ISSN 0970 - 6011

Volume 57, Number 1, 2010

Indian Journal of Fisheries

Indian Council of Agricultural Research

Published by
The Director
Central Marine Fisheries Research Institute
Cochin - 682 018, Kerala, India
www.cmfri.org.in

Indian J. Fish., 57(1) : 39-43, 2010

Comparative regeneration of excised mantle tissue in one year and seven year old Indian pearl oyster, *Pinctada fucata* (Gould) grown under land-based culture system

G. SYDA RAO, PHALGUNI PATTNAIK* AND BISWAJIT DASH*

Central Marine Fisheries Research Institute, Post Box No. 1603, Ernakulam North P. O. Kochi - 682 018, Kerala, India

**Visakhapatnam Regional Centre of Central Marine Fisheries Research Institute Visakhapatnam - 530 003, Andhra Pradesh, India*

e-mail: gsydarao@gmail.com

ABSTRACT

Excised mantle tissue (saibo) from the donor oyster is one of the important factors determining quality of cultured pearls. The present study was conducted to compare the process of regeneration of excised mantle tissue in one year and seven year old donor oysters, *Pinctada fucata* grown under land-based culture system. Menthol was used as relaxant prior to excision of mantle tissue, which was found to be effective at a concentration of 500 mg l^{-1}. The mantle tissue was found to regenerate within 3 months post-excision in both one year as well as seven year old *P. fucata,* with 100% survival. On gross examination, the regenerated mantle tissue of the 1 year as well as the 7 year old oysters appeared similar to that of the normal mantle tissue of the control group. Histological analysis demonstrated complete regeneration of the mantle tissue and its associated structures in both the groups. This is the first description of *in vivo* mantle regeneration in 7 year old pearl oyster, *P. fucata*. The findings revealed that even aged donor oysters yielding good quality saibo can be recovered after mantle excision and could be further used as saibo donors for quality pearl production.

Keywords: Excised mantle tissue, Indian Pearl oyster, Mantle regeneration, *Pinctada fucata*, Saibo

Introduction

The major species of oysters used for cultured pearl production world over are the Akoya pearl oyster (*Pinctada fucata*, Gould), the silver/gold-lip pearl oyster (*Pinctada maxima*, Jameson) and the black-lip pearl oyster (*Pinctada margaritifera*, Linnaeus) from the family Pteriidae. Of these, *P. fucata* and *P. margaritifera* are of major importance with regard to availability and production of cultured round pearls as well as mabe pearls in India (Alagarswami, 1991; James *et al.*, 1991; Dev and Durairaj, 1993; Kripa *et al*, 2007; Syda Rao, 2005; 2007). Studies were initiated on propagation of cultured round pearls about a century ago (Saville-Kent, 1893; George, 1966; Gervis and Sims, 1992). Cultured round pearls are produced by grafting a round nucleus and a piece of mantle tissue or 'saibo' from a sacrificed donor oyster into the gonad of a recipient oyster (Acosta-Salmon *et al.,* 2004). Subsequent proliferation of the donor mantle tissue forms the pearl sac around the nucleus and continued deposition of nacre from the pearl sac onto the nucleus forms a cultured pearl (Gervis and Sims, 1992; Acosta-Salmon *et al.,* 2004). The quality of cultured pearl depends greatly on the selection of appropriate donor oysters (Taylor, 2002)

Under normal circumstances, donor oysters are sacrificed during excision of mantle tissue. Recently, attempts have been made on *in vivo* regeneration of excised mantle tissue in different species of pearl oysters and the oysters were found to be able to survive after excision of mantle and were also found capable of complete regeneration of the excised mantle and its internal structures (Acosta-Salmon *et al.,* 2004; Acosta-Salmon and Southgate, 2005, 2006; Mamangkey and Southgate, 2009). Even though, tissue/organ regeneration is a feature of every phyla in the animal kingdom, it varies among taxa and affected by several factors including age and cell complexity (Goss, 1969). So far, no attempt has been made to assess the *in vivo* regeneration of mantle tissue in pearl oysters grown for more than 5 years. The present work is a maiden attempt to evaluate the *in vivo* regeneration capability of the excised mantle tissue in response to age, in *P. fucata* grown under land-based culture system.

Materials and methods

The pearl oysters (*P. fucata*), used in this study were hatchery produced following the techniques of Alagarswami *et al.* (1983) and were cultured in land-based system (Syda Rao, 2001; 2007) at the Regional Centre of the Central Marine Fisheries Research Institute, Visakhapatnam, India. Briefly, the oysters were grown in specially designed concrete tanks having 10 t water holding capacity. They were maintained on the bottom of the tanks provided with suitable substratum to facilitate attachment of oysters. Pearl oysters were fed on a mixed diet of three species of algae *viz*., *Chaetoceros calcitrans*, *Isochrysis galbana* and *Nanochloropsis salina* by a specially designed drip method, so that the algal cell concentration in the tanks was continuously and automatically maintained at the desired rate (Syda Rao, 2001).They were gradually thinned as they grew, to a stocking rate of 60–70 nos. per m^2 by the time they reached implantation size. Growth recorded under land-based conditions was faster as compared to sea-based culture, owing to continuous availability of feed at required levels (Syda Rao, 2005). Mortality recorded under such land-based culture system was negligible.

Ten numbers each of 7 year old (7Y) oysters grown under land-based system as described above during the period 1998 to 2005, having a mean (± S.D.) dorso-ventral measurement (DVM) of 82.77 ± 12.68 mm and one year old (1Y) hatchery produced oysters with mean DVM of 56.17 ± 6.93 mm were used for the study. Studies in our laboratory have shown that menthol (-2-Isopropyl-5-methyl cyclohexanol, $C_{10}H_{20}O$) at a concentration of 500 mg l^{-1} induced relaxations in *P. fucata* over a short period of time *i.e*., within 15 to 30 min of exposure allowing rapid recovery within 30 to 60 min post-excision, without any mortality. The same method was used in the present study prior to mantle excision. The oysters were anesthetized with 500 mg l^{-1} menthol for relaxation. Excision of mantle tissue was made from either the left or the right mantle lobe. The mantle tissue removed was approximately 5 mm x 20 mm in size. Following mantle excision, the two groups of oysters were returned to separate culture tanks immediately, maintained as described earlier and the survival was recorded over a period of 3 months post-excision. Control groups of 7Y oysters without mantle excision were also maintained under the same environmental conditions for comparison. The oysters were subjected to gross examination of the healed mantle, subsequently they were anaesthetized using menthol as described earlier and samples of mantle tissue were excised from normal 7Y oysters as well as from the regenerated mantle of both 7Y and 1Y oysters for histological examination. The fresh tissue samples were fixed in Bouin's fluid for 24 h. They were then dehydrated in graded series of alcohol, embedded in paraffin, sectioned to 5 µm thickness and stained with Periodic acid Schiff-Light Green (PAS-LG), Alcian blue-PAS and Azan as per the procedures described by Humason (1967) and Bancroft (1975).

Results

Application of menthol at a concentration of 500 mg l^{-1} resulted in relaxation in 50% of oysters within 10 min and in the rest 30% within 15-30 min. There was relaxation without body collapse while mantle collapse was observed in 3% of the oysters, in the 7Y group. In both the treatment groups as well as in the control group, survival recorded was 100% during the period of 3 months observation. There was complete regeneration of the mantle within 58 days after mantle excision in 1Y oysters while it took 82 days to complete the process in 7Y oysters. Byssal secretion was noticed only in 1Y oysters while 7Y oysters had lost the power of byssal secretion even at the onset of the experiment. However, they were active in feeding.

Histological analysis of 7Y oysters showed complete regeneration of the mantle tissue (MT), 82 days post-excision of mantle. The regenerated and the normal MT showed typical bivalve mantle morphology with the marginal (with the inner, middle and the outer folds), pallial and central zones and secretion of conchiolin, in both 1Y and 7Y groups (Fig. 1, 2 and 3). The inner and outer surfaces of the mantle epithelium were well defined with ciliated epithelial cells. The inner fold of the regenerated mantle tissue was found to be larger in size than the other folds with large amount of muscle fibers. There were secretory cells in both inner and outer epithelia in the regenerated tissue (Fig. 3). The growth of tissue at the point of excision proceeded in the typical manner and there was no difference in the tissue aggregation and differentiation into respective layers of the regenerated MT of 1Y and 7Y groups. Both the longitudinal and radial muscles at the pallial zone were well developed in both the cases. There was no appreciable microscopic differentiation in the pallial zones, the outer epithelium of the secretory basophilic cells and the mucous secretory cells, either between the regenerated mantle of 1Y and 7Y groups or between the normal MT and regenerated MT of 7Y oysters. There was no abnormality observed in the anatomy of the mantle regeneration process in 7Y oysters as compared to 1Y oysters. However, the prismatic layer appeared darker in 1Y MT than that of the 7Y MT. The new nacre secreted on the inner surface of the shell adjacent to the wound area appeared thinner and irregular in 7Y regenerated oysters as compared to that of 1Y group and control 7Y group.

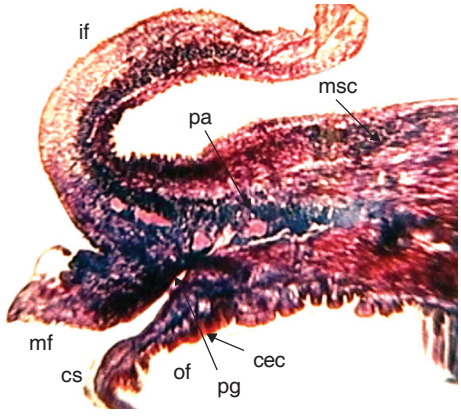

Fig. 1. Histological section showing regenerated mantle folds in 1 year old *Pinctada fucata*, 58 days after mantle excision (Azan; X 200).
pa: artery, cec: columnar epithelial cells, msc: muscle, cs: conchiolin secretion, if: inner fold, mf: middle fold, of: outer fold, pg: periostracal groove.

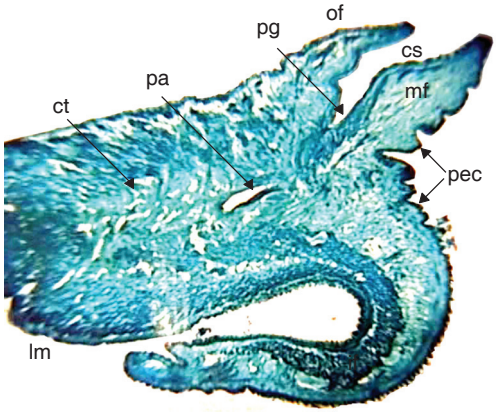

Fig. 2. Photomicrograph of normal mantle tissue of 7 year old *P. fucata* (PAS-LG; X 200)
if: inner fold, mf: middle fold, of: outer fold, cs: conchiolin secretion, pec: pigmented epithelial cells, lm: longitudinal muscles, ct: connective tissue, pa: pallial artery, pg: periostracal groove.

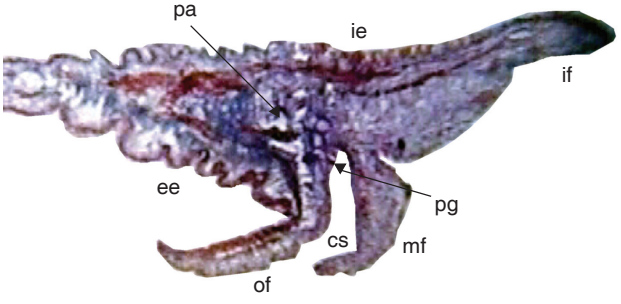

Fig. 3. Histological section of regenerated mantle tissue of 7 year old *P. fucata*, 82 days after mantle excision (Alcian blue-PAS; X 100).
if: inner fold, mf: middle fold, of: outer fold, cs: conchiolin secretion, ee: external epithelium, ie: internal epithelium, pa: pallial artery, pg: periostracal groove

Discussion

Relaxants have been used with pearl oysters to facilitate internal inspections, seeding operations for pearl production and also to obtain saibo tissue from the donor oysters (Norton *et al*., 2000; O'Connor and Lawler, 2003; Acosta-Salmon *et al*., 2004; Acosta-Salmon and Southgate, 2005). The use of an appropriate relaxant facilitates removal of saibo tissue from live oysters without killing the oysters. A number of chemicals have been used to relax pearl oysters with varying success and limitations (Norton *et al*., 1996; Acosta-Salmon and Rangel-Devalos, 1997; Saucedo *et al*., 2001; O'Connor and Lawler, 2003). Norton *et al.* (2000) evaluated the quality of pearls produced using relaxed oysters. However, they did not use relaxed mantle tissue as saibo. Acosta-Salmon *et al.* (2004) used 500 mg l^{-1} benzocaine for relaxing *P. fucata* with appreciable results. During the present study, it was found that menthol at a concentration of 500 mg l^{-1} can be used safely for relaxation of both 7Y as well as 1Y oysters. There was relaxation without body collapse while mantle collapse was observed in 3% of the oysters, mostly in the 7Y group, which is quite acceptable when the number of oysters taken into account is high. Acosta–Salmon *et al.* (2005) opined that oysters showing body collapse but no mantle collapse could still be used as saibo donors. Tranter (1957) used menthol crystals on *P. maxima* and Dev (1994) used powdered menthol in *P. fucata*. But so far, effective concentration of menthol has been scarcely reported for relaxation of oysters without mantle collapse.

Our studies demonstrated the regeneration process of excised mantle tissue in 7Y and 1Y oysters and also the morphological similarity between regenerated mantle tissue and the normal mantle tissue in 7Y oysters. The regeneration process was found to be complete within 3 months as indicated by the restoration of physical as well as functional tissue structure characterized by the three mantle folds, secretary cells, muscle fibres and conchiolin secretion. Similar results were reported in young *P. fucata* by Acosta–Salmon and Southgate (2005). However, Carlson (2007) reported incomplete restoration of mantle tissue possibly caused by nutritional or other types of deficiency.

Gervis and Sims (1992) reported that the age of a pearl oyster influences the speed of mantle regeneration. For cultured pearl production, older pearl oysters are generally preferred to be used as saibo donors than young ones owing to their slower growth rate. The saibo tissue from such oysters secrete nacre at a slower rate resulting in better quality pearls (Grevis and Sims, 1992). Garcia-Gasca *et al.* (1994) reported that large secretory basophilic cells play an important role in nacre layering. From the fact that in 7Y oysters the mantle tissue took about 24 days more to grow back to its normal size than that of 1Y oysters, it is

quite evident that mantle regeneration is rather a normal process but the time of recovery may differ in accordance to the age of the oyster. This may be attributed to the difference in efficiency in the nutrient storage and utilization. Studies have shown that mantle tissue functions as a site for storage of nutrients in bivalves (Barber and Blake, 1981; Mathieu and Lubet, 1993; Pekkarinen, 1994; Acosta-Salmon *et al.*, 2004). Carlson (2007) stated that nutritional deficiency influences the mantle regeneration process. In the land-based culture conditions as used in the present investigation, the physico-chemical environment and the nutrient availability were kept nearly optimal (Syda Rao, 2007). This might have lead to early mantle regeneration in 1 Y oysters *i. e.*, in 58 days as compared to that of 60 days or more (Acosta-Salmon and Southgate, 2005) recorded under sea based culture conditions. Further, the size of excised mantle tissue also influences the time required for complete healing.

Following mantle excision, pearl oysters are susceptible to the ambient environmental conditions. It has been observed that the feeding process slows down up to 2 days after mantle excision. This may be due to significant loss of mantle tissue or due to the excision shock. Earlier studies have recognized that the rate of wound healing in pearl oysters is likely to be influenced by their physiological state (Acosta-Salmon and Southgate, 2005; 2006) and by seasonal patterns of nutrient storage which have been described for marine bivalves including pearl oysters (Saucedo *et al.*, 2002). Mamangkey and Southgate (2009) found that wound healing with epithelialization was completed within 3-4 days of mantle excision while morphogenesis took 3 months. Carlson (2007) stated that complete regeneration requires all cellular functions to be in place and this process takes much longer than the wound healing. Acosta-Salmon and Southgate (2005) reported that following the mantle excision, both the ends rolled inwards to the centre reducing the size of wound, minimizing hemorrhaging. Regeneration was rapid and complete, once sealing of wound has occurred. Similar pattern was reported during siphon regeneration in *Scrobicularia plana* (Hodgson, 1982) and in *Macoma balthica* (Pekkarinen, 1994). Acosta-Salmon *et al.* (2004) opined that damage of mantle tissue and the energetic cost of the healing and regeneration process do not interfere with other body functions. They reported the regeneration of mantle tissue *in vivo* in *P. fucata* cultured in long lines and have shown that oysters used as saibo donors need not be killed and could be used as future brood stock and as future saibo donors. Maintaining a pool of 3-4 year old population will be promising for continuous availability of desired saibo of suitable quality.

The present study is the first description of *in vivo* mantle regeneration in 7 year old pearl oyster, *P. fucata*. The results indicated that oysters can regenerate the excised mantle tissue in less than three months irrespective of age under land-based culture conditions. Though, a few of the earlier studies have reported regeneration of mantle tissue in pearl oysters, until now no study has been done on comparative regeneration process of mantle tissue with respect to age. The findings of the present study make a significant contribution to our knowledge on tissue regeneration process in pearl oysters with respect to age. As the breeding efficiency declines sharply after 4 years of growth in *P. fucata* (Syda Rao, 2007), oysters above 4 years producing high quality pearls could possibly be used as saibo donors repeatedly, without sacrificing the animal. Demonstration of regeneration of MT even in the aged donor *in vivo* under captive conditions in the present study indicate that a pool of high quality donor oysters can be maintained as future saibo donors. The regeneration capacity of the excised mantle tissue, also makes it possible to make use of good quality saibo donors in selective breeding programmes.

Acknowledgements

We thank Prof. (Dr.) Mohan Joseph Modayil, former Director, Central Marine Fisheries Research Institute, Kochi for constant encouragement. Thanks are also due to our colleagues, Mrs. S. Padmaja Rani and Mr. T. Nageswara Rao, Visakhapatnam RC of CMFRI for the technical support.

References

Acosta-salmon, H. and Rangel-Davalos, C. 1977. Benzocaine (ethyl-p-aminobenzoate) as anesthetic for surgical implantation of nucleus in the pearl oyster *Pteria sterna* (Gould, 1851). *World Aquaculture'97 Book of Abstracts*, Seattle, WA, USA, Feb 19-23. World Aquaculture Society, Baton Rouge LA, USA, p. 3.

Acosta-Salmon, H., Martinez-Fernandez, E. and Southgate, P. C. 2004. A new approach to pearl oyster brood stock selection: can saibo donors be used as future broodstock? *Aquaculture*, 231: 205-214.

Acosta-Salmon, H. and Southgate, P. C. 2005. Mantle regeneration in the pearl oysters *Pinctada fucata* and *Pinctada margaritifera*. *Aquaculture,* 246: 447-453.

Acosta-Salmon, H. and Southgate, P. C. 2006. Wound healing after excision of mantle tissue from the Akoya pearl oyster, *Pinctada fucata*. *Comp. Biochem. Physiol.,* 143: 264–268.

Acosta-Salmon, H., Martinez-Fernandez, E. and Southgate, P. C. 2005. Use of relaxants to obtain saibo tissue from the black-lip pearl oyster (*Pinctada margaritifera*) and the Akoya pearl oyster (*Pinctada fucata*). *Aquaculture,* 246: 167-172.

Algarswami, K. 1991. *Production of cultured pearls.* Indian Council of Agricultural Research, New Delhi, p. 1-11.

Algarswami, K., Dharmaraj, S., Velayudhan, T. S., Chellam, A., Victor, A. C. C. and Gandhi, D. 1983. Larval rearing and production of spat of pearl oyster *Pinctada fucata* (Gould). *Aquaculture*, 34: 287-301.

Bancroft, J. D. 1975. *Histochemical techniques*. Buttersworth, London.

Barber, B. J. and Blake, N. J. 1981. Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (Say). *J. Exp. Mar. Biol. Ecol*., 52(2-3): 121-134.

Carlson, B. M. 2007. *Principles of regenerative biology*. Elsevier, Michigan.

Dev, D. S. 1994. Pearl culture project in India. *SPC Pearl Oyster Info. Bull.* (South Pacific Commission, Noumea), 7: 5-6.

Dev, D. S. and Durairaj, S. 1993. On commercial aspects of cultured pearl production from pearl oyster *Pinctada fucata* (Gould) in India. *Seafood Export J*., 30(8): 25-38.

Garcia-Gasca, A., Ochoa-Baez, R. L. Betancourt, M. 1994. Microscopic anatomy of the mantle of the pearl oyster, *Pinctada mazatlanica* (Hanley, 1856). *J. Shellfish Res.,* 13: 85-91.

George, D. 1966. The cultured pearl, its history and development to the present day. *Aust. Gemmol.,* 8: 10-26.

Gervis, M. H., and Sims, N. A. 1992. The biology and culture of pearl oysters (Bivalvia: Pteriidae). *ICLARM Study Review,* Manila, Philippines, p. 49.

Goss, R. J. 1969. *Principles of Regeneration*. Academic Press. New York, USA, 287 pp.

Hodgson, A. N. 1982. Studies on wound healing and an estimation of the rate of regeneration of the siphon of *Scrobicularia plana* (da Costa). *J. Exp. Mar. Biol. Ecol.*, 62: 117-128.

Humason, H. 1967. *Animal tissue techniques*. W. H. Freeman & Company, London.

James, P. S. B. R., Narasimham, K. A., Victor, A. C. C., Dharmaraj, S., Velayudhan, T. S. and Chellam, A. 1991. *The Indian marine pearls - a culture technique for their production.* Central Marine Fisheries Research Institute, Cochin, p. 1-23.

Kripa, V., Mohamed, K. S., Appukuttan, K. K. and Velayudhan, T. S. 2007. Production of Akoya pearls from the south-west coast of India. *Aquaculture*, 262: 347-354.

Mamangkey, N.G. F., Southgate P. C. 2009. Regeneration of excised mantle tissue by the silver-lip pearl oyster, *Pinctada maxima* (Jameson). *Fish Shellfish Immunol.,* 30: 1-11.

Mathieu, M., and Lubet, P. 1993. Storage tissue metabolism and reproduction in marine bivalves - a brief review. *Invert. Reprod. Dev.*, 23(2-3): 123-129.

Norton, J. H., Deshorst, M., Lansky, T. M., Mayer, R. J. 1996. An evaluation of some relaxants for use with pearl oysters. *Aquaculture*, 144: 39-52

Norton. J. H., Lucas, J. S., Turner, I., Mayer, R. J., Newnham, R. 2000. Approaches to improve cultured pearl formation in *Pinctada margaritifera* through use of relaxation, antiseptic application and incision closure during bead insertion. *Aquaculture*, 184: 1-17.

O' Connor, W. A. and Lawler, N. F. 2003. Propylelne phenoxetol as a relaxant for the pearl oysters *Pinctada imbricate* and *Pinctada albino*. In: O' Connor, W. A., Lawler, N. F. and Heasman, M. P. (Eds.), *Trial farming the Akoya pearl oyster, Pinctada imbricate, in Port stephens*. NSW Fisheries, Australia, p. 600-667.

Pekkarinen, M. 1994. Regeneration of the inhalant siphon and siphonal sense organs of brackish water (Baltic Sea) *Macoma balthica* (Lamellibranchiata, Tellinacea). *Ann. Zool. Fenn.,* 21: 29-40.

Saucedo, P., Racotta. I. S., Bevera, H., Villareal, H. and Monteforte, M. 2001. Differential gonad development of grafted and ungrafted specimens of the Calafia mother of pearl oyster, *Pinctada mazalantica* (Hanley 1856). *Invert. Reprod. Dev.,* 39: 183-193.

Saucedo P., Racotta, I., Villarreal, H. and Monteforte, M. 2002. Seasonal changes in the histological and biochemical profile of the gonad, digestive gland and muscle of the Calafia mother-of-pearl oyster, *Pinctada mazatlanica* (Hanley, 1856) associated with gametogenesis. *J. Shellfish Res.,* 21(1) : 127–35.

Savile-Kent, W. 1893. *The Great Barrier Reef of Australia - its products and potentialities*. John Curey, O'Neil, Melbourne, Australia, 387 pp.

Syda Rao, G. 2001. Eco-friendly onshore marine pearl culture - an overview. In : Menon, N. G. and Pillai, P. P. (Eds.). *Perspectives in Mariculture*, The Maine Biological Association of India, Cochin, p. 259-264.

Syda Rao, G. 2005. Onshore (land-based) culture of pearl oysters for pearl production. *Fishing Chimes*, 25(1): 142–146.

Syda Rao, G. 2007. Growth and biometric relationship of the Indian pearl oyster *Pinctada fucata* (Gould) under long term onshore rearing system. *J. Mar. Biol. Ass. India*, 49 (1): 51-57.

Taylor, J. J. U. 2002. Producing golden and silver south sea pearls from Indonesian hatchery reared *Pinctada maxima*. *World Aquaculture 2002*, 23rd – 27th April, 2002, Beijing, PR China. World Aquaculture Society, Baton Rouge LA, USA, 754 pp.

Tranter, D. J. 1957. Pearl culture in Australia. *Australian J. Sci.*, 19: 230-232.

Date of Receipt : 05/08/2009
Date of Acceptance : 01/03/2010

INDIAN JOURNAL OF FISHERIES
Volume 57 Number 1 (2010)

CONTENTS

Indexed/Abstracted in: Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Fish and Fisheries Worldwide (NISC), Science Citation Index Expanded, SCOPUS and Zoological Records