.* 35:307-313.
- Fitt, W., R. Fisher and R. Trench. 1984. Larval biology tridacnid clams. *Aquaculture* 39:181-195.
- Genin, A., J. Jaffe, R. Reef, C. Richter and P. Franks. 2005. Swimming against the flow: a mechanism of zooplankton aggregation. *Science (Wash.)*. 308(5723): 860-862.

- Gwyther, J. & J. Munro. 1981. Spawning induction and rearing of larvae of tridacnid clams (Bivalvia: Tridacnidae). *Aquaculture* 24: 197-217.
- Hardy, J. and S. Hardy. 1969. Ecology of *Tridacna* in Palau. *Pacific Science* 23:467-472.
- Hawkins, A. and D. Klumpp. 1995. Nutrition of the giant clam *Tridacna gigas* (L.). 2. Relative contributions of filter-feeding and the ammonium-nitrogen acquired and recycled by symbiotic alga towards total nitrogen requirements for tissue growth and metabolism. *J. Exp. Mar. Biol. Ecol.* 190:263-290.
- Hempel, G. and C. Richter. 2002. The Red Sea Programme: sailing a nutshell of hope in the Red Sea waters. *Mar. Ecol. Prog. Ser.* 239:231-232.
- Heslinga, G., F. Perron and O. Orak. 1984. Mass culture of giant clams (F. Tridacnidae) in Palau. *Aquacult.* 39:197-215.
- \_\_\_\_\_, T. Watson and T. Isamu. 1990. Giant clam farming. Honolulu, Hawaii: Pacific Fisheries Development Foundation (NMF/NOAA).
- Hesselman, D., D. Barber and N. Blake. 1989. The reproductive cycle of adult hard clams *Mercenaria* spp. in the Indian River Lagoon, Florida. *J. Shellfish. Res.* 8:43-49.
- Hilton-Taylor, C. 2000. 2000 IUCN Red List of Threatened Species. Gland, Switzerland and Cambridge, UK:IUCN.
- Himmelman, J. 1975. Phytoplankton as a stimulus for spawning in three marine invertebrates. *J. mar. Biol. Ecol.* 20:199-214.
- Himmelman, J. 1980. Synchronization of spawning in marine invertebrates by phytoplankton. In: Clark, J. and T. Adams (eds.) *Advances in Invertebrates Reproduction*, p. 9-19.
- Hulings, N. 1989. A review of marine science research in the Gulf of Aqaba. *Publications of the Marine Science Station Aqaba, Jordan*, Vol. 6, 267 p.
- Juinio, A., R. Menez and C. Villanoy. 1987. Use of giant clam resources in the Philippines. *Naga* 10:7-8.
- \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_ and E. Gomez. 1989. Status of giant clam resources of the Philippines. *J. Moll. Stud.* 55:431-440.
- Kawaguti, M. 1991. Boring clam (*Tridacna crocea*). In: *Aquaculture in Tropical Areas*, pp. 257-272.
- Kilada, R., S. Zakaria and M. Mohallel. 1998. Reproductive biology of the giant clam *Tridacna maxima* (Bivalvia: Tridacnidae) in the northern Red Sea. *Bull. Nat. Inst. of Oceanogr. and Fish., A.R.E.* 24:241-262.
- Klinker, J., Z. Reiss, C. Kropach, I. Levanon, H. Hatpaz and Y. Shapiro. 1978. Nutrients and biomass distribution in the Gulf of Aqaba (Eilat), Red Sea. *Mar. Biol.* 45:53-64.
- Klumpp, D. and C. Griffiths. 1994. Contributions of phototrophic and heterotrophic nutrition to the metabolic and growth requirements of four species of giant clam (Tridacnidae). *Mar. Ecol. Prog. Ser.* 115:103-115.

- \_\_\_\_\_ and J. Lucas. 1994. Nutritional ecology of the giant clams *Tridacna tevoroa* and *T. derasa* from Tonga: influence of light on filter-feeding and photosynthesis. *Mar. Ecol. Prog. Ser.* 107:147-156.
- \_\_\_\_\_, B. Bayne and A. Hawkins. 1992. Nutrition of the giant clam *Tridacna gigas*(L.). I. respiration and growth. *J. Exp. Mar. Biol. Ecol.* 155:105-122
- Knop, D. 2004. Giant Clam Buyer's Guide. *Coral - The Marine Aquarium Magazine* 1(3):36. Ecosystem Publications, California 92705, USA.
- La Barbera, M. 1975. Larval and post larval development of the giant clams *T. maxima* and *T. squamosa* (Bivalvia: Tridacnidae). *Malacologia* 15: 69-79.
- Laing, I. and Lopez-Alvarado. 1994. Effect of dried algal diets on conditioning and fecundity of Manila clam, *Tapes philippinarum* (Adams and Reeve). *Aquaculture and Fisheries Management* 25:157-166.
- Lindell, D. and A. Post. 1995. Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea. *Limnology and Oceanography* 40:1130-1141.
- Lindsay, R., E. Ledua and J. Stanley. 2004. Regional assessment of the commercial viability for marine ornamental aquaculture within the Pacific Island (giant clams, hard and soft corals, finfish, live tock and marine shrimp). SPC Aquaculture Technical Papers. Secretariat of the Pacific Community: Noumea.
- Longhurst, A. and D. Pauly. 1987. *Ecology of tropical oceans*. Academic Press, San Diego CA, USA.
- Lucas, J. 1988. Giant clam description, distribution and life history. Pages 21-33 in J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- Lucas, J. 1994. The biology, exploitation, and mariculture of giant clams (Tridacnidae). *Rev. Fish. Sci.* 2(3):181-223.
- \_\_\_\_\_ and J. Gwyther. 1981. Growth rates and maricultural potential of tridacnid clams. *Proc. 4<sup>th</sup> Int. Coral Reef Symp.* 2: 633-636.
- \_\_\_\_\_ and G. Heslinga. 1983. Prospects for the commercial cultivation of giant clams (Bivalvia: Tridacnidae). *Proc. Gulf Caribb. Fish. Inst.* 35:122-134.
- Manahan, D. 1983. The uptake and metabolism of dissolved amino acids by bivalve larvae. *Biol. Bull.* 164:236-250.
- MacDonald, B and R. Thompson. 1986. Influence of temperature and food availability on the ecological energetics of the giant scallops *Placopecten magellanicus*. IV: Reproductive effort, value and cost. *Oecologia* 72:550-556.
- Murakoshi, M. 1986. Farming of the boring clam, *Tridacna crocea* Lamarck. *Galaxea* 5: 239-254.
- \_\_\_\_\_ and S. Kawaguti. 1986. Sexual maturation and harvest regulations in the shell length of the boring clam *Tridacna crocea*. *Bull. Jap. Soc.Sci. Fish./Nissuishi.* 52(10): 1701-1707.
- Nash, W., R. Pearson & W. Westmore. 1988. Histological study on reproduction in the giant clams *Tridacna gigas* in the North Central Great Barrier Reef. In: In: Copland, J. & J. Lucas (eds.), *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, pp. 89-94.

- Niemann, H., C. Richter, H. Jonkers and M. Badran. 2004. Red Sea gravity currents cascade near-reef phytoplankton to the twilight zone. *Mar. Ecol. Prog. Ser.* 269:91-99.
- Newell, R., T. Hilbish, R. Koehn and C. Newell. 1982. Temporal variation in the reproductive cycle of *Mytilus edulis* L. (Bivalvia:Mytilidae) from localities on the east coast of the United States. *Biol. Bull.* 162:299-310.
- Pandolfi, J., R. Bradbury, E. Sala, T. Hughes, K. Bjorndal, R. Cooke, D. McArdle, L. McClenachan, M. Newman, G. Paredes, R. Warner and J. Jackson. 2003. Global trajectories of the long-term decline of coral reef ecosystems. *Science Wash.* 301 (5635):955-958.
- Pearse, J. 1988. Patterns of reproductive periodicities in 4 species of Indo-Pacific Echinoderms. *Proceedings of the Indian Academy of Science.* 67:247-279.
- Rasheed, M., M. Badran, C. Richter and M. Huettel. 2002. Effect of reef framework and bottom sediment on nutrient enrichment in a coral reef of the Gulf of Aqaba, Red Sea. *Mar. Ecol. Prog. Ser.* 239:277-285.
- Reiss, Z. and L. Hottinger. 1984. *The Gulf of Aqaba (Eilat) – ecological micropaleontology.* Springer, Berlin, 354 p.
- Richter, C. and M. Wunsch. 1999. Cavity-dwelling suspension feeders in coral reefs – a new link in reef trophodynamics. *Mar. Ecol. Prog. Ser.* 188:105-116.
- \_\_\_\_\_ and A. Abu-Hilal. 2004. Seas of the Arabian Region. In: Robinson, A. & K. Brink (eds) *The Sea*, Vol. 14, pp. 1371-1410.
- Roa, H. 1997. The reproductive conditioning of *T. crocea* (Tridacnidae) using algae and dissolved inorganic nitrogen as nutritional supplements. MS Thesis submitted to the University of the Philippines, Diliman, Quezon City, Philippines.
- Roa-Quiaoit, H., C. Richter and M. Al-Zibdah. 2004. Towards a sustainable aquaculture of giant clams (Tridacnidae) in the Jordanian sector of the Gulf of Aqaba. Presented at the 6<sup>th</sup> International Aquarium Congress, Monterey CA USA, Dec. 5-10, 2004.
- \_\_\_\_\_ and C. Richter. Manuscript Submitted. Distribution, abundance, growth and size of giant clams (Tridacnidae) in the northern Gulf of Aqaba, Red Sea. Submitted to *Bulletin of Marine Science*.
- \_\_\_\_\_ C. Richter and M. Al-Zibdah. In prep. The culture of giant clams in the northern Gulf of Aqaba: a detailed protocol.
- Rosewater, J. 1965. The Family Tridacnidae in the Indo-Pacific. *Indo-Pacific Mollusca* 1(6):347-395.
- Sastry, A. 1979. Pelyceopoda (excluding Ostreidae). Pages 113-292 in A. Giese and J. Pearse, eds. *Reproduction of Marine Invertebrates (Volume 5)*. John Wiley & Sons, Inc. New York.
- Shang, Y., L. PingSun and J. Brown. 1994. Test marketing of giant clams for food in Honolulu, Guam and Saipan and observations in Okinawa and Taiwan. Pages 69-87 in *Economics of commercial giant clam mariculture*.

- Shelley, C. and P. Southgate. 1988. Reproductive periodicity and morphometry of *Hippopus hippopus* and *Tridacna crocea*. Pages 86-88 *in* J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- Solis, E., J. Onate and M. Naguit. 1988. Growth of laboratory-reared giant clams under natural and laboratory conditions. Pages 201-206 *in* J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- Southgate, P. 1993. Growth and survival of *Tridacna gigas* larvae: the role of exogenous nutrients. Pages 46-49 *in* W. Fitt, ed. *Biology and mariculture of giant clams*. ACIAR Proceedings No. 47, Canberra, Australia.
- \_\_\_\_\_, R. Lee and J. Lucas. 1992. Development of artificial diets for bivalve larvae. In *Proceedings of the Aquaculture nutrition workshop, Salamander Bay, Australia*. NSW Brackish Water Fish Culture Research Station.
- Svane, I. 1996. Some recent advances in studies on the biology of giant clams (*Tridacnidae*). *Spec. Publ. Phuket Mar. Biol. Cent.* 16:221-241.
- Trinidad-Roa, M. 1988. Spawning and larval rearing of giant clams in Pangasinan, Philippines. Pages 120-124 *in* J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- UNEP/IUCN. 1988. Coral Reefs of the world. Pages 145-151 *in* UNEP Regional Seas Directories and Bibliographies, IUCN, Gland, Switzerland and Cambridge, UK, and UNEP, Nairobi.
- Usher, G. and J. Munro. 1988. ICLARM Coastal Aquaculture Center: current facilities and progress. Pages 106-109 *in* J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- Wada, S. 1952. Protandric functional hermaphroditism in the tridacnid clams. *Oceanographic Magazine (Tokyo)*. 4:25-30.
- Wells, S., R. Pyle and N. Collins. 1983. Giant clams. Pages 97-107 *in* *The IUCN Invertebrate Red Data Book*, Gland, Switzerland.
- Yamaguchi, M. 1977. Conservation and cultivation of giant clams in the tropical Pacific. *Biol. Conserv.* 11:13-20.

**Chapter 4: Morphological, Reproductive and Molecular Genetic  
Evidence for a New Species of Giant Clam (Tridacnidae)  
in the Northern Red Sea**



Figure 16. *Tridacna* nov. sp. showing deep radial folds (Courtesy of J. Sprung).

## Abstract

In this paper, we provide separate independent lines of evidence for the existence of a prominent, yet overlooked, new species of giant clam in the northern Gulf of Aqaba, Red Sea. Molecular mitochondrial rDNA analysis, morphological taxonomic classification, and, reproductive periodicity distinguish the new species, *Tridacna* nov. sp. from its sympatric congeners *T. squamosa* and *T. maxima*, as well as other reported tridacnids from the wider Indo-Pacific region. The molecular analysis (16s rDNA) placed *Tridacna* nov. sp. as a separate clade within the subgenus *Chametrachea* (bootstrap value, MP 98% and NJ 100%). Furthermore, it is supported by the amended taxonomic classification which places *Tridacna* nov. sp. in the same subgenus. *Tridacna* nov. sp. as part of *Chametrachea* is strongly supported by its similar morphological and behavioural characteristics with *T. maxima* and *T. squamosa*, the two of three members of the subgenus and the only two extant species in the Red Sea. *Tridacna* nov. sp. shows a species-specific reproductive seasonality with a shorter summer spawning (May-July), smaller ova diameter ( $75 \pm 2 \mu\text{m}$ ) and earlier ovarian maturation (August) as compared to the other two species. Reliction in giant clams (Newman and Gomez, 2002), includes some extinction and range reduction in the Indo-West Pacific. We suggest the possibility of *Tridacna* nov. sp. in the Red Sea as relic population at the northernmost limit of its geographic distribution based on the following arguments: First, spot-checking in other sites suggests that *Tridacna* nov. sp. is geographically restricted at the tip of the Gulf of Aqaba, which is hydrographically distinct from the central and southern Gulf and Red Sea proper. Second, the marginal occurrence suggests a cold-water tolerance of this species relative to its wide-ranged congeners, *T. maxima* and *T. squamosa*. Third, it seems to have close resemblance with the Red Sea fossil species found in the Gulf of Suez, *Tridacna aegyptiaca* (Chenu, 1845). These indications support the assumption that *Tridacna* sp. nov. may be a glacial relic species. Similar glacial relic forms have been invoked in relation to other organisms enduring in marginal environments, e.g. the unusually large species of *Rhincalanus* sp. inhabiting the oxygen minimum layer in the Red Sea (Wiekert, 1982).

## Introduction

The Family Tridacnidae, commonly known as giant clams, is a small but conspicuous family of bivalved molluscs whose number of recognized species has increased in the past two decades (Rosewater, 1965, 1982; Lucas et al., 1991). The interest on giant clams is not just from the commercial exploitation of its meat, shell and the live organism, but, also on its apparent marked decline throughout their Indo-Pacific range (Brown and Muskanofola, 1985; Lucas, 1988; Juinio et al., 1989) resulting in local (Gomez and Alcalá, 1988) and ecological extinction (Pandolfi et al. 2003) of the larger species in some areas.

Tridacnids are presently concentrated in the Indo-Malayan region of its Indo-West Pacific range, showing withdrawal of its range (Newman and Gomez, 2002) instead of radiations found in other family of reef organisms (Ross and Newman, 2002; Veron, 2000). There are three fossil genera and 15 extinct species belonging to the two recent genera, *Hippopus* and *Tridacna*. Reliction in giant clams, including the total extinction in the tropical Atlantic and some extinction and range reduction in the Indo-West Pacific, happened as recently as the Holocene period (Newman and Gomez, 2002). This was associated with the climatic change concomitant with the Tethys Sea break-up, but, the process has been presently intensified with increasing human pressure and environmental deterioration (Newman and Gomez, 2002; Pandolfi et al., 2003). The Convention of International Trade of Endangered Species Giant (CITES) includes the species of giant clams (Wells et al., 1983; Hilton-Taylor, 2000).

Presently, there are eight extant recognized species of giant clams belonging to two genera, and, three subgenera under *Tridacna*: *H. hippopus* Linnaeus, 1758 and *H. porcellanus* Rosewater, 1982; and, subgenus *T. sensu stricto* (*T. gigas* Linnaeus, 1758); subgenus *Chametrachea* (*T. maxima* Roding, 1798, *T. squamosa* Lamarck, 1819, and, *T. crocea* Lamarck, 1819); subgenus *Persikima* (*T. derasa* Roding, 1798 and *T. tevoroa* Lucas, Ledua and Braley, 1991) (Rosewater, 1965, 1982; Lucas et al., 1991).

Two of these eight species were recently discovered in restricted geographic ranges, *H. porcellanus* in the South China Sea by Rosewater (1982) and *T. tevoroa* in Fiji and Tonga by Lucas, Ledua and Braley (1991). There is one reported species in the Indian Ocean, *T. rosewateri* by Sirenko and Scarlati (1991), whose present status as a tridacnid species is still ambiguous. This species has been suggested to be a junior synonym of *T. squamosa* (Benzie & Williams, 1998), but may be a distinct species endemic to the Mascarene Plateau (Newman and Gomez, 2002).

The widely accepted taxonomic classification of the family Tridacnidae is by Rosewater (1965) based on morphology. An amendment of this phylogeny was done by Lucas (1991) to include the

newly discovered species, *T. tevoroa*, which was then evaluated using protein electrophoresis by Benzie and Williams (1998). The resulting phylogenetic classification confirmed the currently recognized major groupings based on the morphologic characteristics, but, suggested that some characters are not reliably diagnostic of the genera, subgenera or species levels. The first DNA molecular phylogenetic analysis of giant clams was done by Schneider & Foighil (1999), which recognized the two genera but showed inconsistencies in the previous classifications in the placement of the sister taxa within the Subgenera *Chametrachea* and *Persikima*.

Giant clams are widely distributed in the Indo-Pacific region (Rosewater, 1965; Lucas, 1988). But, only two species have longer range, namely, *T. squamosa* and *T. maxima*, extending from the southeast Pacific up north in the Red Sea. The two Red Sea species exist up to the northeastern extension of the Red Sea, the Gulf of Aqaba marking the northwestern limit of giant clams (Chapter 2).

In this paper, we describe a possible new species of giant clam (*Tridacna* nov.sp.) in the Gulf of Aqaba, northern Red Sea. The status as a *Tridacna* nov.sp. as a new species is based on morphology, reproduction and molecular phylogenetic analysis from specimens at the Jordanian coast of the Gulf of Aqaba. Here we present an amended taxonomic classification of the Tridacnidae to include the new species based on morphological and behavioral characters. We show the phylogenetic relationships of the *Tridacna* nov.sp among its congeners based on molecular analysis (mitochondrial rDNA), and, described its reproductive periodicity based on gonad extracts.

## **Methods**

### *Taxonomic classification*

Morphological and behavioral characteristics of specimens of *Tridacna* nov.sp. were compared to the diagnostic features of the taxonomic classification of Tridacnidae, using the most recent taxonomic key by Lucas (1991).

### *Reproductive periodicity*

*In situ* biopsy of the gonad was done on natural stocks of *Tridacna* nov. sp. (n = 5) on the reef flat (<3m) of the marine reserve, Aqaba Marine Science Station, Jordan from March 2003 to June 2004 (16 months). Collected gonad samples were placed in 2% buffered formalin. Three parameters (egg stage, egg size and gonad state) were determined for each broodstock in each month. Detailed procedure and data analysis were followed after the reproductive study in Roa-Quiaoit et al. (in

prep.). The reproductive periodicity of *Tridacna* nov. sp. was compared to the two Red Sea species, *T. maxima* and *T. squamosa* (Roa-Quiaoit et al., in prep.).

#### *Molecular Phylogenetic Analysis*

Small tissue clips (~1 cm<sup>2</sup>) from the mantle were collected from specimens of *Tridacna* nov. sp. at the Marine Science Station in Aqaba, Jordan. Samples were preserved in absolute ethanol and stored at -20 °C.

The DNA extraction, polymerase chain reaction (PCR), sequencing and phylogenetic analysis were patterned after Kochzius et al. (2003). DNA was extracted using the Chelex method (Walsh et al., 1991; Soller et al., 2000). Amplification of a partial sequence from the 16S rDNA gene with the primers 16sar-L and 16sbr-H (Palumbi et al., 1991) was conducted for a 50 µl PCR reaction: 10mM Tris-HCl buffer, 4 mM MgCl<sub>2</sub>, 0.2 mM dNTP, 0.2 mM each primer, 20 mM BSA and 1 U Taq polymerase. Thermal cycling profile was at 94 °C (5 min), subsequently 40x of 94 °C (1.30 min), 50 °C (1 min), 72 °C (1.30), with a final step 72 °C (5 min.). PCR products were purified using QIAquick PCR Purification kit (Qiagen, Hilden). Partial sequence of about 450 bp was amplified with the same primers as above. Both strands were sequenced using BigDye Terminator Cycle Sequencing Kit (ver.1.0, PE Biosystems, Foster City) and an ABI Prism 310 automated sequencer (Applied Biosystems, Weiterstadt).

Both strands were initially aligned using Sequence Navigator (ver. 1.0.1, Applied Biosystems), and, multiple alignment done using Clustal V implemented by Sequence Navigator. Phylogenetic analysis was based on 390 bp 16S rDNA representing 7 *Tridacna* sp. and 1 *H. hippopus* as outgroup. Download of *Tridacna* and *Hippopus* sequences were done from GenBank accession numbers AF 122971 through AF 122980 (Schneider and Ó Foighil, 1999). Phylogenetic analysis was done with the program PAUP\* (ver 4.0b10, Swofford, 1998), using maximum-parsimony (MP) with inferred gaps, maximum likelihood (ML), and neighbour-joining (NJ). Evaluation of statistical confidence in nodes was based on 1000 non-parametric bootstrap replicates.

## **Results**

#### *Phylogenetic analysis*

The most parsimonious tree (Fig. 17) from the strict consensus MP analysis placed the *Tridacna* nov. sp. as a separate clade, supported by a statistically robust branch (bootstrap value, MP 98%). The tree supported the monophyly of all specimens of *Tridacna* nov. sp. within the subgenus *Chametrachea*. The phylogenetic positions of the rest of the giant clam species under the two genera *Tridacna* and *Hippopus* were similar with that of Schneider and Ó Foighil (1999).

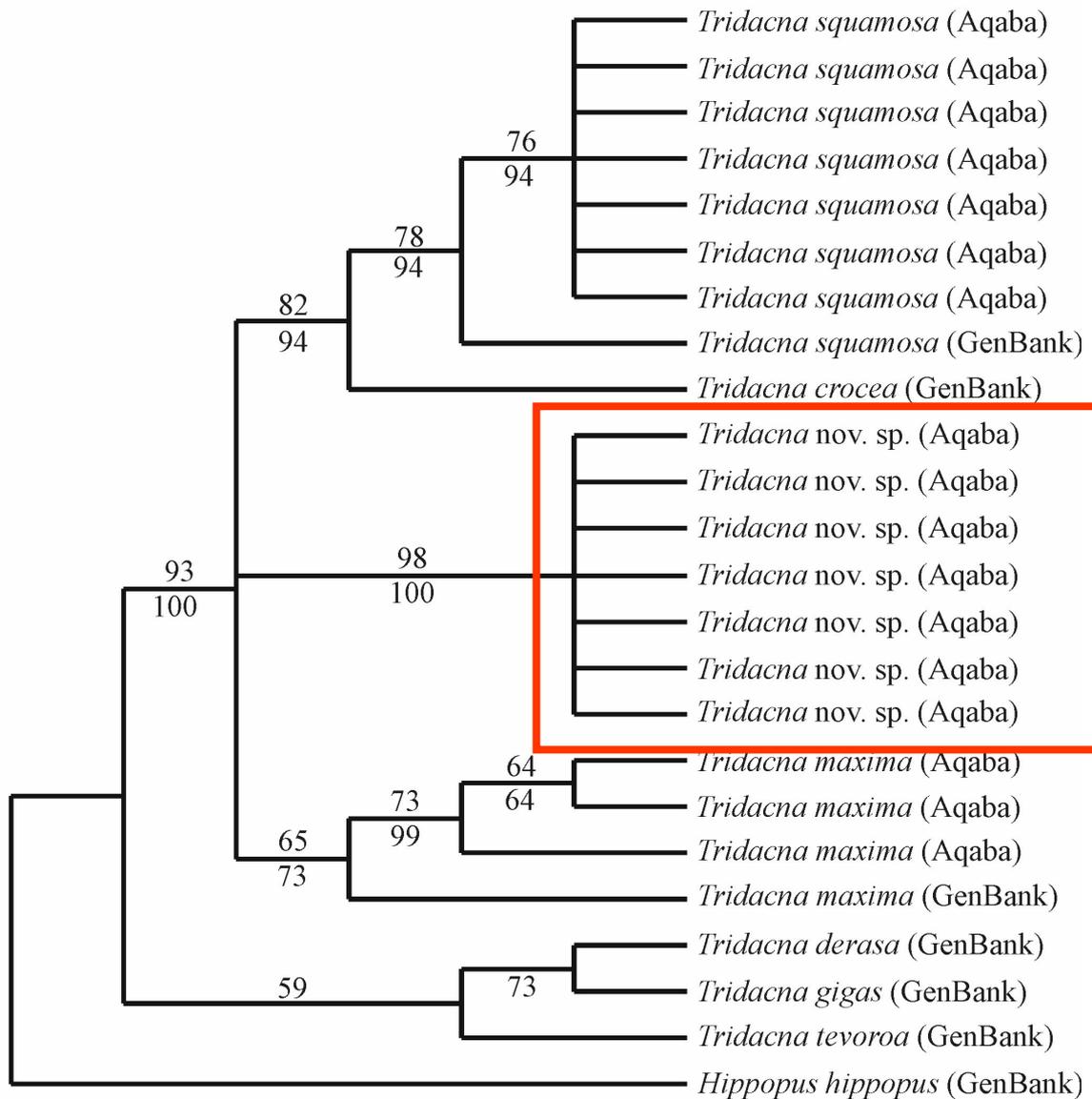


Figure 17. Strict consensus Maximum Parsimony (MP) cladogram from 2 trees of a 383 bp fragment of the mitochondrial 16s rDNA; outgroup: *Hippopus hippopus*; number of parsimony-informative characters: 48; consistency index (CI): 0.759; retention index (RI): 0.839; bootstrap analysis with 1000 replicates, bootstrap branch values from MP (above) and Neighbor-Joining (below).

<i>Tridacna</i> nov. sp.	GGCGGTGTTA	AACCTTAAAG	TAGCGTGATA	AGTCGGCCTT	TAATTGGGGT	CTGGTATGAA
<i>T. maxima</i>	.....	.....	.....	.....	.....	.....
<i>T. maxima</i> _GB	.....	.....	.....	.....	.....	.....
<i>T. squamosa</i>	.....	.....	.....	.....	.....	.....
<i>T. squamosa</i> _GB	.....	.....	.....	.....	.....	A.....
<i>T. crocea</i> _GB	.....	.....	.....	A.....	.....	.....
<i>T. tevoroa</i> _GB	.....	.....	.....	.....	.....	.....
<i>T. derasa</i> _GB	.....	.....	.....	.....	.....	.....
<i>T. gigas</i> _GB	.....	G.....	.....	T.....	.....	.....
<i>H. hippopus</i> _GB	.....	.....	.....	GA.T.....	.....	.....
<i>Tridacna</i> nov. sp.	GGGGTTGACG	TCGGAAAAGC	TGCTCAGAA	AGACTAAGTG	AAATTTACTT	TTAAGTGAAA
<i>T. maxima</i>	A.....	.....	.....	T...A..	.....	.....
<i>T. maxima</i> _GB	A.....	GA.....	.....	A.T...A..	.....	.....
<i>T. squamosa</i>	A.....	A.....	G.....	GA.....C..	.....	.....
<i>T. squamosa</i> _GB	A.....	A.....	G.....	GA.....	.....	G.AAG.....
<i>T. crocea</i> _GB	A.....	A.....	G.....	GA.T.G.....	.....	.....
<i>T. tevoroa</i> _GB	.....	G.....	A.....	GA.T.TT.....	.....	.....
<i>T. derasa</i> _GB	.....	G.A.....	G.....	A.TATG.....	.....	CT.....
<i>T. gigas</i> _GB	G.....	G.A.....	G.....	TA.A.....	.....	.....
<i>H. hippopus</i> _GB	T.....	T.....	A.....	A.TAT.A.....	.....	C.....
<i>Tridacna</i> nov. sp.	AGGCTTAAAT	AAGGATAAAA	GACGAGAAGG	CCCTGTCGAG	CTTGAGGGAT	TTGAATGTTT
<i>T. maxima</i>	.....	G..AG.....	.....	.....	.....	.....C.....
<i>T. maxima</i> _GB	.....	G..AG.....	.....	.....	.....	.....C...C
<i>T. squamosa</i>	.....	A G..AAG.....	A.....	.....	.....	.....
<i>T. squamosa</i> _GB	.....	T..AAG.....	A.....	.....	.....	.....
<i>T. crocea</i> _GB	.....	T..AAG.....	A.....	.....	.....	.....
<i>T. tevoroa</i> _GB	.....	A.....	TAT.....	A.....	.....	.....T...A
<i>T. derasa</i> _GB	.....	GAAG.....	.....	.....	.....	.....G.....
<i>T. gigas</i> _GB	.....	G.G..GGCTG.....	A.....	.....	.....	A.....
<i>H. hippopus</i> _GB	.....	G.....TT..AG.....	A.....	A.....	.....	CT.....G
<i>Tridacna</i> nov. sp.	AGCAA- CGTG	ATTAGGAGTT	TGGCTGGGGC	AGCAATGGAG	ATAGCCTCC	TTTTTAAAGC
<i>T. maxima</i>	..T..A.....	.....	.....	.....	.....	.....
<i>T. maxima</i> _GB	..T..A.....	.....	.....	.....	.....	.....
<i>T. squamosa</i>	..TT..A.....	.....	.....	.....	.....	.....T..
<i>T. squamosa</i> _GB	..TT..A.T.....	.....	.....	.....	.....	.....T..
<i>T. crocea</i> _GB	..T..A.....	.....	.....	.....	G.....	.....GC..
<i>T. tevoroa</i> _GB	CAA..A.A.....	.....	A.....	.....	G.....	.....T..
<i>T. derasa</i> _GB	TAAT..A.A.....	.....	.....	.....	G.....	.....
<i>T. gigas</i> _GB	TAAC..A.A.....	.....	.....	.....	G.....A.....	.....
<i>H. hippopus</i> _GB	TAA..C...T..	GA.....	.....	C.....	G..A.....	.....GT..
<i>Tridacna</i> nov. sp.	AAAGATCCAT	TATGTTCAAA	ATAATGAAAA	AAGGAAAAAG	TTACCGCAGG	GATAACAGCA
<i>T. maxima</i>	.....	.....GG.....	.....	.....	C.....	.....
<i>T. maxima</i> _GB	.....	.....G.....	.....	.....	C.....	.....
<i>T. squamosa</i>	.....	.....AC.T.....	.....	A.....	C.....	.....
<i>T. squamosa</i> _GB	.....	.....AC.T.G.....	.....	A.....	C.....	.....
<i>T. crocea</i> _GB	.....	.....T.A.CT..G.....	.....	A.....	C.....	.....
<i>T. tevoroa</i> _GB	..T.....	.....A..T.....	T.....	.....	C.....	.....
<i>T. derasa</i> _GB	.....	.....AA.G.TG.....	T.....	.....	.....	.....
<i>T. gigas</i> _GB	.....	.....GAAAG.G.....	T.....	.....	.....T.....	.....
<i>H. hippopus</i> _GB	G.....	GA..GGT-.....	T.....	G.....	.....T.....	.....
<i>Tridacna</i> nov. sp.	CAAGACCGCC	TCAGAGGTCG	TATCGAAGGC	GGTAAGTGTG	ACCTCGATGT	TGGATTAGGG
<i>T. maxima</i>	.....	.....	.....	.....	.....	.....
<i>T. maxima</i> _GB	.....	.....	.....	.....	.....	.....
<i>T. squamosa</i>	.....	.....	.....	.....	.....	.....
<i>T. squamosa</i> _GB	.....	.....	.....	.....	.....	.....
<i>T. crocea</i> _GB	.....	.....	.....	.....	.....	.....A..
<i>T. tevoroa</i> _GB	.....	C.....	.....	G.....	.....	.....
<i>T. derasa</i> _GB	.....	.....	.....	G.....	.....	.....
<i>T. gigas</i> _GB	.....	.....	.....	G.....	.....	.....
<i>H. hippopus</i> _GB	.....	.....C.....	.....	A.G.....	.....	.....C.C..
<i>Tridacna</i> nov. sp.	TAAACCTCTT	TGTGCAGGAG	C-A			
<i>T. maxima</i>	.....	.....	.....			
<i>T. maxima</i> _GB	G.....	.....	.....			
<i>T. squamosa</i>	.....	.....	.....			
<i>T. squamosa</i> _GB	.....	.....	.....			
<i>T. crocea</i> _GB	.....	.....	.....			
<i>T. tevoroa</i> _GB	G.....AT..	G.....	G.....			
<i>T. derasa</i> _GB	G.....CT..	C.....	G.....			
<i>T. gigas</i> _GB	G.....CT..	C.....	G.....			
<i>H. hippopus</i> _GB	G.....GCA..	G.....	T.....			

Figure 18. Alignment of the 383 bp fragment of the mitochondrial 16s rDNA. Dots indicate nucleotide identity to the genetic sequence of *Tridacna* nov. sp. and sequences from Gene Bank are indicated with GB after the species name.

As shown in Fig.17, the relationship of *Tridacna* nov. sp. between its subcongeners in the subgenus *Chametrachea* was not clear. However, genetic distance estimates showed a closer affinity of *Tridacna* nov. sp. to *T. maxima* (genetic distance, 0.037) than to *T. crocea* and *T. squamosa* (0.06, for each species). The genetic distance between the two distinct clam species, *T. crocea* and *T. squamosa* is similar to the above (0.035). *Tridacna* nov.sp. showed distant molecular relationships with all of its other congeners (Table 8).

Table 8. Genetic distance estimate (HKY85) among the six *Tridacna* species of giant clams, including *Tridacna* nov.sp., with *H. hippopus* as outgroup for a 383-mitochondrial 16s rDNA fragment.

	1	2	3	4	5	6	7	8
<i>T. nov.sp.</i>								
<i>T. maxima</i>	0.037							
<i>T. squamosa</i>	0.060	0.055						
<i>T. crocea</i>	0.065	0.068	0.035					
<i>T. tevoroa</i>	0.106	0.116	0.119	0.114				
<i>T. derasa</i>	0.084	0.094	0.086	0.087	0.084			
<i>T. gigas</i>	0.125	0.114	0.123	0.127	0.112	0.067		
<i>H. hippopus</i>	0.158	0.156	0.166	0.156	0.160	0.144	0.163	

Phylogenetic analysis of the aligned 383 bp fragment of the mitochondrial 16s rDNA (Fig. 18) showed 260 constant characters, 48 of which were parsimony informative characters.

#### *Taxonomic classification based on morphological and behavioural characters*

*Tridacna* nov.sp. shared morphological and behavioral characteristics with *T.squamosa*, *T. maxima* and *T. crocea*, all belonging to the subgenus *Chametrachea*. Lucas (1991) used seven diagnostic criteria (Table 9) within the *Chametrachea* group to separate *T. squamosa* from *T. maxima* + *T. crocea*. However, these criteria were not able to clearly delineate the *Tridacna* nov.sp.. among the other three species. Based on these criteria, the similar features (see Fig. 19) on shell symmetry, hinge length, scutes (crowded) and byssal orifice positioned *Tridacna* nov.sp. with *T. maxima*, but, the features on the mantle color, incurrent tentacles and well-spaced scutes placed this species also with *T. squamosa*.

Therefore, to accommodate the new species, we had to restructure and modify the taxonomic key within the *Chametrachea* group, used 4/7 of the diagnostic criteria and added one more feature, shell radial folds. The revised taxonomic classification (Fig. 20) placed the *Tridacna* nov. sp. together with *T. maxima*, and *T.squamosa* with *T. crocea*.

Table 9. The diagnostic criteria within the Chametrachea from Lucas (1991) showing characteristic feature of each member in the group. Features with (+) are used to separate species at the subgenera *Chametrachea* level in the amended taxonomic key to include *Tridacna* nov. sp..

Diagnostic criteria	<i>T. squamosa</i>	<i>T. maxima</i>	<i>T. crocea</i>	<i>T. nov. sp.</i>
Shell symmetry+	symmetrical	asymmetrical	slightly asymmetrical	asymmetrical
Hinge length+	= half shell length	< half shell length	< half shell length	< half shell length
Scutes+	large, well spaced entire shell	low, crowded upper half of shell	low, crowded shell margins	crowded & well-spaced upper- & lower-half
Byssal Orifice+	narrow	moderate-wide	wide	wide
Embedded on substrate	attached on surface	partially embedded	fully embedded	attached on substrate
Mantle pattern	subdued mottled	colored	colored	subdued
Incurrent tentacles	distinct	indistinct	indistinct	distinct
Additional:				
Radial folds+	4-6 pointed to bluntly rounded	usually 5 sharply triangular	4 - 5 blunt to slightly triangular	5 - 6 deeply triangular

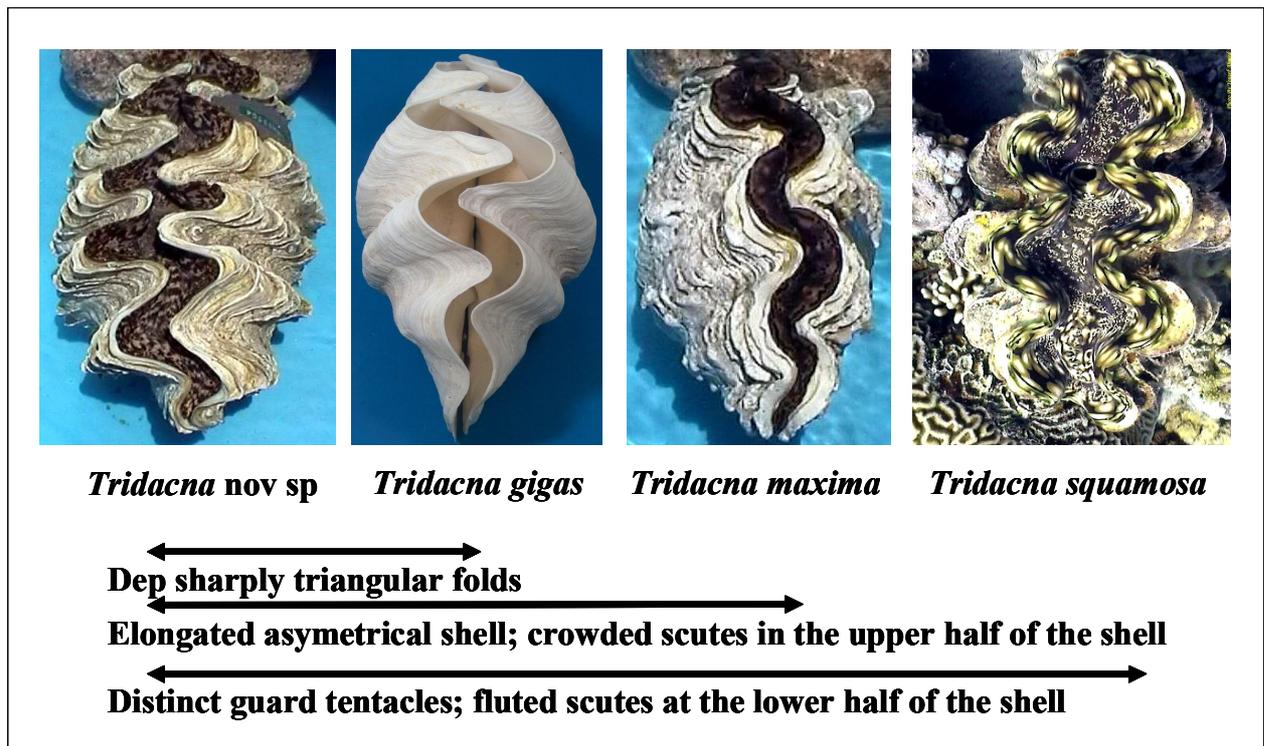


Figure 19. Morphological similarities of *Tridacna. nov.sp.* with three other giant clam species.

KEY TO THE TRIDACNID SPECIES

- 1 Byssal orifice region of opposed valves with interlocking teeth; distinct region of shell around byssal orifice, outlined by ventral-most pair of prominent radial ribs; mantle, when fully extended, not projecting laterally beyond shell margins  
.....*Hippopus*.....2
- Byssal orifice of opposed valves without interlocking teeth; no distinct ventral region of shell outlined by prominent radial ribs; mantle, when fully extended, usually projecting laterally beyond shell margins  
.....*Tridacna*.....3
- 2(1) Shells thick and strongly ribbed, with reddish blotches in irregular bands; incumbent apertures without guard tentacles  
.....*H. hippopus*
- Shells in specimens less than about 200 mm shell length not thick nor strongly ribbed and with only faint reddish blotches; incumbent apertures with guard tentacles  
.....*H. porcellanus*
- 3(1) Shell length of large specimens >550 mm, sometimes greater than 1 m; with about four elongate, interdigitating projections of each distal shell margin, being most elongate and acute in large specimens; shell without scutes, except for some tubular projections near umbo in very small juveniles; mantle brownish, with numerous iridescent blue-green circles  
.....*T. gigas*
- Shell length rarely >550 mm; without elongate, interdigitating projections on each distal shell margin; mantle variably colored, without iridescent blue-green circles  
.....4
- 4(3) Shell length up to 550 mm, occasionally larger; upper region of large shells plain, without scutes or ribs; hinge usually longer than half shell length  
.....5
- Shell length usually <400 mm; upper shell region with scutes or eroded scutes; hinge equal to or less than half shell length  
.....6
- 5(4) Rib-like radial folds on shell without colored patches; mantle without protruberances; incumbent aperture with inconspicuous guard tentacles  
.....*T. derasa*
- Rib-like radial folds on shell usually striped with colored patches near umbo; mantle with protruberances; incumbent aperture with conspicuous guard tentacles  
.....*T. tevoroa*

Figure 20. Taxonomic key to the Tridacnidae, expanded and modified from Lucas (1991) to incorporate *Tridacna*. nov.sp. from the northern Red Sea.

6(4)	Shell approximately symmetrical about umbo in lateral view, with hinge about or equal half shell length; crowded scutes usually none or only at margins and well-spaced both within and between the radial rows; byssal aperture narrow to wide; usually with 4-5 pointed to bluntly rounded radial folds	7
	Shells usually asymmetrical about umbo in lateral view, with hinge less than half shell length; crowded scutes usually at upper half of shell and set close together both within radial rows and between rows; byssal aperture moderately wide to wide; usually with 5-6 sharp to deep radial folds	8
7(6)	Shell approximately symmetrical about umbo in lateral view, with hinge about half shell length; scutes large and well-spaced both within and between the radial rows; lateral distance between scutes in adjacent rows usually about the same as scute width; byssal aperture narrow to moderately wide; not embedded into substrate; mantle usually of subdued and mottled color; incurrent aperture with distinct guard tentacles; shell length >150 mm	<i>T. squamosa</i>
	Shells not strongly asymmetrical about umbo in lateral view, with hinge less than half shell length; scutes eroded away except near shell margin; byssal aperture wide; occurs deeply embedded in reef substrate; mantle usually colorful; incurrent aperture with indistinct guard tentacles; shell length <150 mm	<i>T. crocea</i>
8(6)	Byssal aperture moderately wide to wide; crowded scutes present in substantial part of upper shell region, with usually no scutes on lower region; occurs partially embedded in reef substrate; mantle colorful; incurrent aperture with indistinct guard tentacles; usually 5 sharp but not so deep radial folds	<i>T. maxima</i>
	Byssal aperture wide; crowded scutes present in substantial part of upper shell region, with well-spaced large scutes in lower region; usually occurs attached on reef substrate; mantle subdued; incurrent aperture with distinct guard tentacles; usually 5-6 deep radial folds	<i>T. nov.sp</i>

Figure 20. ...continuation from preceding page.

### *Reproductive periodicity*

*Tridacna* nov. sp. showed a short summer spawning starting early in May until July, with a major spawning in June (Fig. 21a), as shown by the low gonad state indices during this period. Gonad state was positively related to the frequencies of mature eggs ( $r = 0.64$ ) and inversely correlated with spent gonads ( $r = -0.35$ ) (Fig. 21b). The build-up of mature eggs in August was immediately followed by high frequencies of mature ova sustained over winter to spring (Fig. 21c). This was supported by the drop in the ova sizes in September which was indicated by eggs at early maturity stage. The ova diameter increased in October and showed similar range over winter to spring.

*Tridacna* nov.sp. showed smaller egg sizes ( $75 \pm 2 \mu\text{m}$ ) compared to *T. squamosa* and *T. maxima* ( $89 \pm 10 \mu\text{m}$  and  $87 \pm 9 \mu\text{m}$ , respectively).

We found a distinct species-specific reproductive seasonality among the three species in Jordan. *T. squamosa* showed the longest spawning period extending from June to November with two spawnings peaks, while, *T. maxima* showed a shorter unimodal spawning in June-September (Roquoit et al., in prep.). *Tridacna* nov.sp. showed a brief spawning period from May-July, with smaller ova diameter and earlier ovarian maturation compared to the other two species.

## **Discussion**

Our data from the mitochondrial rDNA analysis, morphological and behavioural classification, and a distinct reproductive periodicity showed a very strong indication of a new species in the northern Red Sea, *Tridacna* nov.sp..

Both the phylogenetic analysis and the taxonomic classification placed the *Tridacna* nov.sp. as a separate species, within the subgenus *Chametrachea*. The position of *Tridacna* nov.sp. in *Chametrachea* is strongly supported by the fact that the other two member species in the group, *T. maxima* and *T. squamosa*, are the only long-range tridacnid species and the only extant species in the Red Sea. Morphological and behavioral characteristics of *Tridacna* nov.sp. are shared between these two species, but, species-specific variability in the reproductive seasonality is evident among these species in this marginal sea.

*Tridacna* nov.sp. has closer affinity to *T. maxima* within the subgenus *Chametrachea*. However, there has been considerable movement among the member species in this group (Fig. 22). The position of *T. maxima* as sister taxa of (*T. crocea* + *T. squamosa*) would be the most supported by molecular and stratigraphic data (Schneider and Foighil, 1999). The fossil records show that *T. maxima* appeared in the Late Miocene while *T. squamosa* and *T. crocea* appeared in the Late to

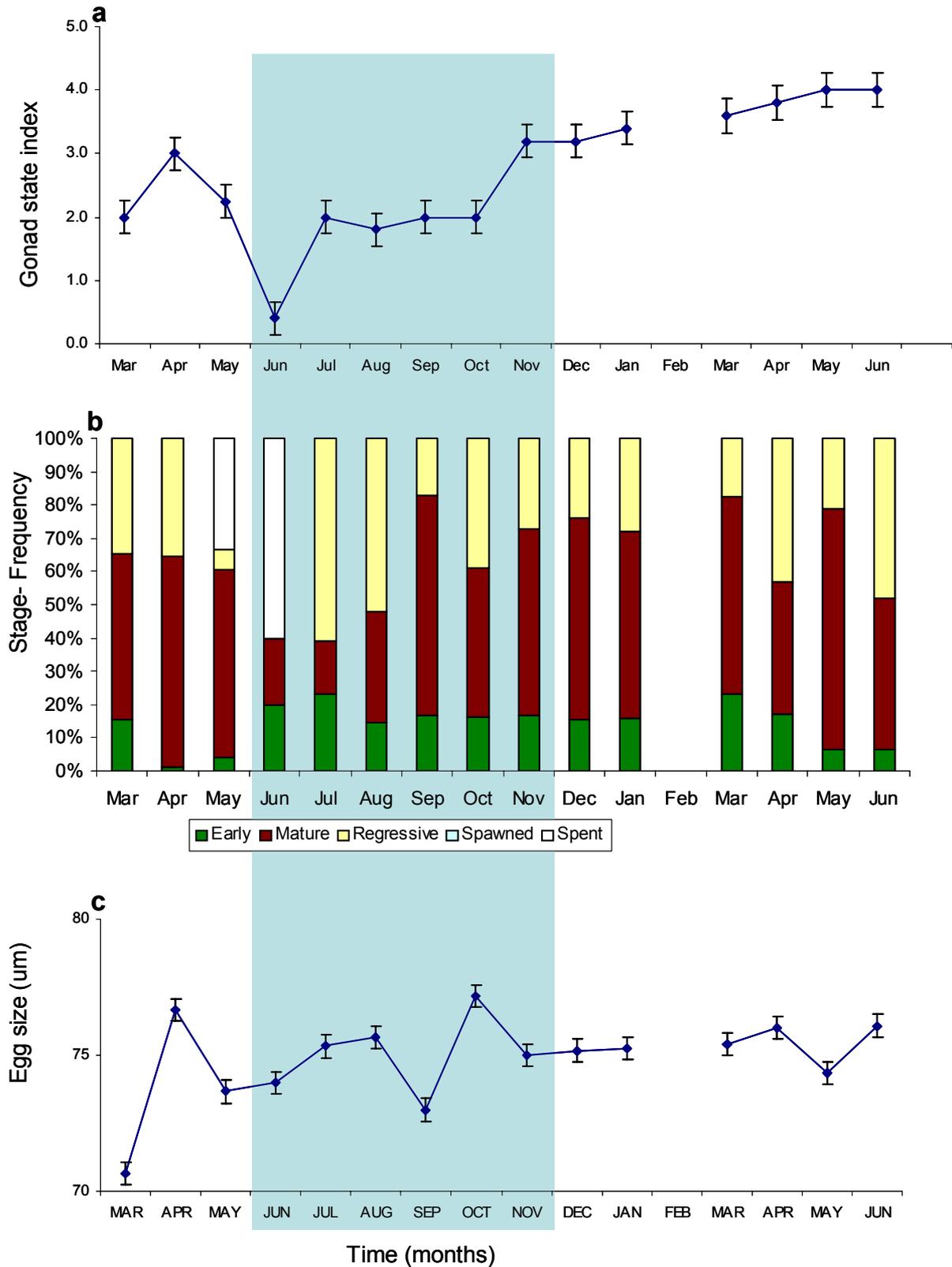


Figure 21. The gonad state index (a), frequencies of the different egg stages (b) and egg size (c) from the gonad biopsy extracts of *Tridacna nov. sp.* (n=5), showing progressive maturity of eggs (Aug-May) culminating in spawning (May-Jul), no bar indicates no data.

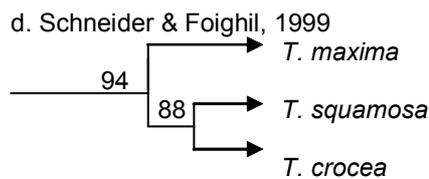
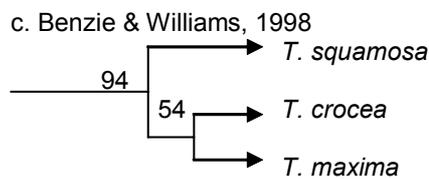
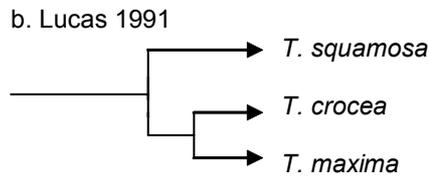
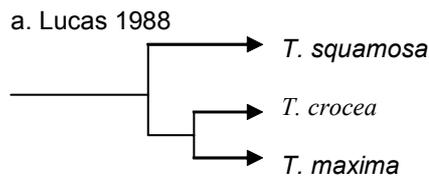
Early Pleistocene and in the Holocene, respectively (Schneider and Foighil, 1999; Newman and Gomez, 2002).

The existence of two groups within the subgenus *Chametrachea* is well-supported by molecular phylogenetic analysis (Benzie and Williams, 1998; Schneider and Foighil, 1999). The most likely position of the *Tridacna* nov. sp. in the subgenus would be together with *T. maxima*, considering its lower genetic distance and more shared characters.

Among the two extant Red Sea species, *T. maxima* has a wider range and is more common in the Red Sea proper up to its north-western limit in the northern Gulf of Aqaba (Chapter 2). This species also has the highest morphological variation among all tridacnid species (Rosewater, 1965). In the Red Sea, a *Tridacna elongatissima* (Bianconi, 1856) described by Rosewater (1965), having an elongate shell and one or two additional radial folds has been reported as a junior synonym of *T. maxima* (Bonfido et al., 1994). Albeit, the similar features with some of *T. maxima* variants with regards to morphological, behavioural and distribution patterns, our data on molecular analysis strongly support the separation of *Tridacna* nov. s.p. from *T. maxima* (bootstrap value of 98% MP and 100% NJ, Fig. 17). Furthermore, both species have distinct reproductive seasonality even at close proximity on the reef flats. *T. squamosa* adults are mostly seen in the deeper forereef, while, adults of both *T. maxima* and *Tridacna* nov. sp. are found on the shallow reef flats (Chapter 2).

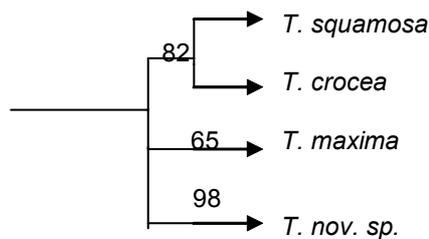
The two recently discovered species, *H. porcellanus* and *T. tevoroa*, with their restricted geographic ranges have been proposed as ancient relic lineages of Miocene origin (Schneider and Foighil, 1999). And, indeed *T. tevoroa* has been found to be a synonym of the fossil species in Fiji, *T. mbalavuana* (Ladd, 1934) living survivors at its eastern limit (Newman and Gomez, 2002). Newman and Gomez (2002) suggested Tridacninae as a relic subfamily contributing to the high endemism in the Southwest Pacific and Australia.

Is the *Tridacna* nov. sp. in the Red Sea a relic population at the northernmost limit of its geographic distribution? The absence of fossil specimens at present provides no evidence to this supposition, however, a survey at the fossil reefs along the coastline of Aqaba would provide a clue (T. Felis, pers. comm.). We can, however, speculate on this possibility based on following arguments: First, *Tridacna* nov. sp. seems to be restricted at the northwestern limit of the Red Sea. Although, we only have personal confirmation of the absence of specimens in the Red Sea proper (M. Hassan, pers. comm.), this species could possibly be limited to the tip of the Gulf of Aqaba, the northernmost fringing reefs in the IndoPacific. The northern Gulf of Aqaba is hydrographically distinct from the central and southern Gulf and Red Sea proper. Second, this new species occurs at the northwestern margin of the geographical range of its subcongeners, *T. maxima* an



e. Roa-Quiaoit, Kochzius & Richter, this paper

e.1. from molecular phylogeny of mitochondrial 16s rDNA



e.2. from morphological taxonomic classification

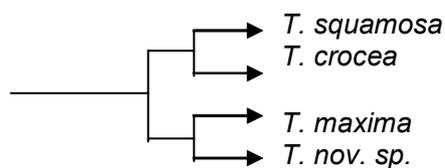


Figure 22. The different relationships of the giant clam species in the subgenus *Chametrachea* produced from literature, analysis from molecular phylogenies are indicated with bootstrap values (MP) (c-e).

*T. squamosa*, but still comes out as a distinctly separate species based on morphology, behavioral, reproductive and molecular phylogenetic analysis, as shown herein. The marginal occurrence suggests a cold-water tolerance of this species relative to its wide-ranged congeners. Third, it seems to have close affinity to the only one reported fossil species in the Red Sea, *Tridacna aegyptiaca* (Chenu, 1845) found in the Gulf of Suez whose single striking feature are the ‘deeply fold interstices’ (Rosewater, 1965). The deep radial folds distinguishing *Tridacna* nov. sp. from its subcongeners are only found in *T. gigas*, which fossil records show is of Miocene origin. *T. gigas*, however, are presently restricted in the Indo-Malayan region. This would support the suggestion of Benzie and Williams (1998) of a closer relationship between *Chametrachea* and *T. gigas*. These indications support the assumption that *Tridacna* sp. nov. may be a glacial relic species. Similar glacial relic forms have been invoked in relation to other organisms enduring in marginal environments, e.g. the unusually large species of *Rhincalanus* sp. inhabiting the oxygen minimum layer in the Red Sea (Wiekert, 1982).

Comparison of morphological and behavioural characteristics, mitochondrial rDNA analysis and reproductive study showed a strong indication of a new species of giant clams in the northwestern limit of its distribution. We can only speculate at this point that this new species could be a relic population on the northern border of its former range or a synonym of a fossil species. The detailed taxonomic description and diagnosis of *Tridacna* nov. sp is beyond the scope of this present study.

## **Acknowledgements**

We thank the Director and staff of the Marine Science Station, Aqaba for their support, especially M. Al-Zibdah, and Y. Ahmed; to C. Jantzen, S. Luna and M. Malik for the sample collection; to the Biotechnology and Molecular Genetics group, J. Timm, N. Silkenburmer, S. Todt, F. Meiyerjuren, R. Kotenkar, M. Marco and especially to Prof. Dr. Blohm; special mention to A. Nuryanto and T. Faroukh who have patiently guided the first author in the laborious genetic work. This research is part of the doctoral dissertation of H. Roa-Quiaoit, funded by the German Federal Ministry of Education and Research (BMBF) Grant No. 03F0356A-B.

## **Literature Cited**

- Benzie, J. & S. Williams. 1998. Phylogenetic relationships among giant clam species (Mollusca: Tridacnidae) determined by protein electrophoresis. *Marine Biology*, 132: 123-133.
- Benzie, J., S. Williams & J. Macaranas. 1993. Allozyme electrophoretic methods for analyzing genetic variation in giant clams (Tridacnidae). ACIAR Technical Report No. 23, 48 p.
- Bodoy, A. 1984. Assessment of human impact on giant clams (*Tridacna maxima*) near Jeddah, Saudi Arabia. *Proc. Symp. Coral Reef Environ. Red Sea, Jeddah* (Jan. 1984). 472-490.

- Bonfito, A., B. Sabelli, S. Tommasini and D. Herbert. 1994. Marine molluscan taxa from Mozambique described by G. G. Bianconi and preserved in the Zoological Museum of the University of Bologna. *Ann. Natal Mus.* 35:133-138.
- Brown, J. & M. Muskanofola. 1985. An investigation of stocks of giant clams (Family Tridacnidae) in Java and of their utilization and potential. *Aquacult. Fish. Manage.* 16(1):25-39.
- Gomez, E. and A. Alcalá. 1988. Giant clams in the Philippines. Pages 51-53 in J. Copland and J. Lucas, eds. *Giant Clams in Asia and the Pacific*. ACIAR Monograph No. 9, Canberra.
- Hilton-Taylor, C. 2000. 2000 IUCN Red List of Threatened Species. Gland, Switzerland and Cambridge, UK:IUCN.
- Juinio, A., R. Menez and E. Gomez. 1989. Status of giant clam resources of the Philippines. *J. Moll. Stud.* 55:431-440.
- Kilada, R., S. Zakaria & M. Farghalli. 1998. Distribution and abundance of the giant clam *Tridacna maxima* (Bivalvia: Tridacnidae) in the northern Red Sea. *Bull. Nat. Inst. of Oceanogr. & Fish., A.R.E.* 24:221-240.
- Kochzius, M., R. Soller, M. Khalaf and D. Blohm. 2003. Molecular phylogeny of the lionfish general *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 28:396-403.
- Lucas, J., E. Ledua & R. Braley. 1991. *Tridacna tevoroa* Lucas, Ledua and Braley: a recently described species of giant clam (Bivalvia: Tridacnidae) from Fiji and Tonga. *Nautilus*, 105:92-103.
- Mergner, H. & Schuhmacher H. 1974. Morphologie, Ökologie und Zonierung von Korallenriffen bei Aqaba, (Golf von Aqaba, Rotes Meer). *Helgolander wissenschaftliche Meeresuntersuchungen*. 26: 238-358.
- Newman, W. and E. Gomez. 2002. On the status of giant clams, relics of Tethys (Molusca: Bivalvia: Tridacnidae). *Proc. 4th Int. Coral Reef Symp.* (2), pp. 927-936.
- Palumbi, S., A. Martin, S. Roman, W. McMillan, L. Stice and G. Grabowski. 1991. The simple fool's guide to PCR. (Version 2) (unpubl.)
- Pandolfi, JM, Bradbury, RH; Sala, E; Hughes, TP; Bjorndal, KA; Cooke, RG; McArdle, D; McClenachan, L; Newman, MJH; Paredes, G; Warner, RR; Jackson, JBC. 2003. Global trajectories of the long-term decline of coral reef ecosystems. *Science (Washington [Science (Wash.)])* 301 (5635):955-958.
- Roa-Quiaoit, H., M. Al-Zibdeh and C. Richter. In prep. Reproductive periodicity of giant clams (Tridacnidae) in the northern Gulf of Aqaba, Red Sea.
- Rosewater, J. 1965. The family Tridacnidae in the Indo-Pacific. *Indo-Pacif Mollusca*, 1:347-394.
- Rosewater, J. 1982. A new species of *Hippopus* (Bivalvia: Tridacnidae). *Nautilus*, 96: 3-6.
- Ross, A. and W. Newman. 2002. Coral barnacles: Cenozoic decline and extinction in the Atlantic/East Pacific versus diversification in the Indo-West Pacific. *Proc. 4th Int. Coral Reef Symp.* (2), pp. 179-184.

- Sirenko, B. and O. Scarlati. 1991. *Tridacna rosewateri* sp. n., a new species of giant clam from the Indian Ocean. *La Conchiglia* 22(261):4-9.
- Schneider, J and O. Foighil. 1999. Phylogeny of giant clams (Cardiidae: Tridacninae) based on partial mitochondrial 16s rDNA gene sequences. *Molecu. Phylogen. Evol.* 13(1):59-66.
- Soller, R.,W. Warnke, U. Saint-Paul and D. Blohm. 2000. Sequence divergence of mitochondrial DNA indicates cryptic biodiversity in *Octopus vulgaris* and supports the taxonomic distinctiveness of *Octopus mimus* Cephalopoda: Octopodie. *Mar. Biol.* 136:29-35.
- Swofford, D. 1998. PAUP\*. Phylogenetic analysis using parsimony (\*and other methods). Version 4 Sinauer, Sunderland.
- Veron, J. 2002. Corals of the World, 3 volumes, Australian Institute of Marine Science, Townsville, Australia.
- Walsh, P., D. Metzger and R. Higuchi. 1991. Chelex100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10(4):506-513.
- Wiekert, H. 1982. The vertical distribution of zooplankton in relation to habitat zones in the area of the Atlantis II Deep, Central Red Sea. *Mar. Ecol. Prog. Ser.* 8:129-143.
- Wells, S., R. Pyle & N. Collins.1983. Giant clams. In: *The IUCN Invertebrate Red Data Book*, pp. 97-107. IUCN, Gland, Switzerland.

## Chapter 5: Giant Clam Culture Protocol for Jordan, Gulf of Aqaba, Red Sea

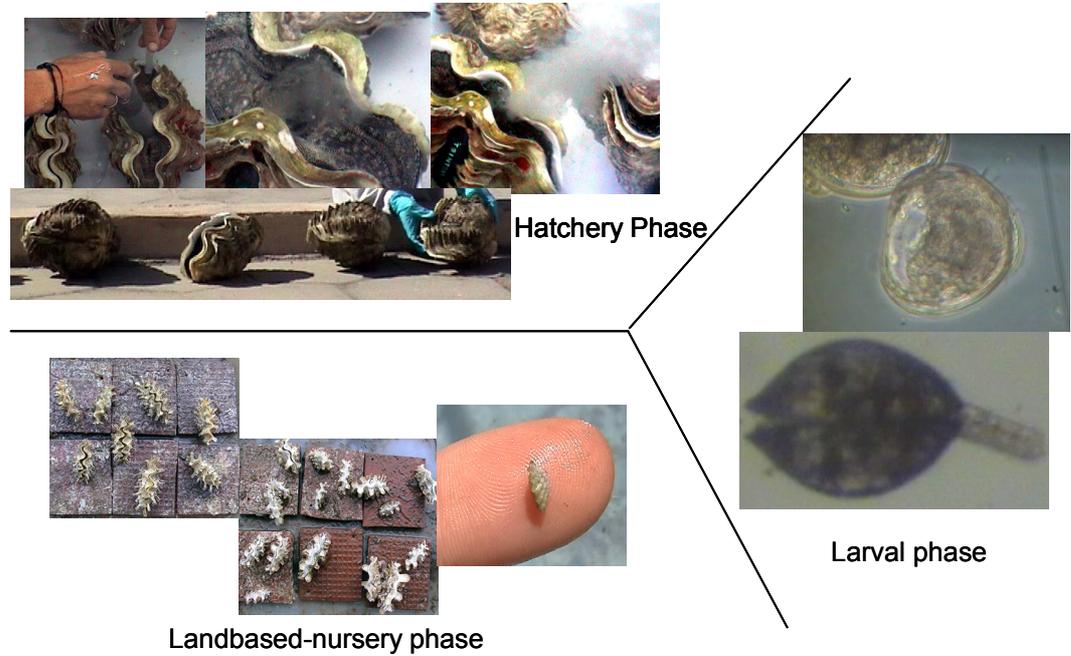


Figure 23. Giant clam culture: spawning of gametes, hatching into larvae and metamorphosing to visible clam.

# Giant Clam Culture Protocol

## for Jordan, Gulf of Aqaba, Red Sea

### I. Spawning Preparation

Day 0

#### 1.1 Prepare the following:

- spawning tank (white plastic, 800 L)
- hatching tank (1- 3 white plastic tanks, 1000 L)
- holding tank for BS (flowing water)
- fertilization bins (3- 4 blue barrels)
- drying rack/ table
- brush
- chlorine and chlorine bath tank
- filter cartridges (5, 10, 25  $\mu\text{m}$ ) or
- filter bags: (5, 10, 25  $\mu\text{m}$  screen) (fine & coarse linen bags)
- data sheet A

#### 1.2 Land-based:

1. Clean spawning tank and fill with seawater up to water depths of 20- 30 cm. Make sure the water is not flowing but with aeration.
2. Fill fertilization bins with 5  $\mu\text{m}$  filtered seawater (FSW), aerate and keep under the shade.
3. Fill hatching tanks with 5  $\mu\text{m}$  FSW, aerate but no flow. Cover with clean plastic.
4. Fill one tank with chlorine and dilute with tap water (chlorine-bath).
5. Dip all materials for spawning in the chlorine tank and rinse with freshwater (refer to list of materials).
6. Dry on racks/ table near the spawning tank.

#### 1.3 Clam Broodstocks (BS):

**Note:** Collect broodstocks for spawning from the field, one day prior to spawning and transport to the station by laying them on their valves. Hold in separate tank with flowing seawater. Due to high temperatures in Aqaba from late spring to early autumn (May-September), heat stress is the change in temperature from one-hour sun-exposure (50°C) to immersion in cold water (24-26°C), instead of warm water as in the tropics. This modification has been found effective in stimulating the clams to spawn.

7. Brush the clams (around 10 am of the spawning day) and place under direct sun, upright on their umbos (emersion) for an hour (heat stress).
8. Transfer broodstock to spawning tank (static water), wash shell, put in water with umbo up (or mantle facing down), turning upright at the tank bottom to release trapped air bubbles (which are harmful to the clams).
9. List tag and order of the clams in the tank to facilitate recording of spawning later.
10. Make sure the excurrent siphon faces not towards tank center but towards the collector for easy sperm collection.

## **II. Spawning Procedure**

### 2.1 Prepare the following:

- serotonin (2 mM → 0.0136 g in 20 ml FSW)
  - alcohol
  - distilled water
  - injections
  - pipette (500 µl and 1000 µl)
  - buckets
  - flow-through screen or flow-through canisters
  - drain hose
  - microscope
  - counting cells/ rafter counter
  - eyepiece micrometer
  - counters
  - plunger
  - data sheet B
11. Inject the clams intragonadally, at least 1" from the exhalant siphon. Needle vertical – not pointing toward direction of kidney (which is under excurrent siphon). If in doubt, inject at 1.5" from the siphon.
  12. When clams start releasing sperm (Fig. 23) after 1- 3 min, collect at least 2 liters from each clam and dispose the rest. Keep the sperm under shade.
  13. Collection: catch sperms by buckets or use long airhose with funnel over the excurrent siphon to concentrate sperm into 10-20L pails. Make sure pails are labeled to prevent mixture of sperms.

14. When a clam starts releasing egg (Fig. 23), remove it from the tank, rinse with FSW and place in the fertilization bin where the eggs will be further released.
15. To facilitate visual of eggs, use magnifying glass or bottom of glass beakers to examine water column.
16. At least after 15 minutes from spawning, fertilize the eggs with sperm from different clams. Exclude the sperm of the egg-releasing clam!
17. Add freshly released sperm only, not refrigerated sperm from a day- earlier spawning.
18. The amount of sperm to be added to egg-water depends on density of sperm in the sperm water – if very cloudy, add 1 ml/ l egg-water; if less cloudy, up to 4- 5 ml/l egg-water.

### **III. Estimate of egg count**

19. Fill fertilization bin to a known volume.
20. Mix the water evenly by using a plunger and take 6- 10 x 1 ml samples using pipettor and sedgewick rafter counter/ tissue culture plate (0,5 ml volume; hence, x 2 when calculating for mean density per ml).
21. Count eggs through compound or dissecting microscope.
22. Compute for number of eggs (data sheet B).
23. Measure 30- 50 eggs.

### **IV. Stocking of eggs (20- 25 eggs/ ml)**

24. Transfer egg water of known volume to hatching tank (water temp= 26 – 30 °C).
25. Add moderate aeration to keep eggs in suspension.
26. For flowing through hatching tank, add flow-through screen or flowing canister (55 µm) (see Figs. x) and relatively slow flow. Regularly check tanks for overflows, in cases where the screens are clogged (e.g. by mucus or algae).

### **V. Fertilization rate**

27. Two to three hours after, check fertilization rate (data sheet C).
28. Take 3 x 1 ml samples, if density is high. If low density, filter 1- 2 L to a 55 µm sieve, collect and placed in a petri dish.
29. Count 100 eggs and note down the number of eggs that divided. Take 3 counts of 100 minimum and then average. The number is equal to percentage and represents the fertilization rate.

## **VI. Check-up**

**Day 1**

30. Next day, check the bottom tank for bacterial growth (small colored patches) or dense aggregations of unfertilized eggs or other materials.
31. If present, turn off air for 30 min to an hour. Afterwards, siphon bottom.
32. Chlorine-rinsed all materials after use.

## **VII. Retrieving veligers (1- 3 veligers/ ml)**

**Day 2**

7.1 Prepare the following:

- chlorine bath
  - bins
  - siphon hoses
  - filter sieves (50 & 80  $\mu\text{m}$ )
  - filter cartridges or filter bags (5 $\mu\text{m}$ ) (10  $\mu\text{m}$ ) (fine) (coarse)
  - flow-through screen/ -canister
  - drain hose
  - overflow bucket
  - buckets
  - tray
  - aerators/ airstones
  - plunger
  - pipette
  - counting cells/ rafter counter
  - microscope
  - eyepiece micrometer
  - counter
  - data sheet D
33. Prepare chlorine-cleaned larval tanks and fill with FSW. Install the flow-through nets or canisters. Put the settling substrates (after 1day conditioning in flowing seawater) on the tank bottom.
  34. In the hatching tanks, siphon water column 5 cm from the bottom into 50  $\mu\text{m}$  sieve in the overflow bucket (bucket with drain pipe). Make sure the veligers are immersed in water in the sieves while draining.
  35. When sieve is clogged or filled with veligers, wash sieve into the bins through a 200  $\mu\text{m}$  sieve using strong water flow

36. Get 6- 10 x 1 ml samples (same procedure as in egg count) and count in the microscope (data sheet D).
37. Measure 30- 50 veligers.
38. Stock veligers (1- 3 veligers/ ml) into the larval tanks with moderate aeration. Inlet hose should be connected to a filter-cartridge or filter bags (both down to at least 5 µm).
39. Check regularly for tank overflow. Change nets daily if dense or just clean net (by hand) if not too dense for at least 2 days, then change.

## **VIII. Algal feeding**

**Day 3**

40. Start algal feeding (1,000 – 15,000 cells/ml)
41. Live algal culture (e.g. *Isochrysis* sp., *Nanochloropsis* sp.)
42. Freeze dried algae/ off-the-shelf algae (\*if available)
43. Feed daily or every two days (days 03, 05, 08, 10, 12)

## **IX. Zooxanthellae**

**Day 8**

44. Clip mantle of adult clams of the same species or use zooxanthellae of a more common species which can be sacrificed (in case of high larval batch).
45. Blend in FSW for 1 min. using ordinary blender or homogenizer.
46. Pass solution through a 20 µm sieve to remove mantle tissues.
47. Count zooxanthellae density of the filtrate using haemocytometer. Preferred density in the larval tank is 20 – 100 cells/ml or more.
48. Stock known volume to the larval tank, stop water flow but continue aeration.
49. Preferably, add zooxanthellae again after settlement, days 14 onwards.

## **X. Retrieving pedi-veligers (optional only)**

**Day 9**

50. Prepare materials for retrieval (refer to VII).
51. Siphon tank bottom into 50 µm sieves.
52. Stock veligers in settlement tanks with low aeration for 1 day, to ensure attachment on the bottom.
53. Following day, increase aeration to moderate. Start moderate water flow. Put filter bags or filter cartridge down to 5 µm.

## **XI. Post-settlement**

**Day >14**

54. Remove the flow-through screens over the tank drain on day 14.
55. Make sure water circulates at the bottom, inlet water on bottom and drain at mid-water column.

56. Use filter bags over inlets, 5  $\mu\text{m}$  <1-month and 10-25  $\mu\text{m}$  1-3 months.

## **XII. Harvesting juveniles**

**2-3 months**

57. Harvest juveniles after 2 – 3 months when visible to the eye.
58. Leave settled clams on old substrates as is.
59. Scrape bottom with sharp blade and siphon out juveniles (to prevent clams from being pulled by water current) into overflow bucket.
60. Sort bottom of bucket for clams.
61. Measure 30-50 clams and put on new substrates, with slow aeration for 1 day for attachment..
62. Start spiking water with 20-50  $\mu\text{M}$  dissolved inorganic nitrogen, 3x/ week.



**DATASHEET B: ESTIMATE OF EGG COUNT**

Species: ..... Total N:.....eggs

Day:..... Date: ..... SD:

Time: ..... Stocking Rates:

By: ..... Tank(s) #:

Sample # #:..... (ml):.....	Bin/Tank #..... Volume (ml):..... [n/ml]	Bin/ Tank #:..... Volume (ml):..... [n/ml]	Bin/Tank Volume [n/ml]
1	= .....	= .....	= .....
2	= .....	= .....	= .....
3	= .....	= .....	= .....
4	= .....	= .....	= .....
5	= .....	= .....	= .....
6	= .....	= .....	= .....
7	= .....	= .....	= .....
8	= .....	= .....	= .....
9	= .....	= .....	= .....
10	= .....	= .....	= .....
<b>Mean:</b>	.....	.....	.....
<b>Total eggs:</b>	.....	.....	.....

Size Measurement (n = 50)      Average Size:

Calibration Factor: .....

Remarks:

**DATASHEET C: FERTILIZATION RATE**

Species: ..... Spawning Date: .....

Day:.....

Stage:.....

Bin/Tank #: .....	Sample	Fertilized Eggs	Total N	FRate (%)
	1	.....	.....	.....
	2	.....	.....	.....
	3	.....	.....	.....
	mean:			.....

Bin/Tank #: .....	Sample	Fertilized Eggs	Total N	FRate (%)
	1	.....	.....	.....
	2	.....	.....	.....
	3	.....	.....	.....
	mean:		.....	

Bin/Tank #: .....	Sample	Fertilized Eggs	Total N	FRate (%)
	1	.....	.....	.....
	2	.....	.....	.....
	3	.....	.....	.....
	mean:			.....

Bin/Tank #: .....	Sample	Fertilized Eggs	Total N	FRate (%)
	1	.....	.....	.....
	2	.....	.....	.....
	3	.....	.....	.....
	mean:			.....

Remarks:

**DATASHEET D: ESTIMATE OF VELIGER COUNT**

Species: ..... Total N:.....eggs

Day:..... Date: ..... SD:

Time: ..... Stocking Rates:

By: ..... Tank(s) #:

Sample #	Bin/Tank #:..... Volume (ml):..... [n/ml]	Bin/ Tank #:..... Volume (ml):..... [n/ml]	Bin/Tank #:..... Volume (ml):..... [n/ml]
1	= .....	= .....	= .....
2	= .....	= .....	= .....
3	= .....	= .....	= .....
4	= .....	= .....	= .....
5	= .....	= .....	= .....
6	= .....	= .....	= .....
7	= .....	= .....	= .....
8	= .....	= .....	= .....
9	= .....	= .....	= .....
10	= .....	= .....	= .....
<b>Mean:</b>	.....	.....	.....
<b>Total eggs:</b>	.....	.....	.....

Size Measurement (n = 50)      Average Size:

Calibration Factor: .....

Remarks:

## **Chapter 6: Contributions**

In all instances, the main contribution in terms of data acquisition, analysis, and writing has been by me, with contributions from Dr. C. Richter (marine biology and ecology), Dr. M. Al-Zibdah (culture facilities) and Dr. M. Kochzius (molecular genetics). The specific contributions are as follows:

**1. Distribution, Abundance, Size And Growth Of Giant Clam (Tridacnidae) In The Northern Gulf Of Aqaba, Red Sea**

The conceptualization of the study was done by Dr. C. Richter and myself. I did the field surveys with logistic support from the Aqaba Marine Science Station (MSS), did the data processing and analysis, and writing of the manuscript. The manuscript was revised with additional inputs from Dr. C. Richter.

**2. Reproductive periodicity of giant clams (Tridacnidae) in the northern Gulf of Aqaba, Red Sea**

I developed the concept of the study with improvements from Dr. C. Richter. I did the in situ gonad biopsy with logistic support from MSS, carried out the microscopic examination, data processing and analysis. The culture work was assisted by MSS staff under the supervision of Dr. M. Al-Zibdah. Data collection was done by myself. The manuscript was improved by Dr. C. Richter.

**3. Morphological, Reproductive and Molecular Genetic Evidence for a New Species of Giant Clam (Tridacnidae) in the Northern Red Sea**

The concept was developed by Dr. C. Richter and myself. I did the morphological and behavioral classification, and, in situ gonad biopsy including microscopic work, data processing and analysis on these aspects. I carried out all aspects of the genetic work under the supervision of Dr. M. Kochzius at the Biotechnology and Molecular Genetics laboratory. The molecular data analysis was done by myself with advice by Dr. M. Kochzius. I wrote the manuscript with revisions and improvements from Drs. Kochzius and Richter.

**4. Culture manual of giant clams in the northern Red Sea**

The manual was conceived, developed and written by me. The culture work was supported by MSS staff under the supervision of Dr. M. Al-Zibdah.

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Erklärung:

Hiermit erkläre ich, dass ich die vorliegende Dissertationsschrift selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet habe.

Bremen, den 30. August 2005

Hilly Ann Roa-Quiaoit