REPRODUCTION AND DEVELOPMENT OF \textit{HOLOTHURIA} 
AND \textit{ACTINOPYGA} SPECIES IN SOLOMON ISLANDS: 
IMPLICATIONS FOR AQUACULTURE

Christain Ramofafia

A thesis submitted in fulfilment of the requirements for the degree of Doctor of 
Philosophy in the Department of Anatomy and Histology. 
The University of Sydney, Australia. 
December 2001
The data contained in this thesis is the result of my own work except where specifically acknowledged.

Christain Ramofafia
To

Angie, Philley and Cecy

The girls in my life
General Abstract

The bêche-de-mer fishery that supports the livelihood of many coastal communities in the Indo-Pacific is based on the exploitation of about 20 sea cucumber species belonging to the order Aspidochirotida (Echinodermata: Holothuroidea). Unsustainable exploitation of these species is widespread, and in many countries severe depletion of wild populations is reported. Current management strategies to promote sustainable exploitation are ineffective due to limited human resources. The focus now in many countries in the region, including Solomon Islands, is to artificially culture sea cucumbers and to restore depleted wild populations through the release of juveniles. A major constraint to the successful implementation of restocking programmes is the lack of the biological and fisheries data essential for aquaculture of the most valuable species.

This thesis documents reproduction and development of three tropical aspidochirote sea cucumbers, *Holothuria fuscogilva*, *H. scabra* and *Actinopyga mauritiana*. All three species are commercially important for the production of bêche-de-mer, a dried body wall product. Depletion of wild stocks and interest in aquaculture of these species prompted the current investigation of aspects of their biology essential for artificial culture. The study was conducted over a 5 year period and represented the first detailed study of holothurians from Solomon Islands.

*H. fuscogilva* and *A. mauritiana* had annual reproductive cycles while *H. scabra* spawns year-round. Based on the macroscopic appearance of the gonad tubules, reproductive cycles for each species progressed through five maturity stages: Indeterminate, Growing, Mature, Partly-spawned and Spent. These maturity stages corresponded to discrete stages of gametogenesis identified by histology. *H. fuscogilva* spawned from August through to October with increased spawning activity in October. Spawning in *A. mauritiana* occurred from October to December. In *H. scabra*, a period of enhanced spawning activity occurred from September to November with the greatest activity in September. Histology revealed that gamete release was partial in all three species. In *H. fuscogilva* and *A. mauritiana*, spawning occurred before the maximum gonad indices (GI) were reached. Maximum GI values were variable among months and among years. Once gamete release commenced the gonads of all three species contained spawned and unspawned tubules. Both these types of tubules were resorbed during the spent stage. Spawning in all three species
coincided with longer day length and increased water temperature. Successful induction of spawning during the breeding period in all three species corroborated the GL and histological data. For *H. scabra* and *A. mauritiana*, stress due to transportation or temperature shock was used to induce spawning. Spawning in *H. fuscogilva*, required temperature shock and addition of dried algae.

Gametogenesis was initiated in March–April in *H. fuscogilva* and *A. mauritiana* with oogenesis and spermatogenesis occurring in parallel within each species. Initiation of gametogenesis coincided with the change from longer to shorter days in Solomon Islands, and was marked by the appearance of previtellogenic oocytes in females and spermatocytes in males. The mature stage was reached in August and October for *H. fuscogilva* and *A. mauritiana*, respectively. Histology revealed that re-initiation of gametogenesis occurred in partly-spawned gonads but the rationale for this gametogenic renewal were unclear as the tubules containing these new gametes were subsequently resorbed in the spent stage. In *H. scabra*, gametogenesis was asynchronous across the population. Individuals with advanced gametes were encountered throughout the year. Females with partly-spawned ovaries and males with mature testes were encountered most frequently.

The gonads of the three species comprised a single cohort of numerous tubules that developed uniformly to maturity from the gonad basis. Tubule growth involved an increase in the size and branching of tubules. Branching always occurred by bifurcation. Gonads in the mature and the partly-spawned stages were the largest, occupying the posterior regions of the coelomic cavity. Ovary tubule morphology of the three species, coupled with histological evidence showed that ovary development did not conform to the ‘tubule recruitment model’ suggested for oogenesis in the Holothuroidea.

In *H. fuscogilva* and *A. mauritiana* examination of gonad biopsies could be used to assess gonad maturity due to the synchronous nature of gametogenesis in these species. For *H. scabra* this tubule biopsy method can be used for selective harvesting of mature broodstock from the wild.

Development of the three species was investigated under hatchery conditions. All three species have planktotrophic development similar to most aspidochirote sea cucumbers. *H.*
scabra was reared successfully to settlement at 26 to 28°C on a mixed microalgal diet. The larvae developed through the feeding auricularia and nonfeeding (lecithotrophic) doliolaria and pentactula stages. Settlement occurred at the pentactula stage after 14 – 17 days of development. H. fuscogilva and A. mauritiana larvae were raised to the late auricularia and doliolaria stages, respectively. In all three species, the early auricularia stage was reached by day 3 and the late auricularia was reached on days 10 – 12. The mean length of the late auricularia ranged from 700 to 900 µm for all three species. Transition of the larvae from the late auricularia stage to the doliolaria stage resulted in a reduced larval size. The development of hyaline spheres, potential nutritive stores, during the late auricularia stage appeared to be a good indicator of larval competence. Larvae of H. scabra lacking hyaline spheres did not settle. Although the percent survival of H. scabra by the settlement stage was low, thousands of juveniles were produced for grow-out experiments. The larvae of H. fuscogilva and A. mauritiana lacked or had poor hyaline sphere development. This indicates that the diet failed to promote hyaline sphere development in these species. The development of the three species is discussed in terms of their potential for aquaculture.
Publications

*Papers arising from this thesis


ACKNOWLEDGMENTS

I thank my supervisor Associate Professor Maria Byrne for taking me on board and for continual support, encouragement, guidance and enthusiasm during these four years of my candidature. Dr Stephen Battaglene co-supervised my candidature and led the Sea Cucumber Project at ICLARM Coastal Aquaculture Centre (CAC), Solomon Islands of which I was a member. Thanks mate for everything since 1996! I thank ICLARM – the World Fish Center for recruiting me to do the work which is the story contained in this thesis, for financial support rendered to my family and me in Solomon Islands and in Sydney and for funding my family’s travel expenses to and from Sydney. I thank Dr Meryl Williams and Dr Johann Bell for the continual support my family received from ICLARM.

I thank everybody at the ICLARM Coastal Aquaculture Centre (CAC). Dr Johann Bell for supporting me in securing my AusAID Scholarship and for continual support during my candidature. Dr Stephen Battaglene and the Sea Cucumber Team (Evizel Seymour, Maxwell Sau, Joseph Olisia and Henry Rota) for contributing to all aspects of this study. Without you all, this thesis would have not materialised. I thank all the hatchery boys (you know who you are!) under the leadership of Rayner Pitt and Cletus Oengpepe for all assistance received during this study. Half of this thesis would never be completed without the commitment and dedication of Idris Lane and his team at ICLARM Nusa Tupe Field Station at Gizo, Western Solomons. A big thank you to you all. Susan Dance, Phil Clark, Rayner Pitt and Cletus Oengpepe for standing in for me to oversee hatchery work during the height of the ethnic crisis on Guadalcanal. Dr Anthony Hart, Hugo Tafea, Feral Lasi, Max Sau, Joe Borule, Henry Rota, Paul Mercy, Cletus Oengpepe, Evizel Seymour and Dr Stephen Battaglene for being dive buddies. Idris and Clay for answering the last minute call for more sea cucumber specimens. For office support, I thank the CAC office staff Stephanie Pallay, Kathy Launa, Moana Pelu, Beverley Monica and Aniel.

Dr J-F Hamel and Dr Annie Mercier for friendship and discussions about sea cucumbers. Thank you for all the encouragement and support along the way. It was good to know you both.
I thank the Marau Sound community, particularly late David Adilamo and Martin Ukaria for assistance with the 4 years of research in Marau. The New Mala, Savo, Gela communities for allowing your reefs to be researched for this study.

The Byrne Lab comrades, Dr Francis Chee, Dr Paula Cisternas, Dr Suzy Renn, Dr Anna Cerra, Suzanne Long, Paulina Selvakumaraswamy, Inke Falkner, Franca Mazzone, Demian Koop and Jamie Potts for friendship, support and intellectual discussions (whatever that is!). Thank you all for the numerous ‘short’ coffee breaks that lasted...oops! roughly 0.5 of an hour plus or minus ......whatever the standard error is! Suzanne, thank you for checking the references and Matt for proof-reading the thesis.

The staff at Electron Microscope Unit, especially Tony Romeo, Dennis Dwarte, Tom Joyce and Ellie Kable for photographic support. R. Smith and C. Jeffery of the Department of Anatomy and Histology also provided photographic assistance.

Scholarship to undertake this study was funded by AusAID to whom I am grateful. Australian Centre for International Research provided financial assistance for the work undertaken at the CAC.

I thank the Bebeu, Fakaia and the Funifaka families for support and understanding. Clay and Placida, tagio tumas for supporting Cecy and the girls in Honiara. My family and I appreciated your support to us. Steve and Jenny, thanks bro for all the support. Tagio nao fo stori kam lo email olowe.

I thank Stephen Gura’au, Abert Carlot and Daniel Koroi at Forum Fisheries Agency (FFA) for providing the map on Solomon Islands.

Pastor Bernard and the All Nations Christian Assembly congregation at Granville for providing family support for these last two years. I am grateful to James and Hiroko, Esther and the Fernando family for family support. Christiana, Kimberley and Rachel, Katy and Jens for being our ‘wantoks’ here in Sydney. Tina and Katy, thanks for all the support.

Most of all, Angie, Philley, Cecy and the Lord for providing love, strength, faith and life.
# Table of Contents

General Abstract .................................................................................................................. i  
Publications ......................................................................................................................... iv  
Acknowledgements ............................................................................................................... v  
Table of Contents ............................................................................................................... vii  

**CHAPTER 1. General Introduction** ................................................................. 1  
1.1 Historical and Recent Perspectives............................................................... 1  
1.2 Reproduction and Development of Tropical Aspidochirotes ....................... 2  
1.3 The Bêche-de-mer Fishery and Trade ................................................................. 3  
1.3.1 Solomon Islands bêche-de-mer fishery ....................................................... 4  
1.4 Stock Management .............................................................................................. 5  
1.5 Sea Cucumber Culture ......................................................................................... 6  
1.6 Thesis Aims .......................................................................................................... 8  

**CHAPTER 2. General Materials and Methods** ................................................. 9  
2.1 Collection, Transportation, Dissection and Fixation ......................................... 9  
2.2 Processing of Gonad ............................................................................................ 9  
2.2.1 Assessment of reproductive activity .......................................................... 9  
2.2.2 Assessment of reproductive condition ....................................................... 10  
2.3 Gonad Histology ................................................................................................ 10  
2.4 Culture of Larvae ............................................................................................... 11  
2.5 Day Length Data ................................................................................................. 11  
2.6 Data Analysis ....................................................................................................... 11  

**CHAPTER 3. Reproductive Biology of *Holothuria fuscogilva*** ......................... 12  
Abstract ......................................................................................................................... 13  
3.1 Introduction ......................................................................................................... 14  
3.2 Materials and Methods ...................................................................................... 15  
3.3 Results .................................................................................................................. 16  
3.3.1 Gonad morphology ....................................................................................... 16  
3.3.2 Gonad index ................................................................................................... 17  
3.3.3 Histology ........................................................................................................ 18  
3.3.3.1 Females: ................................................................................................... 18  
3.3.3.2 Males ......................................................................................................... 19  
3.3.4 Reproductive cycle ....................................................................................... 20  
3.3.5 Induction of spawning ................................................................................. 20  
3.4 Discussion ............................................................................................................. 21
CHAPTER 4. Reproductive Biology of *Holothuria scabra*................................. 25

Abstract........................................................................................................................... 26
4.1 Introduction.............................................................................................................. 27
4.2 Methods and Materials ........................................................................................ 28
4.3 Results...................................................................................................................... 29
  4.3.1 Gonad morphology ................................................................................ 29
  4.3.2 Histology........................................................................................................ 30
    4.3.2.1 Oogenesis .................................................................................. 30
    4.3.2.2 Spermatogenesis ........................................................................ 31
  4.3.3 Gonad index (GI) and tubule size.................................................................. 32
  4.3.4 Reproductive cycle ......................................................................................... 32
4.4 Discussion................................................................................................................ 33

CHAPTER 5. Reproductive Biology of *Actinopyga mauritiana*............................. 37

Abstract........................................................................................................................... 38
5.1 Introduction.............................................................................................................. 39
5.2 Materials and Methods ........................................................................................ 40
5.3 Results...................................................................................................................... 41
  5.3.1 Distribution.................................................................................................... 41
  5.3.2 Gonad index.................................................................................................... 41
  5.3.3 Macro- and micro- examination of gonad development............................... 41
  5.3.4 Histology of gametogenesis ........................................................................ 43
    5.3.4.1 Oogenesis .................................................................................. 43
    5.3.4.2 Spermatogenesis ........................................................................ 43
  5.3.5 Cycle of gametogenesis ................................................................................. 44
  5.3.6 Environmental factors .................................................................................... 44
5.4 Discussion................................................................................................................ 45

CHAPTER 6. Assessment of the ‘Tubule Recruitment Model’ for Ovary Development in *Holothuria fuscogilva*, *H. scabra* and *Actinopyga mauritiana* .... 48

Abstract........................................................................................................................... 49
6.1 Introduction.............................................................................................................. 50
6.2 Materials and Methods ........................................................................................ 51
6.3 Results...................................................................................................................... 51
  6.3.1 Gonad morphology ................................................................................ 51
    6.3.1.1 Tubule organisation ................................................................... 51
    6.3.1.2 Tubule growth ........................................................................... 51
    6.3.1.3 Gametogenesis .......................................................................... 52
6.4 Discussion................................................................................................................ 53
CHAPTER 7. Development of *Holothuria scabra*, *H. fuscogilva*, and *Actinopyga mauritiana* in Hatchery Culture ................................................. 55

Abstract ........................................................................................................................... 56
7.1 Introduction .............................................................................................................. 57
7.2 Materials and Methods ............................................................................................ 58
  7.2.1 Collection of samples .................................................................................... 58
  7.2.2 Induction of spawning ................................................................................... 58
  7.2.3 Collection, fertilisation and stocking of eggs ................................................ 59
  7.2.4 Rearing of larvae ............................................................................................ 59
  7.2.5 Collection and analysis of data ...................................................................... 60
7.3 Results ...................................................................................................................... 60
  7.3.1 Induction of spawning ................................................................................... 60
  7.3.2 Embryogenesis and larval development ........................................................ 61
    7.3.2.1 *H. scabra* ........................................................................................... 61
    7.3.2.2 *H. fuscogilva* and *A. mauritiana* ....................................................... 63
  7.3.3 Larval survival of *H. scabra* .......................................................................... 63
7.4 Discussion ................................................................................................................ 64

CHAPTER 8. General Discussion .................................................................................. 69

  8.1 Research Focus ........................................................................................................ 69
  8.2 Reproduction of *H. fuscogilva*, *H. scabra* and *A. mauritiana* ..................... 69
    8.2.1 Reproduction ................................................................................................. 69
    8.2.2 Spawning ....................................................................................................... 70
      8.2.2.1 Proximal cues of spawning ................................................................. 70
      8.2.2.2 Artificial induction of spawning ......................................................... 70
  8.3 Development of *H. fuscogilva*, *H. scabra* and *A. mauritiana* in Hatchery Culture. 71
  8.4 Conclusions and Future Research ............................................................................ 71

REFERENCES ................................................................................................................. 74
CHAPTER 1

General Introduction

1.1 Historical and Recent Perspectives

Current knowledge of echinoderm reproduction and development is largely based on data from the echinoids and asteroids, prompted by their accessibility and ease by which mature gametes can be obtained (Byrne, 1999). In contrast, holothuroids, the subject of this thesis, have received comparatively little attention because they are less accessible and because of the difficulty of obtaining gametes for developmental studies (McEuen, 1987). Among echinoderms, the Holothuroidea, commonly known as sea cucumbers, encompasses 1200 extant species (Smiley et al., 1991). Sea cucumbers inhabit diverse marine habitats, from coral reefs to soft sediments in shallow and deep waters of both tropical and temperate regions (Smiley et al., 1991). Despite their abundance and diversity, the reproductive biology and development of sea cucumbers are poorly understood and this is particularly true for tropical species.

This thesis focuses on the reproduction and development of tropical holothuroids, order Aspidochirotida, with emphasis on the commercial species, *Holothuria fuscogilva* (Selenka, 1867), *Actinopyga mauritiana* (Quoy & Gaimard, 1833) and *H. scabra* (Jaeger, 1833) (Fig. 1.1). Aspidochirotidae are the familiar large sea cucumbers in tropical regions where they live as deposit feeders. Early work on the biology of these holothurians focussed on reproductive anatomy, larval morphology and aspects of reproductive periodicity (Mortensen, 1921, 1937, 1938). Most holothuroids are gonochoric and their gonads consist of numerous branched tubules that arise from the gonad basis. Hermaphroditic sea cucumbers have ovotestes (Frick, et al., 1996).

Over the past three decades new information has emerged concerning holothuroid reproductive patterns and modes of development (Smiley et al., 1991; Sewell et al., 1997). Most recent studies utilise the gonad index method and/or histology to determine details of gametogenesis, reproductive activities and spawning patterns (Smiley et al., 1991; Sewell et al., 1997). Much of the information gathered on tropical sea cucumbers has been on
Figure 1.1.
Chapter 1. General Introduction

aspidochirote, prompted by their commercial importance as the source of bêche-de-mer, a
dried body wall product (Fig 1.2). Particular emphasis has focussed on the ecology and
fisheries biology of commercial species in order to identify ways to conserve an often
over-exploited resource (Table 1.1). This thesis investigates the reproduction and
development of three commercial holothurians from the Indo-Pacific (Fig. 1.1).

1.2 Reproduction and Development of Tropical Aspidochirote

Reproduction of tropical aspidochirote has been documented in numerous studies (Table
1.1). Overall, most species have an annual reproductive cycle although a few species
display biannual or continuous reproductive patterns. Spawning in most species occurs in
summer. However, H. nobilis has a winter spawning season (Conand, 1993a).

The timing and duration of gametogenesis and spawning are important features of
reproduction and may be influenced by a suite of external environmental factors. For
aspidochirotids, specific cues regulating gametogenesis and/or spawning have not been
identified but many factors have been suggested. These include ambient sea temperature,
photoperiod, water velocity, salinity, day length, lunar cycles, phytoplankton abundance or
combinations of these factors (Tanaka, 1958; Krishnaswamy & Krishnan, 1967; Shelley,
1982; Ong Che & Gomez, 1985; Cameron & Fankboner, 1986; Babcock et al., 1992 and
Battaglene et al., in press; Hamel et al., 2001). Larger scale latitudinal changes in climatic
conditions also affect reproduction. For example, Thorson (1950) indicated that
reproductive cycles become distinctly seasonal with increasing distance from the equator
due to latitudinal restrictions on the optimal periods for phytoplankton growth. In
temperate regions, spring and summer phytoplankton blooms are sharply defined and
correlate with increasing summer sea temperatures and longer day lengths. The
synchronised spawning of sea cucumbers in these regions is suggested to reflect selection
for the co-occurrence of pelagic planktotrophic larvae and high concentrations of
planktonic food (Cameron & Fankboner, 1986; Starr et al., 1990).

Three developmental patterns are recognised for holothurians and echinoderms in general:
planktonic planktotrophy, planktonic lecithotrophy and benthic lecithotrophy with the
latter category including development in benthic egg masses and brooding (Byrne, 1999).
Species with planktotrophic development have feeding larvae while the lecithotrophs have
non-feeding larvae. Aspidochirote are all planktotrophic developers and have a feeding
Table 1.1. Aspects of reproduction of aspidochirotid holothurians (27 N to 27 S latitude).

<table>
<thead>
<tr>
<th>Species</th>
<th>Latitude</th>
<th>Location</th>
<th>Repro. cycle</th>
<th>Spawning season</th>
<th>Egg size (µm)</th>
<th>Developmental mode</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinopyga</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. echinata</td>
<td>21 N</td>
<td>Southern Taiwan</td>
<td>annual</td>
<td>summer (Jun-Jul)</td>
<td>110</td>
<td>planktotrophic</td>
<td>Chao et al., 1995</td>
</tr>
<tr>
<td>A. echinata</td>
<td>20 S</td>
<td>New Caledonia</td>
<td>annual</td>
<td>summer (Dec-Jan)</td>
<td>165</td>
<td>planktotrophic</td>
<td>Conand, 1993a</td>
</tr>
<tr>
<td>A. mauritiana</td>
<td>13 N</td>
<td>Guam</td>
<td>annual</td>
<td>summer (Jun-Jul)</td>
<td>95-150</td>
<td>planktotrophic</td>
<td>Hopper et al., 1998</td>
</tr>
<tr>
<td>A. mauritiana</td>
<td>20 S</td>
<td>New Caledonia</td>
<td>annual</td>
<td>summer (Dec-Jan)</td>
<td>170</td>
<td>planktotrophic</td>
<td>Conand, 1992, 1993a</td>
</tr>
</tbody>
</table>

| Holothuria       |          |                  |              |                      |               |                    |                      |
| H. atra          | 09 S     | Solomon Islands  | -            | summer (includes Sept) | 150?         | planktotrophic     | Ramofasia et al., 1995 |
| H. atra          | 18 S     | Fiji             | annual       | summer (Sept-Dec)    | 200           | planktotrophic     | Seto, 1994           |
| H. atra          | 20 S     | New Caledonia    | biannual     | -                    | 150           | planktotrophic     | Conand, 1993a        |
| H. atra          | 23 S     | GBR, Australia   | biannual     | Jun-Jul/Jan-Feb       | -             | plankto./ asexual  | Harriott, 1980, 1985 |
| H. cinerascens   | 21 N     | Southern Taiwan  | annual       | spring (Apr-Jun)     | 100           | planktotrophic     | Chao et al., 1995    |
| H. difficilis    | 21 N     | Southern Taiwan  | annual       | summer (Aug-Sep)     | 75            | planktotrophic     | Chao et al., 1995    |
| H. difficilis    | 27 N     | Red Sea          | annual       | Summer (include Aug) | -             | planktotrophic     | Mortensen, 1938      |
| H. edulis        | 23 S     | GBR, Australia   | annual       | Aug-Sep              | -             | planktotrophic     | Harriott, 1980, 1985 |
| H. florida       | 25 N     | Florida          | annual       | summer               | 100-125       | planktotrophic     | Engstrom, 1980       |
| H. fuscogilva    | 20 S     | New Caledonia    | annual       | summer (Dec-Jan)     | 170           | planktotrophic     | Conand, 1981, 1993a  |
| H. fuscopunctata | 20 S     | New Caledonia    | annual       | summer (Dec-Jan)     | 210           | planktotrophic     | Conand, 1993a        |
| H. impatiens     | 27 N     | Red Sea          | annual       | Summer (include Aug) | -             | planktotrophic     | Mortensen, 1938      |
| H. impatiens     | 23 S     | GBR, Australia   | continuous   | -                    | -             | planktotrophic     | Harriott, 1980, 1985 |
| H. leucospilata  | 21 N     | Southern Taiwan  | annual       | summer (Jun-Sep)     | 120           | planktotrophic     | Chao et al., 1995    |
| H. leucospilata  | 22 N     | Hong Kong        | biannual     | Sept-Dec/ Mar-May    | -             | planktotrophic     | Ong Che, 1990        |
| H. mexicana      | 25 N     | Florida          | annual       | summer               | 100-250       | planktotrophic     | Engstrom, 1980       |
| H. nigripilosa   | 20 S     | New Caledonia    | annual       | winter (Jun-Jul)     | 150           | planktotrophic     | Conand, 1981, 1993a  |
| H. nobilis       | 27 N     | Red Sea          | annual       | Summer (include Aug) | 100           | planktotrophic     | Mortensen, 1938      |
| H. papilifera    | 27 N     | Red Sea          | annual       | Summer (include Aug) | 100           | planktotrophic     | Mortensen, 1938      |
| H. pardalis      | 27 N     | Red Sea          | annual       | Summer (include Aug) | -             | planktotrophic     | Mortensen, 1938      |
| H. scabra        | 09 N     | Eastern India    | biannual     | July & October       | -             | planktotrophic     | Krishnaswamy & Krishnan, 1967 |
| H. scabra        | 20 S     | New Caledonia    | biannual     | Aug-Sept/Dec-Jan     | 190           | planktotrophic     | Conand, 1993a        |
Table 1.1 continue

<table>
<thead>
<tr>
<th>Species</th>
<th>Lat</th>
<th>Location</th>
<th>Life Cycle</th>
<th>Season</th>
<th>Growth Form</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. scabra</em></td>
<td>27 S</td>
<td>GBR, Australia</td>
<td>annual</td>
<td>summer (Oct)</td>
<td>-</td>
<td>planktrophic</td>
</tr>
<tr>
<td><em>H. scabra</em></td>
<td>27 S</td>
<td>GBR, Australia</td>
<td>annual</td>
<td>summer (Oct)</td>
<td>-</td>
<td>planktrophic</td>
</tr>
<tr>
<td><em>H. scabra vers</em></td>
<td>20 S</td>
<td>New Caledonia</td>
<td>annual</td>
<td>summer (Nov-Jan)</td>
<td>-</td>
<td>planktrophic</td>
</tr>
<tr>
<td><em>Stichopus</em></td>
<td>20 S</td>
<td>New Caledonia</td>
<td>annual</td>
<td>summer Dec-Jan</td>
<td>180</td>
<td>planktrophic</td>
</tr>
<tr>
<td><em>Theonota</em></td>
<td>20 S</td>
<td>New Caledonia</td>
<td>annual</td>
<td>summer Dec-Jan</td>
<td>200</td>
<td>planktrophic</td>
</tr>
</tbody>
</table>
auricularia larval stage followed by the non-feeding (lecithotrophic) doliolaria and pentactula stages (Fig. 1.3). The auricularia is unique to holothurians and is characterised by the presence of a single continuous ciliary band that functions in swimming and feeding (Fig. 1.3). Most sea cucumbers have lecithotrophic development (Smiley et al., 1991; Materia et al., 1991; Sewell 1994; Frick et al., 1996; Sewell and McEuen (2002). Developmental modes seen in the Holothuroidea are typical of many marine invertebrates and appear to reflect evolutionary trade-offs between fecundity and larval life span (Vance, 1973; Strathmann, 1971; Emlet et al., 1987; Hart et al., 1997; Byrne et al., 1999). Planktotrophic developers as exemplified by the Aspidochirotida, produce large quantities of small (<180 μm diameter) oligolecithal eggs while lecithotrophic holothurians (Dendrochirotida, Apodida and Molpadida) produce fewer larger (> 180 μm diameter) macrolecithal eggs (Sewell & Young, 1997; Byrne et al., 1999). The high individual fecundities that characterise aspidochirotes (Conand, 1993a, b) might be taken to indicate a plentiful supply of stocks for the bèche-de-mer fishery (Fig. 1.2). However, due to the vagaries of successful metamorphosis and settlement, this is not the case. Moreover, with the increased market demands for bèche-de-mer products, intense fishing, the sedentary nature of sea cucumbers, ease of capture and simplicity of bèche-de-mer production, commercial aspidochirotes are at risk of over-exploitation. This thesis in part, investigates alternatives for the sustainable use of this resource, through artificial culture of seed stock.

1.3 The Bèche-de-mer Fishery and Trade
The bèche-de-mer fishery in the Indo-Pacific is a multispecies fishery (Fig. 1.2) based on the exploitation of aspidochirote genera Actinopyga, Holothuria, Stichopus and Thelenota (Conand & Byrne, 1993; SPC, 1994, Conand, 1999). The genera Actinopyga and Holothuria belong to the family Holothuriidae while Stichopus and Thelenota belong to Stichopodidae. The Indo-Pacific fishery is sustained by producer countries and territories that include those in the tropical Pacific, Western Central Pacific, Eastern and Western Indian, with the Western Central Pacific the largest producer (Conand & Byrne, 1993; Conand, 1997; Conand, 1999). The temperate fishery is less diverse (Conand & Byrne, 1993) and is supplied from the exploitation of the Stichopus species Stichopus japonicus, S. mollis, S. californicus and S. parvimensis. S. japonicus (Sloan, 1985, 1986; Mladenov &
Figure 1.2.
Bèche-de-mer of tropical aspidochirote. A. Dried body wall of *Thelenota ananas*. B. Multiple species, sundried. (Courtesy of ICLARM).
Gerring, 1991; Conand & Byrne, 1993). Producer countries of the temperate fishery include China, Japan, Korea, Russia and New Zealand in the Western Pacific and Canada and the United States in the east.

 Marketable sea cucumber products include the body wall, longitudinal muscles and the viscera. The most important product, dried body wall is marketed as bêche-de-mer and is also called trepang or hai-som (Conand & Byrne, 1993) (Fig. 1.2). Raw or pickled body wall and viscera are delicacies in Japan and Korea. Bêche-de-mer is supplied to the international markets mostly from the tropical fishery while other products, including frozen body wall are supplied by the temperate fisheries (Sloan, 1985, 1986; Conand & Byrne, 1993).

The markets for bêche-de-mer products are concentrated in Asia, with Hong Kong, Singapore and Taiwan as major export and re-export destinations (Conand & Byrne, 1993; Conand, 1999). Trade data for 1995 and 1996 show Hong Kong to be leader of the three markets (Jaquemet & Conand, 1999). With the opening of the People's Republic of China market in the late 1980s, a large import trade for lower priced bêche-de-mer was initiated. This product is re-exported by Hong Kong from Indonesia, Philippines and recently the South Pacific (Van Eys & Philipson, 1991). Markets for the fishery expanded rapidly and by 1994 the annual world captures were around 120,000 t, valued over US $60 million (Conand, 1999).

1.3.1. Solomon Islands Bêche-de-mer Fishery

Information on the bêche-de-mer fishery in Solomon Islands is limited. The fishery existed prior to World War II and has continued in a 'boom and bust' cycle to date. Aspects of the fishery have been described by McElroy (1972), Cream (1977) and Holland (1994). In total 22 species have been exploited by 1993, with 6 species considered as high-value species (Holland, 1994). These include *H. fuscogilva*, *H. scabra*, *H. scabra var versicolor*, *Thelenota ananas*, *Stichopus chloronotus* and *S. variegatus*. Currently, 23 species are exploited with two species *Actinopyga mauritiana* and *Bohadschia graeffei* now considered as high-value species in addition to the original six (Table 1.2). Several measures including size regulation, closed seasons, introduction of marine reserves and ban of the use of SCUBA have been recommended for the management of the fishery.
Figure 1.3.
Life cycle of aspidochirote, through the auricularia, doliolaria and the pentactula larval stages. Modified from Battaglene (1999).
Auricularia (feeding) -> Spawning -> Pentactula (settlement) -> Doliolaria (non-feeding)
Chapter 1. General Introduction

(Adams (1993). In the last 10 years the export trends have exhibited a decreased landing of stocks, from a record level of 622 t in 1991 (Holland, 1994) to 160 t in 2000, with > 75% of the latter derived from medium and low-value species (Ministry of Fisheries, Solomon Islands Government, unpublished data).

1.4 Stock Management

The re-entry of China into the world market increased demand for bêche-de-mer sharply in the last 15 years (Conand & Byrne, 1993; Conand, 1999). Consequently, global export trends show that wild stocks of commercial aspidochirote sea cucumbers are being over-exploited and that fishery efforts are now shifting to species of comparatively smaller size, lower quality and lower market value (Conand & Byrne, 1993; Conand, 1997) as exemplified by the fishery in Solomon Islands. Such trends present a major challenge to manage the resource and provide the imperative to seek alternatives to reduce pressure on existing wild stocks. This is especially important in the Indo-Pacific where the bêche-de-mer fishery benefits indigenous coastal communities. Although, management measures including minimum size limits, closed seasons, bag limits and restrictions on the use of SCUBA for harvesting have been recommended, these have not been successfully enforced (Preston, 1993; Conand, 1997).

Battaglene & Bell (1999) list several reasons why such management may not be fully effective for tropical sea cucumbers. The three main reasons are: First, data on sea cucumber population dynamics are difficult to collect because of difficulties in measuring and tagging these animals. Second, the remote and the artisanal nature of fisheries make it difficult to collect catch-per-unit-effort, growth, and mortality data essential for management of fisheries on a sustainable basis. Third, developing countries often do not have the resources to enforce the fisheries regulations. The diverse social and coastal tenure system of many countries in the Indo-Pacific also complicate the application of regulations.

Recent developments indicate that the time frame required to rebuild stocks could be reduced greatly by reseeding areas with hatchery produced juveniles (Battaglene & Bell, 1999). This restocking measure overcomes the unpredictable nature of recruitment and settlement of larvae. The release of cultured juveniles could also be used to increase the yields above historical levels thereby overcoming the recruitment limitation that occurs
Table 1.2. Sea cucumber species currently harvested in Solomon Islands for bêche-de-mer production. ? = scientific name not known, *common names used by bêche-de-mer buying agents in Solomon Islands.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High-value</strong></td>
<td></td>
</tr>
<tr>
<td><em>Actinopyga lecanora</em></td>
<td>Stonefish</td>
</tr>
<tr>
<td><em>A. mauritiana</em></td>
<td>Surf redfish</td>
</tr>
<tr>
<td><em>Bohadschia graffei</em></td>
<td>Orangefish</td>
</tr>
<tr>
<td><em>Holothuria fuscogilva</em></td>
<td>White teatfish</td>
</tr>
<tr>
<td><em>H. scabra</em></td>
<td>Sandfish</td>
</tr>
<tr>
<td><em>H. scabra var versicolor</em></td>
<td>Sandfish</td>
</tr>
<tr>
<td><em>Stichopus chloronotus</em></td>
<td>Greenfish</td>
</tr>
<tr>
<td><em>S. variegatus</em></td>
<td>Curryfish</td>
</tr>
<tr>
<td><em>Thelepus ananas</em></td>
<td>Prickly redfish</td>
</tr>
<tr>
<td><strong>Medium-value</strong></td>
<td></td>
</tr>
<tr>
<td><em>A. miliaris</em></td>
<td>Blackfish</td>
</tr>
<tr>
<td><em>B. argus</em></td>
<td>Leopardfish</td>
</tr>
<tr>
<td><em>B. marmorata</em></td>
<td>Chalkfish</td>
</tr>
<tr>
<td><em>H. nobilis</em></td>
<td>Black teatfish</td>
</tr>
<tr>
<td><strong>Low-value</strong></td>
<td></td>
</tr>
<tr>
<td><em>A. echinites</em></td>
<td>Deep water redfish</td>
</tr>
<tr>
<td><em>B. vitiensis</em></td>
<td>Brown sandfish</td>
</tr>
<tr>
<td><em>H. edulis</em></td>
<td>Pinkfish</td>
</tr>
<tr>
<td><em>H. fuscopunctata</em></td>
<td>Elephant's trunk fish</td>
</tr>
<tr>
<td><em>T. anax</em></td>
<td>Amberfish</td>
</tr>
<tr>
<td>?</td>
<td>Hongpoy fish*</td>
</tr>
<tr>
<td>?</td>
<td>Rainbow*</td>
</tr>
<tr>
<td>?</td>
<td>Three sidefish or ripplefish*</td>
</tr>
<tr>
<td>?</td>
<td>Snakefish*</td>
</tr>
<tr>
<td>?</td>
<td>White snake*</td>
</tr>
</tbody>
</table>
even in unexploited stocks, a process known as ‘stock enhancement’ (Munro & Bell, 1997; Battaglene & Bell, 1999). Application of stock enhancement involves the release of sufficient numbers of juveniles to consistently reach the carrying capacity of the habitat for a given species. Sea cucumbers appear to have the necessary biological attributes for successful stock enhancement because they feed low on the food chain, are restricted to inshore habitats, and are relatively sedentary and easy to harvest (Battaglene, 1999). Stock enhancement of temperate sea cucumbers is practised successfully with *S. japonicus* in Japan and China. To date, there has been very little stock enhancement of tropical sea cucumbers. However, a pilot study investigating the reseeding of juvenile *H. scabra* in the Solomon Islands has shown promise (Dance *et al.*, in press). It appears therefore that aquaculture is the most promising means of restoring and enhancing the stocks of depleted species to previous abundance levels (Conand & Byrne, 1993; Battaglene & Bell, 1999). Nevertheless, the success of restocking and enhancement programs depends on a thorough understanding of the life-history strategies of these animals, particularly, data on gametogenesis, spawning and larval biology. These life-history features are central to the present study of three commercial aspidochirote species from Solomon Islands.

### 1.5 Sea Cucumber Culture

Culture of marine invertebrates has been practised for quite some time and includes the mariculture and aquaculture of species of fishes, crustaceans, molluscs and seaweeds. Research into the breeding and cultivation of aspidochirote holothurians is a recent endeavour, although the fishery has been established for some time. Interest to culture sea cucumbers stems from the recognition of the resource’s commercial value and the need for restoration and enhancement of depleting wild populations.

*Stichopus japonicus* is the first aspidochirote species to be cultured. Breeding and cultivation programmes began in the 1930s in Japan with first production of juveniles was recorded in 1950 (Ito & Kitamura, 1997). By 1994, over 2.5 million juveniles had been commercially produced. The Japanese efforts are focused on restoration and enhancement of the red and blue colour morphs of *S. japonicus*, both of which are the only valuable varieties of the species in Japan (Yanagisawa, 1998). China undertook breeding and cultivation programmes on *S. japonicus* in 1970s and by 1985, production of juveniles was achieved. China now produces over 2375 t of bêche-de-mer annually, 1023 t of which is derived from aquaculture (YSFRI, 1991; Ferdouse, 1999).
While breeding and cultivation of *S. japonicus* is successful, hatchery culture of tropical holothurians are presently restricted to *H. scabra* with programs underway in India, Australia, Indonesia, Maldives, Madagascar and the Solomon Islands (James et al., 1994; Yangisawa, 1998; Battaglene, 1999; Morgan, 2000a; Jangoux et al., 2001). Ranching of wild-caught *H. scabra* juveniles in meshed enclosures has also been practised in India and Indonesia for at least two decades (Tiensongrusmee & Pontjoprawiro, 1988; James, 1996).

The artificial culture of tropical aspidochirotes is hampered by the paucity of data available on their reproduction and development. This is a widespread problem in the south Pacific region, despite the region being a major component of the global bêche-de-mer fishery (Table 1.1). Data on holothuroid reproductive biology in region are limited to studies undertaken in New Caledonia and a few reports from countries elsewhere near the equator (Conand, 1981, 1993; Seeto, 1994; Tuwo, 1999).

With the understanding of the significant contribution of the bêche-de-mer fishery to the livelihood of many coastal communities in the Solomon Islands and the Indo-Pacific in general, and the risk of over-exploitation of this resource, the Coastal Aquaculture Centre (CAC) of ICLARM, the World Fish Center, launched a project in 1993 to investigate the mass culture of several commercial aspidochirote species (ICLARM, 1993). Work at the Centre, focussed on *H. fuscogilva, H. scabra* and *A. mauritiana*. Because of the lack of reproductive data on commercially aspidochirote holothurian species in the Solomon Islands, it was clear that determination of reproductive activities and breeding cycles of these species was required as a necessary first step for the culture of the species. My thesis undertaken in the Solomon Islands investigates the reproduction, development and aquaculture potential of these species.

The choice of the three species for this research was dictated by their high commercial value.

*Holothuria fuscogilva:*

*Holothuria fuscogilva* is the most valuable species for bêche-de-mer in Solomon Islands, ranking first amongst the 8 species considered high-value species. Consequently, the species has been over-exploited. In some island and coastal
communities in the Solomon Islands, the bêche-de-mer fishery is based on this single species alone (Cream, 1977; Holland, 1994).

_Holothuria scabra:_

_Holothuria scabra_ is the second most valuable species in Solomon Islands and like _H. fuscogilva_, is over-exploited. The drastic decrease in wild stocks prompted the Ministry of Fishery of the Solomon Islands Government to issue a national ban against the harvesting of this species in 1997 (ICLARM, 1997).

_Actinopyga mauritiana:_

_Actinopyga mauritiana_ is also a high-value species in the Solomon Islands and has been heavily exploited. Recent trends show dwindling stocks of this species (unpublished data, Ministry of Fisheries, Solomon Islands Government).

1.6 Aims of my Thesis

In view of over-exploitation and the potential for stock restoration and enhancement of commercial tropical aspidochirotids through aquaculture, the aims of my research were to (1) document the reproductive traits and the breeding seasons of _H. fuscogilva_, _A. mauritiana_ and _H. scabra_, (2) to describe development of the larvae and (3) develop methods for production of larvae en masse in culture.

The reproductive biology of the three species is presented in Chapters 3, 4 and 5. Chapter 6 uses the results of the preceding three Chapters to assesses the validity of the “Tubule Recruitment Model” which has been proposed as a model for ovary development in the Holothuroidea (Smiley & Cloney, 1985; Smiley, 1988; Smiley et al., 1991). Investigations into the developmental biology of the species under mass culture conditions are examined in Chapter 7. Finally, Chapter 8 discusses the research presented in the preceding chapters with particular emphasis on the aquaculture potential of the three species examined.
Reproduction of *H. fuscogilva*, *H. scabra* and *A. mauritiana* were investigated in the Solomon Islands (Fig. 2.1). Field and laboratory work was undertaken at ICLARM – the World Fish Center, Coastal Aquaculture Centre (CAC) on Guadalcanal, Solomon Islands (Fig 2.1). Histology of gonads, digital photography and data analyses were carried out in the Department of Anatomy and Histology and the Electron Microscope Unit (EMU) of the University of Sydney.

### 2.1 Collection, transportation, dissection and fixation

All specimens were collected by snorkelling or by SCUBA. Each specimen was placed in a plastic bag containing seawater, sealed, packed in fish boxes and transported to the laboratory. An incision was made longitudinally (anterior to the posterior) on the dorsal side of each specimen and the coelomic fluid was drained. The gonad was removed using forceps. Drained body and gonad weights (Conand, 1981) were then measured (0.01 g) before the gonad was fixed in 7% buffered formalin.

### 2.2 Processing of gonad

#### 2.2.1 Assessment of reproductive activity

A gonad index (GI) was used to document the changes associated with gonad development in the three species. Although the effectiveness of the GI method is reduced for samples with a broad size range (Gonor, 1972; Grant & Tyler, 1983), it proved useful for *H. fuscogilva* and *A. mauritiana* because of their strong and consistent seasonal GI pattern over the study period (see Chapters 3 and 5). It was also useful for *H. scabra*, but the GI signal was not as consistent due to the continuous reproduction in this species (see Chapter 4). GI was determined as:

\[
g_{wt}/d_{wt} \times 100
\]
Figure 2.1.
Solomon Islands map (Main Group Archipelago excluding outer islands), showing collection sites for the three species. CAC: ICLARM Coastal Aquaculture Centre; NT: Nusa Tupe.
where \( gwt \) = wet gonad weight and \( dwt \) = wet drained weight

Gonad index was determined for all males and females in each monthly sample and a monthly mean GI was calculated for each sex. These data were plotted to assess the reproductive cycle.

### 2.2.2 Assessment of reproductive condition

Several features of the gonads were used to assess reproductive condition. These included, gonad size, gonad colour, tubule branching, tubule length and the presence of gametes in gonad squash preparations. Based on these features, a five-stage maturity scale was developed (Table 2.1).

Growth of gonad tubules was examined through the measurement of the length and diameters of 15 tubules per specimen to the nearest millimetre. This examination was undertaken to determine if tubule size was a good indicator of reproductive condition.

### 2.3 Gonad histology

The gonads preserved in formalin were rinsed in tap water and stored in 70% ethanol. For histology, five tubules were removed at random from each gonad and cut into 50 mm long sections. Tubule sections were dehydrated, embedded in paraffin, sectioned (6 \( \mu m \) thick), and stained with haemotoxylin and eosin (H/E). Based on the staining response for H/E each gonad was assigned a gametogenic stage, similar to the maturity stage scale. Five gametogenic stages (Recovery, Growing, Mature, Partly-spawned and Spent) were defined similar to the stages used in other studies of holothurian reproduction (Tanaka, 1958; Sewell, 1992).

Diameters of 10 – 30 oocytes from each of the different stages of vitellogenesis (pre-, mid-, and late vitellogenic oocytes) were also measured. The eosinophilic staining response of yolk was used to assess egg development stage. Data from histology was used to assess if the five-stage maturity scale described above based on the macroscopic appearance of tubules (Table 2.1) represented a meaningful reflection of gametogenic state (Table 2.2).
Table 2.1. Five maturity stages based on macroscopic appearance of tubules used to assess reproduction of *H. fuscogilva*, *H. scabra* and *A. mauritiana*.

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Sex identification</th>
<th>Gonad weight (g)</th>
<th>Tubule appearance</th>
<th>Tubule length</th>
<th>Tubule branching</th>
<th>Presence of gametes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Indeterminate</td>
<td>no</td>
<td>&lt; 5</td>
<td>white</td>
<td>shortest (&lt; 20 mm)</td>
<td>generally unbranched</td>
<td>absent</td>
</tr>
<tr>
<td>II. Growing</td>
<td>yes – from gonad smears</td>
<td>10–50</td>
<td>white</td>
<td>long (20–60 mm)</td>
<td>branched</td>
<td>yes</td>
</tr>
<tr>
<td>III. Mature</td>
<td>yes</td>
<td>40–150</td>
<td>colour sex-dependent</td>
<td>longest (40–180 mm)</td>
<td>branched</td>
<td>yes, oocytes visible through thin tubule wall, sperm motile</td>
</tr>
<tr>
<td>IV. Partly-spawned</td>
<td>yes</td>
<td>40–150</td>
<td>presence of spawned and unspawned tubules</td>
<td>presence of long and short tubules (40–150 mm)</td>
<td>branched</td>
<td>as for stage III</td>
</tr>
<tr>
<td>V. Spent</td>
<td>yes</td>
<td>5–50</td>
<td>wrinkled and shrunken</td>
<td>short (10–50 mm)</td>
<td>branched</td>
<td>relict unspawned gametes present</td>
</tr>
</tbody>
</table>
2.4 Culture of larvae

The microalgae *Chaetoceros calcitrans*, *C. muelleri*, Tahitian *Isochrysis* aff. *galbana*, *Rhodomonas salina* and *Tetraselmis chuii* were raised in the laboratory. All algal cultures were produced axenically in 200 ml borosilicate glass flasks and 1 litre Schott bottles. All species were batch cultured in seawater (salinity 34 to 35 %o) using f/2 beta growth medium (Guillard, 1983) at 24±1 °C with a 16:8 h light:dark photoperiod and illumination with cool white fluorescent tubes. Only algae in the logarithmic growth phase were used. Larvae were fed with 0.4 mg l\(^{-1}\) (dry weight) of the combined algal species as a standard ration feeding on an equal dry weight basis. Algal cell dry weights were taken from the literature for the same strains reared under similar conditions (Table 1, Nell & O'Connor, 1991). The standard diet was equivalent to 20 x 10\(^6\) cells l\(^{-1}\) of *Chaetoceros muelleri*.

2.5 Day length data

Data on annual variation in day length were obtained from the Ministry of Meteorology, Solomon Islands.

2.6 Data analysis

Data were archived using Microsoft\textsuperscript® Excel 97. Where mean (\( \bar{x} \)) values were presented, standard error of the mean (se) and the sample size or the number of replicates (n) followed. Where analysis of variance (ANOVA) was performed, homogeneity of variance was tested using Cochran’s Test and the data were transformed to \( \sqrt{x} \) or \( \ln (X + 1) \) to achieve homogeneity of variance. Student-Newman-Keuls (SNK) multiple range tests were used for a posteriori comparisons of ANOVA means that differed significantly. All statistical analyses were performed using NCSS 2000 (Statistical Systems for Windows, Hintze JL & NCSS, Kaysville, Utah).
CHAPTER 3

Reproductive Biology of *Holothuria fuscogilva*

*Status:*
Chapter 3. Reproduction of Holothuria fuscogilva

Abstract

Reproduction of *Holothuria fuscogilva* in the Solomon Islands was investigated over a 4 yr period (1994 to 1998) by macroscopic and microscopic examination of the gonad tubules, the gonad index (GI) method, histological examination of gametogenesis, and spawning-induction trials. The gonad consisted of numerous tubules that dominated the coelom of gravid specimens. New tubules appeared in March, and grew in size and extent of branching until they reached their maximum size in August. Spawning occurred from August to October, with the majority of gametes released during October, although it was partial in many individuals. After spawning, the tubules appeared wrinkled and resorbed into the gonad basis. A five-stage gonad maturity scale based on the macroscopic appearance of the gonad tubules corresponded with discrete stages of gametogenesis identified by histology. Gametogenesis was initiated in mid-March, with oogenesis and spermatogenesis occurring in parallel, followed by the growing stage (May to June), which was marked by active gamete development. Successful induction of spawning during the breeding period corroborated the GI and histological data. An important outcome of this study is that the appearance of gonad tubules removed by biopsy can be used to determine the gonad condition of wild adults or captive broodstock.
3.1 Introduction

Aspidochirote sea cucumbers are a conspicuous component of the macrobenthos of tropical marine environment. The importance of these large, deposit-feeding holothurians in benthic processes is well documented (Yingst, 1982; Coulon & Jangoux, 1993; Uthicke, 1999). In addition to their ecological importance, aspidochirote support numerous artisanal fisheries for bêche-de-mer throughout the Indo-Pacific (Conand & Byrne, 1993; Preston, 1993; Conand, 1997). Bêche-de-mer is derived by processing the body wall of the sea cucumbers, and is exported mainly to China and Singapore. Global export trends indicate that wild stocks of aspidochirote sea cucumbers are currently over-exploited (Conand & Byrne, 1993; Conand, 1997).

In the Indo-Pacific region, where the sea cucumber fishery benefits coastal communities in developing countries, emphasis is now being given to sustainable use of holothurian resources. Possible management measures include minimum size limits, closed seasons, bag limits and restrictions on the use of SCUBA for harvesting (Preston, 1993; Conand, 1997). However, the artisanal nature of the fishery makes implementation of such measures difficult (Conand, 1997; Battaglene & Bell, 1999). Consequently, release of juvenile sea cucumbers reared in hatcheries is being assessed as an alternative method to restore and enhance wild stocks (Preston, 1994; Munro & Bell, 1997; Battaglene & Bell, 1999), and recent studies indicate that some tropical species are particularly suitable for stock restoration and enhancement programmes (Ramofafia et al., 1997; Battaglene, 1999; Battaglene & Bell, 1999; Battaglene et al., 1999). However, the success of such programmes depends on a thorough understanding of the biology and ecology of these animals, including knowledge of the reproductive cycle.

*Holothuria fuscogilva*, commonly known as the “white teatfish”, is the most prized of the commercially important aspidochirote species (Holland, 1994). It occurs throughout the Indo-Pacific and has a patchy distribution in sea grass beds, reef slopes, and in lagoons at depths of 3 - 40 m (Conand, 1981, 1993a, 1998; Reichenbach, 1999). Previous studies report that the annual reproductive cycle of *H. fuscogilva* is variable, with spawning in December and January in New Caledonia, and from December to March during the northeast monsoon season in the Maldives (Conand, 1981, 1993a; Reichenbach, 1999).
Reproduction in *H. fuscogilva* was examined using the gonad index (GI) method, by histological examination of the gonads, and by induction of spawning. The macroscopic appearance of the gonads was examined as a rapid means of assessing reproductive maturity. The findings reported here extend the knowledge of reproduction in tropical aspidochirotes, and provides important information for culture of *H. fuscogilva*.

### 3.2 Materials and Methods

Reproduction of *H. fuscogilva* was investigated in Marau Sound, (Fig. 2.1), Guadalcanal, Solomon Islands (09°50'S; 160°49'E) over a 4 yr period. Specimens were collected at depths of 25 to 30 m from the lagoon floor, using SCUBA. As *H. fuscogilva* display no external sexual dimorphism, an attempt was made to collect at least 20 individuals each month to ensure that both males and females were obtained. Specimens were collected from February 1994 to February 1997, in May 1997, from July to November 1997, and in March 1998. The samples comprised individuals from a broad size range (1000 – 3000 g, drained weight). I was unable to reduce this size range because of difficulty in collecting specimens (density approximated 1/1000 m²).

Dissection of specimens, measurement of drained and gonad weights and fixation of gonads were done in the field according to Chapter 2, Section 2.1. Gonad maturity was assessed according to the five-stage maturity scale described in Chapter 2 (Table 2.1).

A two-way ANOVA was used to assess variation in GI maxima (*n* = 9) between sexes and years (September GI data for 1994, 1995, and 1996). For analysis, the data were √*x*-transformed to achieve homogeneity of variance as described in Chapter 2, Section 2.5.

Histology of gametogenesis was determined for gonad samples collected between May 1996 and November 1997 and in March 1998 following the procedures in Chapter 2, Section 2.3. In addition, ovary sections were also stained with the periodic acid-Schiff (PAS) method, which stains echinoderm yolk and haemal fluid (Byrne, 1992). In three ovaries, oocyte diameter was determined for 10 oocytes representing the different stages of vitellogenesis.

Spawning induction trials were conducted during the 1996, 1997 and 1998 breeding seasons. In 1996, specimens were collected from Marau Sound and in 1997 and 1998...
specimens were collected from Tulagi. In 1996 and 1997, animals were induced to spawn by heat shock, i.e., raising water temperature 2 – 3 °C above ambient (Ramofafia et al., 1995). Specimens collected in 1998 were induced to spawn by the addition of the dried alga, Schizochytrium sp., to holding tanks (Battaglene, 1999). The proportions of animals induced to spawn were recorded.

Water temperature data were obtained from giant clam grow-out farms 3 km west of the Marau collection site at a depth of 3 m in 1995 and 1996.

### 3.3 Results

A total of 724 *H. fuscogilva* were examined from Marau Sound. These consisted of 319 females, 371 males, 11 specimens of indeterminate sex, and 23 individuals lacking a gonad. Most of the indeterminate individuals were encountered in April and individuals lacking gonads were collected in February and April. The mean drained weight of females, males, individuals of indeterminate sex and specimens with no gonad were 1849.35 g (se = 17.97), 1834.65 g (se = 24.61), 1591.36 g (se = 103.76) and 1408.61 g (se = 107.89), respectively. During the breeding season, all *H. fuscogilva* examined had gonads, indicating that the specimens lacking gonads in February to April would have had gonads during the previous breeding season (see below). The sex ratio of the gonochoric *H. fuscogilva* did not differ from unity ($\chi^2 = 3.90; P > 0.05, n = 790$).

#### 3.3.1 Gonad morphology

The gonad of *H. fuscogilva* was a single structure consisting of numerous branched tubules arising from the gonad basis attached to the anterior body wall (Fig. 3.1A). The gonoduct opened externally at the gonopore dorsally above the mouth. During gonad development, branched tubules extended into the perivisceral cavity and dominated the cavity when gravid. The five stages of gonad development based on tubule size and appearance are detailed in Table 3.1.

Gonad growth in *H. fuscogilva* involved formation of new tubules arising from the gonad basis with subsequent increase in tubule length and diameter (Table 3.1). In the initial stage of gonad growth (Stage I), the tubules had a mean length of 17.0 mm (se = 0.40, n =
Figure 3.1.

Gonad anatomy. A. Gonad basis (gb) and tubule attachment in partly-spawned male; B mature branched testis tubule with beaded appearance; C mature ovary tubule with oocytes clearly visible through gonad wall; D mature oocytes released from gravid tubule; E spawned (st) and unspawned (ut) tubules of partly-spawned female; F spent ovarian tubules with wrinkled and shrunken appearance. (Scale bars in A, B, E = 235 μm; in C, D = 897 μm; in F = 200 μm)
150) and, in general, were unbranched. Sex could not be determined at this stage because gametes were not evident in gonad smears. As gonad growth progressed (Stage II), females and males could be identified by the presence of developing eggs and sperm (Fig. 3.1C, B respectively). As the gonads approached maturity, the sex of specimens could be also be determined by gonad colour. Growing testes appeared creamy white and tubules had a uniform appearance. When mature (Stage III), tubules were packed with spermatozoa and tubules with an irregular beaded appearance were occasionally seen (Fig. 3.1B). These beaded tubules were seen prior to spawning and so their irregular appearance was not due to partial release of sperm. However, beaded tubules with variable appearance persisted after spawning. Mature ovaries were mustard in colour, and individual tubules had transparent thin tubule walls through which oocytes were evident (Fig. 3.1C, D). Tubule length was a good indicator of reproductive maturity (Fig. 3.2), with the longest tubules present at the mature stage (females: $\bar{X} = 78.61$ mm long, $se = 2.80$, $n = 112$; males: $\bar{X} = 87.48$ mm long, $se = 3.40$, $n = 102$).

Through the spawning season, the simultaneous presence of both spawned and unspawned tubules (Stage IV) indicated that partial spawning was characteristic of *H. fuscogilva* (Fig. 3.1E). Examination of gonad smears revealed the presence of abundant phagocytes and oocyte debris in unspawned tubules, indicating that the gametes could be reabsorbed. Spent gonad tubules (Stage V) were wrinkled and greatly reduced in size (Fig. 3.1F). They occasionally developed a brown colour because of the presence of brown bodies in the connective tissue of the gonad wall and in the lumen.

### 3.3.2 Gonad index

The mean monthly GI for female and male *H. fuscogilva* displayed a distinct seasonal pattern (Fig. 3.3). In both sexes, the GI displayed synchronous gonad development, marked by a gradual increase in mean GI beginning in May or June, and a maximum in August, September or October. The subsequent sharp decline in the GI indicated that most gamete release was complete by November (Fig. 3.3). During the spawning season, the GI values for male *H. fuscogilva* were consistently lower than those for females (Fig. 3.3). Over the 4 yr period, the maximum GI of females and males ranged from 3.69 to 4.59 and 2.70 to 2.84, respectively. The two-way ANOVA showed that this difference between
<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Gonad wt (g)</th>
<th>Length (mm)</th>
<th>Diam. (mm)</th>
<th>Branching</th>
<th>Condition</th>
<th>Colour</th>
<th>Histological stage (see Histology section in the text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Indeterminate</td>
<td>1–5</td>
<td>5–20</td>
<td>&lt; 0.30</td>
<td>0–1</td>
<td>gametes not evident</td>
<td>white</td>
<td>Recovery</td>
</tr>
<tr>
<td>II Female</td>
<td>10–40</td>
<td>20–50</td>
<td>0.5–1.0</td>
<td>1–3</td>
<td>growing oocytes (30 – 80 μm)</td>
<td>white</td>
<td>Growing</td>
</tr>
<tr>
<td>Male</td>
<td>10–30</td>
<td>20–60</td>
<td>0.5–1.0</td>
<td>1–3</td>
<td>sperm developing</td>
<td>white</td>
<td>Growing</td>
</tr>
<tr>
<td>III Female</td>
<td>50–150</td>
<td>50–120</td>
<td>1–2.5</td>
<td>1–4</td>
<td>oocytes visible through thin tubule wall (140 – 170 μm)</td>
<td>translucent</td>
<td>Mature</td>
</tr>
<tr>
<td>Male</td>
<td>50–100</td>
<td>50–180</td>
<td>1–2.0</td>
<td>1–4</td>
<td>tubules packed with sperm</td>
<td>creamy white</td>
<td></td>
</tr>
<tr>
<td>IV Female</td>
<td>50–150</td>
<td>50–120</td>
<td>1–2.0</td>
<td>1–4</td>
<td>tubules reduced, relict oocytes present, empty lumen visible. Phagocytes present, small oocytes observed</td>
<td>tubules appeared brown</td>
<td>Partly-spawned</td>
</tr>
<tr>
<td>Male</td>
<td>50–100</td>
<td>50–180</td>
<td>1–2.0</td>
<td>1–4</td>
<td>unspawned tubules densely packed with sperm. Spawned tubules empty and reduced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V Female</td>
<td>10–50</td>
<td>10–30</td>
<td>&lt;1.00</td>
<td>1–3</td>
<td>relict oocytes may be present, tubules shrunken and wrinkled and reduced in size</td>
<td>white, or brown</td>
<td>Spent</td>
</tr>
<tr>
<td>Male</td>
<td>10–40</td>
<td>10–50</td>
<td>&lt;0.80</td>
<td>1–4</td>
<td>shrunken and wrinkled tubules, relict sperm present, brown spots occasionally developed.</td>
<td>gonad basis with brown colouration</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.2.
Monthly variation in tubule length and tubule diameter of females (●) and males (O) over 4 yr. $n = 5 - 19$. (Vertical bars standard error of mean)
sexes was significant in all years of the study \((F = 5.30; P < 0.05)\). It also showed that there was no significant variation in GI among years \((F = 1.50; P > 0.05)\).

3.3.3 Histology

Histology revealed that the five gonad maturity stages designated by gonad tubule size and appearance correlated with the five stages of gametogenic development. A description of the histological features of each gametogenic stage is detailed below.

3.3.3.1 Females:

Based on their staining responses to PAS and H/E, the developing oocytes were categorized as previtellogenic \((\bar{X} = 13 \mu m, se = 0.8, n = 30)\), and early \((\bar{X} = 40.10 \mu m, se = 0.70, n = 30)\) mid \((\bar{X} = 60.10 \mu m, se = 1.00, n = 30)\), or late vitellogenic \((\bar{X} = 71.10 \mu m, se = 1.26, n = 30)\). Previtellogenic and vitellogenic oocytes maintained their position along the germinal epithelium (Fig. 3.4A - D). Haemal fluid was not seen in sections stained with PAS. The histological stages of oogenesis are as follows:

**Stage I: Recovery.** Ovaries in the recovery stage (Fig. 3.4A) had thick walls. Previtellogenic oocytes were basophilic and PAS-negative. In some sections, early vitellogenic oocytes were observed along the germinal epithelium and were still strongly basophilic, although presence of yolk was evident among the scattered PAS+ granules. Relict oocytes from the previous reproductive season and phagocytes were occasionally seen.

**Stage II: Growing.** The growing stage (Fig. 3.4B) was characterised by active vitellogenesis. Early and mid-vitellogenic oocytes were abundant. These oocytes had a distinct germinal vesicle which persisted until spawning. Vitellogenic oocytes were surrounded by follicle cells throughout development. The gonad wall was greatly reduced in thickness as oogenesis progressed towards maturity.

**Stage III: Mature.** Mature ovaries were densely packed with PAS+ and eosinophilic late-vitellogenic oocytes and the gonad wall was thin (Fig. 3.4C). The oocytes remained within their follicle and the germinal vesicle started to take up an eccentric position towards the
Figure 3.3.
Monthly variation in Gl of females (●) and males (O) from Solomon Islands over 4 yr. $n = 5 - 19$. (*Vertical bars* standard error of mean)
Chapter 3. Reproduction of Holothuria fuscogilva

basal protuberance (Fig. 3.4D). The protuberance was PAS negative and contained filamentous strands.

**Stage IV: Partly-spawned.** Not all ovarian tubules released gametes during spawning. Partly-spawned ovaries contained both spawned and unspawned tubules (Fig. 3.4E, F). Although phagocytes were present in the spawned and unspawned tubules, there were more of these cells in the latter (Fig. 3.4F). Spawned tubules had a reduced diameter and a wrinkled appearance. The ovary wall gradually increased in thickness. In some specimens, gametogenesis was re-initiated and previtellogenic and early vitellogenic oocytes lined the germinal epithelium. Relict oocytes and debris were occasionally observed in the lumen (Fig. 3.4F, G, H).

**Stage V: Spent.** Spent ovaries were wrinkled and shrunken with relict oocytes occasionally present in the lumen (Fig. 3.4G, H). Phagocytes were evident and the gonad wall was thick.

### 3.3.3.2 Males

**Stage I. Recovery.** Testes in recovery stage could only be identified by histology. A layer of spermatogonia was present along the germinal epithelium and the gonad wall was at its maximum thickness (Fig. 3.5A). The lumen was empty.

**Stage II. Growing.** A striking feature of growing testes was the numerous infolds of the germinal epithelium, which extended for some distance into the lumen (Fig. 3.5B, C). These infolds were lined by a dense layer of spermatocytes organized in short columns. In late growing-stage testes, the germinal infolds were reduced and spermatozoa were abundant in the lumen. The thickness of the gonad wall was greatly reduced.

**Stage III. Mature.** In mature testes, the infolds of the germinal epithelium were reduced or absent and the lumen was packed with spermatozoa (Fig. 3.5D, E). A few spermatocytes may still be present along the germinal epithelium (Fig. 3.5D). The gonad wall was at its minimal thickness.
Figure 3.4.
Oogenesis. A, B Recovering and early growing ovaries with previtelligenic (pv) and early (ev) vitellogenic oocytes. C, D Mature ovary with oocytes enclosed within their follicle (f) and germinal vesicle (gv) located eccentrically towards protuberance (pt) which attached to germinal epithelium. E, F Partly-spawned ovary; many unspawned ova persisted during this stage; phagocytes (p) were especially abundant in unspawned tubules (F). G, H Spent ovaries; intensive shrinkage of tubules occurred and a few relict oocytes persisted; in some sections, sex could be identified only by presence of one or two oocytes (H). (Scale bars in A, D = 84 μm; in B, C, F = 185 μm; in E, G, H = 320 μm)
Stage IV. Partly-spawned. As for the ovary, partly-spawned testes contained tubules that had, and those that had not, spawned. Spawning activity was indicated by a diffuse arrangement of spermatozoa in the lumen. There was also a reduction in the size of the tubules, which were wrinkled and shrunken in appearance. Dense aggregations of spermatozoa and phagocytes were present in the lumen (Fig. 3.5F). Spermatocytes along the germinal epithelium indicated the renewal of gametogenesis in some tubules. Some testes had a distinct gap between this spermatocyte layer and the spermatozoa in the lumen.

Stage V: Spent. Spent tubules were shrunken and generally had an empty lumen except for a few relict spermatozoa (Fig. 3.5G, H). Spermatogonia were scattered along the germinal epithelium. The gonad wall was thick and the germinal epithelium was convoluted in some specimens.

3.3.4 Reproductive cycle
The percentage of individuals at each gametogenic stage in monthly samples collected over 2 yr is shown in Fig. 3.6. By May of each year, most gonads contained new cohorts of developing gametes. Examination of gonads from March 1998 \( (n = 18) \) revealed that both spent (39%) and recovery (61%) gametogenic stages were present, indicating that re-initiation of gametogenesis occurred in March/April. This approximated the time when day become shorter than night (Fig. 3.7). In both sexes, gametogenesis occurred in parallel (Fig. 3.6), resulting in a similar increase in GI (Fig. 3.3). The rapid increase in GI from May to August coincided with active vitellogenesis and spermatogenesis, with maturity reached in August (Figs. 3.3, 3.6). Histology revealed that partial gamete release was underway by August (Fig. 3.6). Partial spawning was also characteristic of the specimens collected in September and October. The marked decrease in GI between September/October and November, and the presence of spent gonads in November, indicated that most spawning activity occurred in September or October. Individuals with spent gonads and low GI were found from November to March/April (Fig. 3.3, 3.6).

3.3.5 Induction of spawning
Over 3 yr, spawning of \( H. \ fuscogilva \) was induced successfully in August, September and October, particularly in males (Fig. 3.8). In 1996 and 1997, heat shock successfully elicited gamete release in 10 % of animals \( (n = 134) \). Addition of the dried alga,
Figure 3.5.
Spermatogenesis. **A** Recovering testis with early spermatocytes lining germinal epithelium. **B, C** Growing testis with infolds of germinal epithelium (*arrow*) and sperm developing spermatocyte columns (*sc*); spermatozoa began to fill lumen as growth progressed. **D, E** Mature testis; in early mature stage (**D**) spermatocytes persist along gonad wall but are absent from fully matured testes (**E**). **F** Partly-spawned testis with spermatozoa in lumen. **G, H** Spent testis with residual spermatozoa or empty lumen. (*Scale bars* in **A, C, D** = 84 μm; in **F, H** = 185 μm; in **B, F** = 320 μm; in **E** = 741 μm)
Chapter 3. Reproduction of Holothuria fuscogilva

Schizochytrium sp., resulted in release of gametes in 36% of individuals \((n = 47)\). Both methods were most successful at inducing spawning in September and October.

The breeding period of *H. fuscogilva* coincided with an increase in day length, which begins in August (Fig. 3.7). There was no clear relationship, however, between spawning and water temperature. Temperature varied annually between 28 and 31°C.

3.4 Discussion

The reproductive cycle of *H. fuscogilva* in Solomon Islands was similar to that in New Caledonia (Conand, 1981, 1993a) and the Maldives (Reichenbach, 1999) in that spawning occurred in summer. However, the onset of spawning differed, starting in August in Solomon Islands, November in New Caledonia and December in the Maldives. In addition, mature individuals were present for 3 to 5 months in the Solomon Islands, whereas they occurred throughout the year in the Maldives, with the greatest numbers recorded from August to December (Reichenbach, 1999).

The seasonal reproduction of *H. fuscogilva* is typical of many tropical holothurians (Conand, 1981, 1982, 1993a, b; Harriott, 1985; Hopper *et al.*, 1998; Reichenbach, 1999) and contradicts the widespread assumption of year-round spawning of marine invertebrates in the tropics (Thorson, 1950), but see Ong Che & Gomez (1985) and Ong Che (1990) for species that do have prolonged reproduction. Water temperature (Tanaka, 1958; Conand, 1981), photoperiod (Conand, 1982; Cameron & Fankboner, 1986), water velocity (Engstrom, 1980), salinity (Krishnaswamy & Krisnan, 1967), a combination of water temperature and photoperiod (Costelloe, 1985) and phytoplankton blooms (Hamel *et al.*, 1993) have all been implicated as factors controlling gametogenesis and/or spawning in sea cucumbers. The onset of gametogenesis by *H. fuscogilva* in Marau coincided with the inflection point in March when light period becomes shorter than the dark period, indicating that photoperiod may entrain gonad development, as documented for sea urchins (Pearse *et al.*, 1986; Byrne *et al.*, 1998; Walker & Lesser, 1998). Spawning may be entrained to a lunar cue, with the crepuscular period defining the time of gamete release, as reported for a variety of echinoderms (Babcock *et al.*, 1992; Byrne *et al.*, 1998).
Figure 3.6.
Gametogenic cycle of females (A) and males (B), collected from May 1996 to November 1998. Histograms showing percentage of individuals with gonads in each of five maturation stages. (n sample size for each month; samples containing < 5 females or males are not included)
Tanaka (1958) suggested that temperature may control spawning in holothurians and Conand (1981, 1982, 1993a, b) demonstrated a consistent relationship between temperature and spawning in some tropical species. In the Solomon Islands there is no clear seasonal changes in sea temperature during the breeding season of *H. fuscogilva*. Spawning was, however, induced by heat-shock indicating that *H. fuscogilva* may release gametes *in situ* in response to short term heat-shock. Although heat-shock is often used to induce spawning in holothurians, the mechanism by which this stimulus promotes oocyte maturation, ovulation and spawning is not known (McEuen, 1987, Ramofafia *et al.*, 1995). Induction of spawning in *H. fuscogilva* in response to introduction of the dried alga, *Schizochytrium* sp., to holding tanks supports the supposition that phytoplankton exudates may influence spawning in the wild. Spawning in response to phytoplankton has been reported for other sea cucumbers and sea urchins (Cameron & Fankboner, 1986; Hamel *et al.*, 1993; Starr *et al.*, 1990).

Histology revealed that many individuals initiated gamete release before GI peaked and that many partially spawned gonads contained numerous phagocytes. This demonstrates that changes in GI alone cannot be used to estimate the timing and duration of gamete release in *H. fuscogilva*. On the other hand, there was good correlation between the maturity stages based on the tubule size and appearance and those based on histology. The fact that tubule morphology can be used to detect release of gametes is of great benefit to breeding programmes. In particular, it means that the biopsy techniques described by Yanigasawa (1998) and Reichenbach (1999), in which tubules can be removed through a dorsal incision in the body wall near the gonopore, can be used to assess the condition of broodstock. This assessment method will be especially important in remote locations or when the animals are used as broodstock and cannot be sacrificed.

Gametogenesis in *H. fuscogilva* was similar to that documented for other holothurians (Smiley *et al.*, 1991; Sewell *et al.*, 1997). Initiation of gametogenesis in the March-April period, and subsequent gametogenic maturation and gamete release, occurred in parallel for both sexes. Like several other sea cucumber species (Sewell, 1992; Hamel *et al.*, 1993), spawning in *H. fuscogilva* is partial and it is not known if unspawned tubules eventually release their gametes. However, as seen for *Psolus fabricii*, the abundance of phagocytes in unspawned tubules suggested a strong phagocytic activity, with potential sequestration of material for storage and eventual use in gametogenesis (Hamel *et al.*, 1993).
Figure 3.7

Annual day-length cycle for Solomon Islands
1993. Reinitiation of gametogenesis in spawned and unspawned tubules resulted in the presence of overlapping generations of immature and unspawned relict oocytes, as documented for other holothurians (Sewell et al., 1997). Despite this gametogenic renewal, all the gonad tubules in *H. fuscogilva*, were resorbed to the basis after cessation of breeding and thus any immature oocytes present in the gonads were also reabsorbed. The rationale underlying reinitiation of gametogenesis just prior to tubule resorption is not clear, but has been reported for several holothurians (Choe, 1963; Cameron & Fankboner, 1986; Sewell, 1992; Hamel et al., 1993).

A feature of testis development in *H. fuscogilva*, the presence of numerous longitudinal folds in the germinal epithelium, appears typical of sea cucumber spermatogenesis (Smiley et al., 1991). Two functions have been suggested for these folds; to increase the surface area for proliferation of spermatogonia (Cameron & Fankboner, 1986), and to provide a reservoir of nutrients in the haemal fluid to support spermatogenesis (Smiley et al., 1991).

The occurrence of *H. fuscogilva* lacking gonads in the February to April samples shows that complete resorption of gonad tubules occasionally occurs at the end of breeding. This result was not a result of sampling individuals below the size at first maturity. This phenomenon appears to be rapid, short lived and variable among the population. As noted for *H. fuscogilva* in the Solomon Islands, individuals with indeterminate gonads also occur in April in New Caledonia (Conand, 1981). Annual gonad resorption has also been reported for *Stichopus mollis* (Sewell, 1992) and *S. japonicus* (Choe, 1963). In *S. mollis*, total resorption occurs in populations at a lower latitude and higher water temperature, whilst populations at higher latitude undergo incomplete resorption, and maintain a resting phase. These contrasting populations subsequently redevelop their gonads in time to spawn in the next season. My data differed from those of Sewell in that individuals lacking gonads or with indeterminate gonads were present in the population at the same time. I suggest that total gonad resorption may occur annually in *H. fuscogilva* followed by rapid gonadal regeneration. This however, is asynchronous among individuals. A weekly sampling programme during the February to April period is required in order to verify this suggestion.

In conclusion, I have shown that the breeding season of *H. fuscogilva* in Solomon Islands occurs from August to October, and that gonad tubule morphology provides a relatively
Figure 3.8.
Percentage of females and males induced to spawn from August to October. Sample size \((n)\) combines data from 1996 to 1998.
rapid and reliable method of assessing reproductive condition without sacrificing valuable and scarce animals. Biopsy of gonad tubules should prove to be particularly effective in assessing the availability of gametes from captive broodstock used in breeding programmes to restore and enhance wild fisheries.
CHAPTER 4

Reproductive Biology of *Holothuria scabra*

*Status:*
Chapter 4. Reproduction of Holothuria scabra

Abstract

Over a 3 yr period (1996 – 1998), reproduction of the commercial sea cucumber *Holothuria scabra* (Jaeger, 1833) was investigated in the Solomon Islands to determine the spawning pattern and if gametogenesis is continuous or seasonal. The single gonad consisted of a single cohort of tubules that developed uniformly. Macroscopic examination of the gonads revealed that mature gametes were present throughout the year. Individuals with gonads at different stages of maturity were present in most samples. Partly-spawned gonads were prevalent in females while mature gonads were prevalent in males. The time at which the peak gonad index was recorded differed among years. Although gametogenesis was continuous with a potential for prolonged gamete release, a period of enhanced spawning activity occurred during the dry season, from September to December. Maximum gonad indices were reached prior to and during this period of enhanced spawning activity. Histology revealed that gametogenesis reinitiated in partly-spawned gonads resulting in the presence of gametes at different stages of development in the gonad. Continuous reproduction in *H. scabra* and prolonged availability of mature gametes indicates this species has potential for aquaculture.
4.1 Introduction

*Holothuria scabra* (commonly known as sandfish) is an aspidochirote holothurian widely distributed in coastal regions throughout the Indo-Pacific region (Conand, 1998). *H. scabra* is often found on inner reef flats and near estuaries, half buried in the silty sand during the day and emerging at night to feed (Mercier et al., 1999). Two colour morphs, black and grey varieties, are known and genetic data have shown that these are a single species (Uthicke & Benzie, 1999). *H. scabra* is among approximately 20 aspidochirote species that constitute the bêche-de-mer fishery in the Indo-Pacific and one of only three species that consistently fetches high prices in the trade (Conand & Byrne, 1993; SPC, 1994). In the last decade, increased demand for bêche-de-mer, has resulted in over-exploitation of *H. scabra* (Conand & Byrne, 1993; Conand, 1997). To ease the pressure on wild stocks, new management strategies have been sought, including the hatchery production of juvenile sea cucumbers to restock and enhance depleted populations (Battaglene & Bell, 1999).

Among the diverse assemblage of tropical aspidochirotids, *H. scabra* exhibits the greatest potential as an aquaculture species and has been raised successfully to the juvenile stage (James et al., 1994; Martoyo et al., 1994; Morgan, 1998; Battaglene, 1999; R. Pitt, pers. comm.). Attempts to culture several other species in Solomon Islands and elsewhere in the tropical Pacific have not been successful (Ramofafia et al., 1995; Battaglene, 1999).

A biannual spawning pattern for *H. scabra* is reported in India, Philippines, New Caledonia and Indonesia (Table 4.1). These studies also reported that mature *H. scabra* can be found year-round, raising the possibility that reproduction may be continuous, a feature that would facilitate the use of this species for aquaculture. In subtropical regions of Australia, by contrast, annual reproduction is reported for *H. scabra* (Harriott, 1980; Morgan, 2000a). A recent review of the biology of *H. scabra* concluded that, despite the investigations conducted over its geographic range, further research is required to gain a better understanding of the reproductive cycle and spawning cues (Hamel et al., 2001). In particular, the suggestion that tropical *H. scabra* populations have continuous gametogenesis has to be assessed through histological examination of gonad condition.
### Table 4.1. *Holothuria scabra*. Reproduction of *Holothuria scabra* over the geographical range 27° N to 27° S.

<table>
<thead>
<tr>
<th>Latitude (°)</th>
<th>Location</th>
<th>Methods used</th>
<th>Study duration (yr)</th>
<th>Gonad Index (GI) Maximum</th>
<th>Reproductive pattern</th>
<th>Spawning period</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>27N</td>
<td>Red Sea</td>
<td>Field Spawning observation</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>Includes June</td>
<td>Mortensen, 1937</td>
</tr>
<tr>
<td>13N</td>
<td>Philippines</td>
<td>GI and histology</td>
<td>2</td>
<td>No obvious peaks</td>
<td>Continuous Mature specimens year-round</td>
<td>Increased activity in June &amp; October (biannual peaks assumed)</td>
<td>Ong Che &amp; Gomez, 1985</td>
</tr>
<tr>
<td>09N</td>
<td>India</td>
<td>GI</td>
<td>1</td>
<td>July &amp; October</td>
<td>Biannual</td>
<td>July - August (main) October - November</td>
<td>Krishnaswamy &amp; Krishnan, 1967</td>
</tr>
<tr>
<td>05S</td>
<td>Indonesia</td>
<td>GI and histology</td>
<td>1</td>
<td>April &amp; November</td>
<td>Biannual Mature specimens year-round</td>
<td>April &amp; November</td>
<td>Tuwo, 1999</td>
</tr>
<tr>
<td>09S</td>
<td>Solomon Islands</td>
<td>GI and histology</td>
<td>3</td>
<td>August &amp; October</td>
<td>Continuous Mature specimens year-round</td>
<td>Enhanced activity from September to December</td>
<td>Present Study</td>
</tr>
<tr>
<td>20S</td>
<td>New Caledonia</td>
<td>GI</td>
<td>2 or more years data. Annual cycle presented</td>
<td>August &amp; December</td>
<td>biannual</td>
<td>August – September December - January</td>
<td>Conand, 1993a</td>
</tr>
<tr>
<td>27S</td>
<td>Australia</td>
<td>GI and histology</td>
<td>1.5</td>
<td>December</td>
<td>Annual</td>
<td>November - January</td>
<td>Harriott, 1980</td>
</tr>
<tr>
<td>27S</td>
<td>Australia</td>
<td>GI and histology</td>
<td>1.5</td>
<td>November</td>
<td>Annual</td>
<td>November - December</td>
<td>Morgan, 2000a</td>
</tr>
</tbody>
</table>

** not documented
Chapter 4. Reproduction of Holothuria scabra throughout the year: most other studies have relied on the gonad index method (Hamel et al., 2001).

This Chapter documented the reproduction of *H. scabra* in Solomon Islands. The aims were to determine the breeding pattern of *H. scabra*, to identify the timing of gamete availability, and to determine if gametogenesis is continuous or seasonal.

### 4.2 Methods and Materials

Samples of *H. scabra* were collected from Kogu Halingi Bay on Kohinggo Island, Vona Vona Lagoon, Western Province of Solomon Islands (8° 10'S: 157° 11'E). Samples were collected by snorkelling or by SCUBA from May 1996 to December 1998. Specimens were transported to the laboratory as described in Chapter 2, Section 2.1. Methods of dissection of specimens, fixation and processing of gonads were described in Chapter 2, Section 2.2.

Histology methods were described in Chapter 2, Section 2.3. The percentage of *H. scabra* in each of the five gametogenic stages (Recovery, Growing, Mature, Partly-spawned and Spent) was determined for all samples collected from May 1996 to December 1998.

To determine if GI differed among months and years, the GI data of both sexes were combined and compared by analysis of variance (ANOVA). Factors were year (Y) and month nested in year (M(Y)); sample size was *n* = 17, the minimum monthly sample size recorded. Where monthly sample size was greater than 17, specimens were picked at random to achieve *n* = 17.

A second ANOVA was also carried out to determine if the GI data of females and males differed. For this ANOVA, factors were sex (S), year (Y) and months nested in year (M(Y)); sample size was *n* = 6. For the ANOVA models to be balanced, the data obtained in 1996 were excluded from the analyses as only 6 monthly samples were collected. The 1997 and 1998 November data were also excluded from the second ANOVA as the male sample size in 1998 was lower than *n* = 6. Data were transformed to ln (*X* + 1) to achieve homogeneity of variance and Student-Newman-Keuls (SNK) multiple range tests were
used for a posteriori comparisons of monthly GI results that differed as described in Chapter 2, Section 2.6.

4.3 Results

A total of 845 *H. scabra* were collected, 414 females (\( \bar{x} \text{dwt} = 644.94 \text{ g}, \text{se} = 11.28 \)) and 428 males (\( \bar{x} \text{dwt} = 655.49 \text{ g}, \text{se} = 11.87 \)). Two specimens with indeterminate gonads (\( \bar{x} \text{dwt} = 384.95 \text{ g}, \text{se} = 105.05 \)) were collected in July 1996 and April 1998 and one specimen that lacked a gonad (\( \bar{x} \text{dwt} = 396.50 \text{ g} \)) was collected in December 1996. The sex ratio did not differ significantly from unity (\( \chi^2 = 0.85; P > 0.05, n = 842 \)).

4.3.1 Gonad morphology

The gonad of *H. scabra* was a single tuft of numerous branched tubules located on the right side of the dorsal mesentery. All the tubules developed uniformly through the gametic maturity stages. The size, colour and shape of tubules and numbers of branches of each tubule depended upon the stage of gonad development. Gonad maturity stages are compared in Table 4.2. All stages, with the exception of indeterminate stage I, were present in samples throughout the year.

**Stage I: Indeterminate.** Indeterminate gonads were small and composed of white unbranched tubules (< 10 mm in length). Sex could not be determined, even by microscopic examination of gonad squash preparations.

**Stage II: Growing.** Growing gonads increased in size with length ranging from 20 to 70 mm. Examination of gonad squash preparations revealed the presence of developing gametes. In growing gonads, the tubules began to bifurcate forming a series of branches. The length of the tubule branches varied within each gonad. Ovaries and testes were orange and glossy white, respectively.

**Stage III: Mature.** Mature gonads had large fecund tubules (30 - 200 mm long) that dominated the coelomic cavity (Table 4.2). During the mature stage the tubules developed additional branches. Ovaries were a red-orange colour and oocytes were apparent through
Table 4.2. Five maturity stages in the reproductive cycle of *Holothuria scabra* based on morphology of the gonad tubules. The column ‘Branching’ refers to the number of times tubule branching had occurred.

<table>
<thead>
<tr>
<th>Maturity stage, Sex</th>
<th>Gonad wt (g)</th>
<th>Tubule Length (mm)</th>
<th>Diameter (mm)</th>
<th>Branching Condition</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Indeterminate</td>
<td>&lt; 5</td>
<td>&lt; 10</td>
<td>&lt; 0.3</td>
<td>0</td>
<td>White</td>
</tr>
<tr>
<td>Female</td>
<td>5-30</td>
<td>30-70</td>
<td>0.7-1.4</td>
<td>Developing oocytes (20-80 μm)</td>
<td>Orange</td>
</tr>
<tr>
<td>Male</td>
<td>5-30</td>
<td>20-60</td>
<td>0.5-1.3</td>
<td>Sperm developing</td>
<td>White</td>
</tr>
<tr>
<td>II: Growing Female</td>
<td>40-200</td>
<td>40-160</td>
<td>1.0-3.0</td>
<td>Oocytes visible through transparent tubule (120-170 μm)</td>
<td>Reddish orange</td>
</tr>
<tr>
<td>Male</td>
<td>30-200</td>
<td>30-170</td>
<td>0.5-2.5</td>
<td>Tubules packed with sperm, tubules have beaded appearance</td>
<td>Creamy white</td>
</tr>
<tr>
<td>III: Mature Female</td>
<td>30-120</td>
<td>30-140</td>
<td>0.5-2.1</td>
<td>Tubules reduced, empty regions of lumen visible, Phagocytes present in spawned and unspawned tubules</td>
<td>Reddish orange</td>
</tr>
<tr>
<td>Male</td>
<td>20-90</td>
<td>20-150</td>
<td>.3-1.9</td>
<td>Spawned tubules reduced, unspawned tubules packed with sperm</td>
<td>Creamy white</td>
</tr>
<tr>
<td>IV: Partly-spawned Female</td>
<td>0.1-40</td>
<td>6-130</td>
<td>0.1-1.8</td>
<td>Tubules reduced and shrunken, relict oocytes present</td>
<td>Pale orange</td>
</tr>
<tr>
<td>Male</td>
<td>0.1-40</td>
<td>10-140</td>
<td>0.1-1.3</td>
<td>Unspawned sperm present in shrunken and wrinkled tubules</td>
<td>White</td>
</tr>
</tbody>
</table>
the thin transparent tubule wall. Testes were creamy white and the tubules took on a beaded appearance. Within each gonad the total length and diameter of the tubules were relatively uniform. Examination of ovary squash preparations showed the presence of oocytes at different stages of development. However, fully grown oocytes were dominant. Squash preparations of testes revealed the presence of highly active sperm.

**Stage IV: Partly-spawned.** Partly-spawned gonads possessed a combination of large fecund tubules and tubules that had released their gametes. Spawned tubules were reduced in size and appeared flaccid. The unspawned tubules were similar in appearance to that described for mature tubules. Phagocytes were evident in the lumen of both tubule types and were particularly abundant in the unspawned tubules indicating that the gametes present were being resorbed. In some ovaries, oocytes in the unspawned tubules were clearly being broken down. Due to presence of spawned and unspawned tubules within the gonads, tubule length and diameter were highly variable. Examination of ovary squash preparations revealed the presence of immature oocytes in both spawned and unspawned tubules, indicating that re-initiation of gametogenesis had occurred. Immature oocytes were particularly conspicuous in spawned tubules.

**Stage V: Spent.** Spent gonads were greatly reduced in size (Table 4.2). Tubules were wrinkled and shrunken in appearance. The weight of these gonads ranged from < 1 g to 40 g. Relict oocytes and unspawned sperm were usually seen in gonads 10 - 40 g in weight. The gonad basis of the smallest gonads (< 1g) typically had a brown colour due to the accumulation of brown bodies, residual deposits characteristic of sea cucumbers (Jans et al., 1996). Examination of squash preparations of these gonads revealed that most tubules were empty.

### 4.3.2 Histology

#### 4.3.2.1 Oogenesis

Ovaries in the recovery or indeterminate stage (I) had previtellogenic oocytes (< 20 μm diameter) developing along the germinal epithelium (Fig. 4.1A). These oocytes were strongly basophilic. Growing stage (Stage II) ovaries were characterised by active vitellogenesis (Fig. 4.1B). The oocytes were in the early (20 - 40 μm diameter) and mid (30 - 80 μm diameter) vitellogenic stages and were increasingly eosinophilic. The oocytes
Figure 4.1.
Oogenesis in *H. scabra*. **A** Recovering ovary with pre-vitellogenic oocytes *(arrow)*. **B** Growing ovary with active vitellogenesis with early *(evo)* and mid *(mvo)* vitellogenic oocytes. **C, D** Mature ovary with fully grown oocytes with large germinal vesicle *(gv)* developed within follicles *(f)* and attached to the germinal epithelium by the protuberance *(arrowhead)*. **E, F, G** Partly-spawned ovary with loosely packed unspawned oocytes and renewed gametogenic activity with pre-vitellogenic *(pv)* and vitellogenic oocytes *(vo)*. Relict oocytes *(ro)* from previous spawning period also persist. **H** Spent ovary with wrinkled and shrunken ovarian tubule containing relict oocytes *(ro)* and oocytes being degraded by phagocytic *(p)* activity. *(Scale bar in A, B = 25 μm; in C = 133 μm; in D = 65 μm; in E, F, G, H = 240 μm).*
developed within a follicle formed by somatic cells. Mature ovaries (Stage III) were densely-packed with eosinophilic late-vitellogenic oocytes (120 – 170 μm diameter) which remained within their follicle (Fig. 4.1C, D). The germinal vesicle of these oocytes was prominent and occupied a central or acentric position near the basal protuberance (Fig. 4.1D). The protuberance attached the oocyte to the germinal epithelium (Fig. 4.1D). Partly-spawned ovaries (Stage IV) were characterised by the presence of unspawned oocytes and aggregations of phagocytes in the lumen. Unspawned oocytes were loosely arranged with vacancies in the lumen left by recent spawning activity (Fig. 4.1E). Renewed gametogenesis in partly-spawned ovaries resulted in the presence of overlapping generations of oocytes (Fig. 4.1F, G). The tubules of spent ovaries (Stage V) had a large lumen largely devoid of contents. A few relict oocytes were occasionally present and phagocytes were also seen in some specimens (Fig. 4.1H).

4.3.2.1 Spermatogenesis
The pattern of testis development was also divided into five stages (Fig. 4.2). Recovery stage (Stage I) testes were characterised by a layer of basophilic spermatocytes lining the germinal epithelium. Testis development then progressed to the growing stage (Stage II) which was characterised by dense layers of spermatocytes in the germinal epithelium (Fig. 4.2A, B). Invaginations of the germinal epithelium projected towards the centre of the tubule, increasing the surface area of the germinal epithelium. As testis growth progressed, spermatozoa accumulated in the lumen (Fig. 4.2C, D). In mature testes (Stage III), the lumen was packed with spermatozoa and the infolds of the germinal epithelium were reduced or absent (Fig. 4.2E). The wall of the mature testes was also at their minimal thickness. In partly-spawned (Stage IV) testes the spermatozoa were less densely packed (Fig. 4.2F, G) and phagocytes were also present in the lumen. In some testes, gaps in the spermatozoal mass indicated the occurrence of gamete release. Gametogenesis reinitiated in partly spawned testes as indicated by the presence of developing spermatocytes and the reappearance of infolds of the germinal layer (Fig. 4.2G). Spent (Stage V) testes were largely devoid of contents, with the exception of a few unspawned spermatozoa (Fig. 4.2H, I). In some spent testes, the germinal epithelium took on a convoluted appearance but renewed gametogenesis was not observed.
Figure 4.2.
Spermatogenesis in *H. scabra*. A, B Late Recovering/Growing testes with infolds of germinal epithelium lined with columns of developing spermatocytes (*arrowheads*). C Late Growing testis with accumulation of spermatozoa (sz) in the lumen. Infolds of the germinal epithelium lined with columns of developing spermatocytes still persist (*arrows*). D An early Mature testis with increased predominance of spermatozoa (sz) in the lumen. Spermatocytes (sc) still evident. E A fully Mature testis with lumen packed with spermatozoa (sz). F, G Partly-spawned testes. The spermatozoa (sz) are less dense (F) and renewed gametogenic production of spermatocytes (sc) occurring (G). H, I Spent testis containing unspawned spermatozoa (sz) (H) and an empty lumen (I) (*Scale bars* in *A, B* = 90 μm; *C, D, G* = 110 μm; *E* = 750 μm; *F, H, I* = 150 μm).
4.3.3 Gonad index (GI) and tubule size

The pattern of gonad growth, as exhibited by the GI data, was variable between sexes and across years (Fig. 4.3A). For most of the study, the GI remained between 2 and 6 with the GIs of female *H. scabra* often being higher than those of males. The largest GIs for females were recorded in October 1997 and August 1998 (Fig. 4.3A). The particularly high mean GI value obtained in October 1997 was due to 4 specimens (total $n = 10$) having unusually large gonads with wet weights ranging from 125 to 210 g. The weight of gravid ovaries encountered during the 3 yrs of sampling more typically ranged between 70 and 100 g. In both 1997 and 1998, the maximum GI values in the females were followed by a decrease to December in 1997 and to October in 1998. The males recorded the largest GIs from August to October 1997 but exhibited no similar pattern in 1998. The lowest GIs for both sexes were recorded in July 1997.

The results of the ANOVA comparing all the combined male and female data revealed GI differed significantly among months and that this depended upon year (Table 4.3A). This was indicated by the nested term (M(Y)) being significantly different (Table 4.3A). Subsequently, SNK tests showed the October GI differed from that of all other months in 1997 and that the August GI differed from all other months in 1998. In the second ANOVA comparing the GI for females and males, the term S x M(Y) was also significantly different (Table 4.3B) and indicated that the GI and the reproductive activity of each sex was also variable among months and between years.

The pattern of the mean tubule size was variable in both sexes and not synchronised with the growth in weight of the gonad as measured by GI data (Fig. 4.3). For instance, in 1997 ovary tubule length was greatest in August, two months before the GI maximum was recorded. Conversely, the ovary tubule diameter was greatest in August 1997 corresponding to the peak GI. In 1998, the longest tubules were recorded in March several months before the maximum GI occurred. The pattern of testis tubule length and diameter followed change parallel that for the ovary (Fig. 4.3B, C).

4.3.4 Reproductive cycle

Gametogenesis was asynchronous in *H. scabra* and did not exhibit a seasonal pattern (Fig. 4.5). For example, most samples contained females with ovaries in at least three different stages of oogenesis (Fig. 4.5). Overall, partly spawned (53 %, $n = 220$) and spent ovaries
Figure 4.3.

A. Gonad index for females (●) and males (O) from Kogu Halingi Bay, Solomon Islands over a 3 year period. B, C tubule length (B) and diameter (C) of females (●) and males (O) over the same period. (Bars are standard error of the mean).
Table 4.3. ANOVA of the GI data. ns—not significant, ** significant at 0.01

<table>
<thead>
<tr>
<th>A. Combined female and male GI data</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sources of Variation</td>
<td>df</td>
<td>MS</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Year = Y</td>
<td>1</td>
<td>0.29</td>
<td>0.24</td>
<td>ns</td>
</tr>
<tr>
<td>Month (Y)</td>
<td>20</td>
<td>1.20</td>
<td>4.42</td>
<td>**</td>
</tr>
<tr>
<td>Residual</td>
<td>352</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>373</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Individual female and male GI data</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sources of Variation</td>
<td>df</td>
<td>MS</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Sex = S</td>
<td>1</td>
<td>0.33</td>
<td>0.53</td>
<td>ns</td>
</tr>
<tr>
<td>Year = Y</td>
<td>1</td>
<td>0.32</td>
<td>0.45</td>
<td>ns</td>
</tr>
<tr>
<td>Month (Y)</td>
<td>20</td>
<td>0.71</td>
<td>3.14</td>
<td>**</td>
</tr>
<tr>
<td>S × Y</td>
<td>1</td>
<td>0.61</td>
<td>0.26</td>
<td>ns</td>
</tr>
<tr>
<td>S × Month (Y)</td>
<td>20</td>
<td>0.62</td>
<td>2.73</td>
<td>**</td>
</tr>
<tr>
<td>Residual</td>
<td>220</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>263</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 4. Reproduction of Holothuria scabra

(30%, \( n = 123 \)) were the most frequently encountered maturity stages in females. Females with growing, mature or recovering ovaries were comparatively rare (Fig. 4.5A). In male \( H. \) scabra, individuals with mature (33 %, \( n = 143 \)), growing (29 %, \( n = 124 \)), and partly spawned testes (25 %, \( n = 108 \)) were common (Fig. 4.5B). Like the females, the presence of testes at 3 or more stages of development also indicated that gametogenesis was asynchronous among the males. Spent testes (12 %, \( n = 53 \)) were less frequent than spent ovaries. The recovery stage was only seen in one male collected in December 1996 (Fig. 4.5B).

The data from gonad index, tubule size and histology suggested that \( H. \) scabra has a continuous and asynchronous pattern of gonad development in Solomon Islands (Figs. 4.3, 4.5). However, reproductive activity did exhibit some consistency among months with maximum gonad growth for the females recorded in the August to October period in 1997 and 1998. This was followed by a decrease in GI due to gamete release. In September and October 1996, some of the \( H. \) scabra underwent spontaneous gamete release before the gonads could be used for analysis.

4.4 Discussion

\( H. \) scabra is one of the most widely distributed tropical sea cucumbers, with a distribution encompassing 54 degrees of latitudes (Table 4.1). Over this broad distribution, \( H. \) scabra exhibits two main basic reproductive patterns: seasonally predictable spawning at high latitudes and aseasonal spawning at low latitudes (Table 4.1). Reproduction of \( H. \) scabra in Solomon Islands was characterised by continuous, asynchronous gametogenesis with mature gametes present year round. Spawning appeared to be episodic through the year with a period of enhanced activity from September to December. In the Philippines, \( H. \) scabra exhibits two periods of enhanced spawning activity superimposed on continual reproductive maturity (Ong Che & Gomez, 1995). The continuous gametogenesis that characterised \( H. \) scabra in Solomon Islands and the Philippines may extend broadly in the Indo-Pacific region (20°N to 20°S) (Table 4.1). At the northern and the southern limits of its range, \( H. \) scabra has an annual pattern of synchronous gametogenesis (Mortensen, 1937; Morgan, 2000a) potentially cued by seasonally predictable factors such as day length.
Figure 4.5.
Gonad histological stage in gametogenic cycle from (A) females and (B) males collected from Kogu Halingi Bay, May 1996 to December 1998. In September and October 1996 the entire sample mass spawned so the gonads were not used for histology. ($n$ sample size examined each month).
Chapter 4. Reproduction of Holothuria scabra

and water temperature, at higher latitudes. The reproductive strategies in *H. scabra*, therefore, support the hypothesis that reproduction of widely distributed tropical marine invertebrates changes from continuous to seasonal with increasing latitude (Thorson, 1950).

Across the Indo-Pacific region, spawning activity in *H. scabra* is temporally variable with biannual and continuous activities recorded (Table 4.1). Even when twice-yearly spawning peaks have been observed, the months differed among years (Table 4.1). Furthermore, the lack of an annually predictable GI pattern as seen in Solomon Islands (the present study) and in the Philippines (Ong Che & Gomez, 1985) also suggests that gonad growth can be temporally variable. This interannual variability in reproduction of populations of *H. scabra* in the Indo-Pacific region (Table 4.1) may be influenced by variable local environmental conditions, particularly those associated with the coastal mangrove and sea grass habitats occupied by this species. Spawning activity of *H. scabra* in the region may be opportunistic with some flexibility to respond to ephemeral favourable conditions.

The presence of the black and grey morphs of *H. scabra* could be taken to suggest that variable reproduction across the Indo-Pacific has an underlying genetic influence. However, there was no apparent genetic differences between the morphs of this species of the east coast of Australia (Uthicke & Benzie, 1999). None-the-less, genetic differences between populations in geographical regions were large, compared to other marine broadcast spawners (Uthicke & Benzie, 2001).

Although *H. scabra* spawns year-round, the presence of a period of enhanced spawning activity (September to December) was consistent over the 3 yrs of study in Solomon Islands, suggesting some role for exogenous cues. This period of spawning coincided with the dry season, before the monsoon begins in late December. It also coincided with periods of increased day length and water temperature (Chapters 3, 5). Temperature, salinity and photoperiod have been suggested to influence the biannual spawning pattern of *H. scabra* in India, Philippines, New Caledonia and Indonesia (Krisnaswamy & Krishnan, 1967; Ong Che & Gomez, 1985; Conand, 1993a; Tuwo, 1999). Gamete release is also likely to be influenced by other environmental factors, such as the lunar cycle which appears to stimulate aggregation and spawning in *H. scabra* around the new moon (Hamel et al.,
Synchronous spawning of _H. scabra_ and other aspidochirote species in the field and in captivity has also been noted to coincide with different lunar phases, depending on location (Shelley, 1982; Babcock et al., 1992; Morgan, 2000b; Battaglene et al., in press). Hamel et al., (2001) provide further details of the influence of various environmental factors and the timing of spawning.

Asynchronous gametogenesis and the year round availability of mature gametes in _H. scabra_ is not typical of other tropical aspidochirote species. For example, _H. fuscogilva_ and _Actinopyga mauritiana_ have synchronous and seasonal gonad development suggested to be cued by seasonally predictable changes in environmental factors (Chapters 3, 5). Further work is required to determine factors that modulate gametogenesis in _H. scabra_ in tropical regions. Gametogenic renewal occurred in partly-spawned gonads in _H. scabra_ resulting in the presence of gametes of different stages of development in the gonads. This renewed gametogenesis in _H. scabra_ provided the mechanism for continuous spawning. In contrast, the fate of new gametes in partly-spawned tubules is not known for _H. fuscogilva_, _A. mauritiana_ and several other holothurians, because the gonad tubules subsequently become reduced or are resorbed completely (Chapters 3, 5). Total gonad resorption is comparatively rare in _H. scabra_ in Solomon Islands and difficult to distinguish macroscopically from animals, which may have eviscerated.

Partly-spawned gonads were common in female _H. scabra_ while mature gonads were common in males. Due to presence of gonads at different stages of maturity in the population throughout the year, examination of gonad tubule biopsies from a few _H. scabra_ cannot be used as a tool to assess the reproductive cycle as has been done for other species (Chapters 3, 5). None-the-less, the biopsy method may be useful for selective harvesting of mature individuals in the field for use in breeding programmes.

The ability to breed year round is a great advantage for aquaculture of _H. scabra_. Moreover, spawning induction is relatively easy in mature _H. scabra_ compared to other tropical aspidochirote species (Ramofafia et al., 1995; Morgan, 2000b; Battaglene et al., in press). In agreement with our findings, spawning can be induced in _H. scabra_ year-round in Solomon Islands with the greatest response observed from September to December (Battaglene et al., in press). _H. scabra_ is relatively easy to culture compared with other tropical holothurian species and outplanted juveniles survived well (Dance et al., in press).
Our assessment of gonad development in *H. scabra*, together with results of other studies (Table 2), leads us to suggest that continuous and asynchronous reproduction as seen here for the species in Solomon Islands may be characteristic of this species in the tropical Indo-Pacific.
CHAPTER 5

Reproductive Biology of *Actinopyga mauritiana*

*Status:*

Chapter 5. Reproduction of Actinopyga mauritiana

Abstract

Over a four-year period, gonad index, examination of gonad tubules, and histology were used to study the reproduction in the sea cucumber Actinopyga mauritiana. This species reproduced annually during the warmer months of the year in Solomon Islands. This annual reproductive cycle progressed through five maturity stages: Recovery, Growing, Mature, Partly-spawned and Spent. The Recovery stage (March – May) was marked by initiation of gametogenesis and coincided with decreasing day length. The Growing stage (June – September) was characterized by development of gametes through to the Mature stage (October – December). Spawning occurred October through December and coincided with increased water temperature and day length. Spent gonads (January – March) contained a few unspawned gametes or completely lacked gametes. Although A. mauritiana had annual reproduction, individuals lacking or having indeterminate gonads were present year-round. Macroscopic examination of gonad tubules after biopsy provides a practical tool for assessing maturity condition of broodstock in the field during the reproductive season.
5.1 Introduction

The commercial importance of many tropical aspidochirote holothurians (Conand & Byrne, 1993) has generated considerable research interest in these sea cucumbers. In particular, emphasis has been placed on reproductive biology of several Indo-Pacific species to provide essential information for sustainable management of fisheries (Conand, 1993a, b; Reichenbach, 1999). Management practices have been established to regulate stocks at the local level in many Indo-Pacific countries (Dalzell et al., 1996; Conand, 1997) but limited resources make regulatory enforcement difficult. The continuing decrease in bèche-de-mer catches in many Indo-Pacific countries highlights this problem (Conand & Byrne, 1993).

Recently, emphasis has been placed on the possible use of hatchery-reared juveniles to restore and enhance wild stocks (Yanagisawa, 1998; Battaglene & Bell, 1999). Stock enhancement holds particular promise for increasing the productivity of sea cucumber fisheries by allowing the density of animals to reach the carrying capacity of the habitat. To realize this potential, cost-effective methods must be developed for the mass-release of juveniles (Battaglene & Bell, 1999). A thorough understanding of the reproduction of the species of interest, particularly the availability of quality gametes, is a necessary first step in this process.

Actinopyga mauritiana, commonly known as surf redfish, is an important component of fisheries for bèche-de-mer in the Indo-Pacific region (Conand, 1998). This species differs from most tropical aspidochirote in being found almost exclusively in high-energy reef crest zones (Conand, 1993a, 1997). An annual reproductive cycle has been reported for this species in New Caledonia and in Guam, where it spawns in summer (Conand, 1993a; Hopper et al., 1998). In this Chapter, I document the reproduction of A. mauritiana in Solomon Islands by the gonad index method, macroscopic examination of gonad tubules, and histological examination of the gonads over a 4-year period.
5.2 Materials and Methods

*A. mauritiana* were collected monthly from reef flats near Aruligo, Guadalcanal Island (09°18'S, 159°47'E) between May 1995 and December 1997. Due to dwindling coastal stocks near Aruligo, additional specimens were obtained from reef flats at Epannga, in the Western Province of Solomon Islands (08°05'S, 156°30'E) from January to November 1998. Due to the patchy distribution of *A. mauritiana*, monthly sample sizes varied from 5 to 38 adults with a drained weight range of 300.00 to 600.00 g. Conand (1993) reported that the drained weight at first maturity was 250 g for this species. Specimens were brought to the laboratory as described in Chapter 2, Section 2.1.

Gonad condition was assessed following the methods in Chapter 2, Sections 2.2 & 2.3. Diameters of 30 oocytes from each of the different stages of vitellogenesis (pre-, mid-, and late vitellogenic stages) were also measured.

The annual gametogenic cycle was illustrated from combined gametogenic data for 1996 and 1997 from Aruligo because of limited sample size. Data for Epannga in 1998 were not pooled with those from Aruligo, nor plotted, due to small sample size. However, frequencies of each gametogenic stage in the samples from Epannga were evaluated.

Surface seawater temperatures were recorded daily at the CAC and day length data were obtained as described in Chapter 2, Section 2.5.

A 2-way ANOVA was used to test the null hypothesis that reproductive output during peak breeding time (October) was the same between sexes (S) and among the years (Y). The untransformed GI data from 6 females and 6 males from Aruligo each year used in the analysis had homogeneous variances (Cochran's Test). Because the test statistic for the S x Y interaction was not significant at \( P = 0.25 \), the sum of squares for this term and the residual were pooled and the null hypothesis was tested using the pooled MS (Underwood, 1981). Statistical comparisons could not be made between Aruligo and Epannga due to limited samples collected from Epannga during the breeding season.
5.3 Results

5.3.1 Distribution
A total of 441 and 135 adult *A. mauritiana* were collected from Aruligo and Epanngga, respectively. Each sample contained males and females, individuals with indeterminate gonads, and those that lacked gonads (Fig. 5.1). The samples from Aruligo consisted of 131 females, 151 males, 62 specimens of indeterminate sex and 97 individuals lacking a gonad. The Epanngga samples consisted of 33 females, 48 males, 43 individuals of indeterminate sex and 11 specimens lacking a gonad. The mean drained weight of females, males, individuals with indeterminate sex and specimens lacking gonads for Aruligo were 485.57 g ($se = 16.69$), 480 g ($se = 13.51$), 414.25 g ($se = 10.05$) and 389.83 g ($se = 10.05$), respectively. The corresponding specimens from Epanngga measured 506 g ($se = 20.93$), 475.59 g ($se = 13.51$), 407.99 g ($se = 12.26$) and 398.79 g ($se = 12.26$), respectively. The sex ratio of *A. mauritiana* in both locations did not differ from unity ($\chi^2 = 1.42$ and 2.76, $P > 0.05$).

5.3.2 Gonad Index
A consistent annual cycle in gonad index was observed for male and female *A. mauritiana*, with the maximum GI peaks recorded in October, November or December (Fig. 5.2A). The GI seasonal pattern was similar for both Aruligo (1995 to 1997) and Epanngga (1998). Gonad squash preparations showed the presence of mature gametes in October, November or December at both locations. During periods of low GI, developing gametes were evident. At the maximum GI, the index of females appeared to be higher than that of males. However, ANOVA results indicated that peak GI was not significantly different between sexes ($F = 0.26$, $P > 0.05$, df = 1, 32), or among years ($F = 0.63$, $P > 0.05$, df = 2, 32).

5.3.3 Macro- and Micro- Examination of Gonad Development
The gonad of *A. mauritiana* is a single tuft of highly branched tubules arising from the gonad basis attached to the anterior body wall. During gonad development, the tubules extended into the perivisceral cavity and dominated the cavity when gravid. Growth of ovaries and testes was synchronous and similar in both sexes (Fig. 5.2B, C). The 5-stage maturity scale based on macroscopic observations is described below.
Figure 5.1.

Percentages of *A. mauritiana* with sexable, indeterminate and no gonads at Aruligo (1995 to 1997) and at Epannga (1998). Sample sizes ranged from 5 to 38.
Recovery (Stage I). *A. mauritiana* with recovering gonads could not be sexed by microscopic examination of squash preparations. Sex could only be identified by histology. The gonad consisted of fine thread-like tubules that had mean length of 8.8 mm ($se = 0.8, n = 30$) and a mean weight of 0.2 g ($se = 0.03, n = 30$). The thread-like tubules were white and unbranched.

Growing (Stage II). The tubules of growing gonads were longer, thicker and branched. Mean tubule lengths for females and males were 35.2 mm ($se = 3.0, n = 10$) and 42 mm ($se = 10.8, n = 10$), respectively. Sex could be determined microscopically by the presence of developing gametes. Testes were creamy white and smears showed low sperm activity. Ovaries were also white but were more translucent than testes. Oocytes were at a similar stage of development along the tubules. The weight of growing gonads ranged from 2 to 20 g ($\lambda = 8.30$ g ($se = 1.50, n = 16$).

Mature (Stage III). Tubules attained their maximum size and weight during the mature stage (October to December, Fig. 5.2). Mean tubule length in females was 77.60 mm ($se = 13.30, n = 15$) and 99.80 mm ($se = 16.80, n = 15$) in males. In both sexes, weight of mature gonads ranged from 30 to 110 g, with mean gonad weight of females and males being 63.50 g ($se = 5.40, n = 10$), and 44.10 g ($se = 8.10, n = 10$), respectively. Oocytes of 110 to 140 µm diameter ($\lambda = 123.40$ µm ($se = 0.80, n = 50$) were also visible through the thin transparent ovary wall. In many mature testes, spermatozoa were distributed unevenly along the tubule length, giving the testes tubules a beaded appearance.

Partly-spawned (Stage IV). In partly-spawned gonads, the tubules were flaccid as a result of gamete release, although a considerable part of the tubule volume was still occupied by gametes. Tubule size was reduced to a mean length of 57.8 mm ($se = 0.6, n = 10$) in females and 58.7 mm ($se = 8.2, n = 10$) in males. Gonad weight of partly-spawned *A. mauritiana* ranged from 9 to 30 g, with the mean weight of ovaries and testes being 16.6 g ($se = 1.70, n = 10$) and 17.40 g ($se = 1.60, n = 10$), respectively.

Spent (Stage V). Spent gonads were further reduced in size and had a mean weight of 8.80 g ($se = 1.50, n = 20$), and mean tubule length for females and males of 30 mm ($se = 7.90, n$...
Figure 5.2.
(A) Variation in mean gonad index for female (●) and male (O) A. mauritiana from Aruligo (1995 to 1997) and Epannga (1998). (B) Mean tubule length and (C) diameter for female (●) and male (O) A. mauritiana from Aruligo (1995 to 1997) and Epannga (1998). Bars are standard error of the mean.
10) and 51.00 mm ($se = 21.40, n = 10$), respectively. Tubule diameter was reduced to less than 1 mm (females: $\lambda = 0.90$ mm ($se = 0.30, n = 10$); males: 0.80 mm ($se = 0.10, n = 10$).

5.3.4 Histology of Gametogenesis

Gametogenesis in *A. mauritiana* could also be divided into five stages: Recovery, Growing, Mature, Partly-spawned and Spent (Figs. 5.3 & 5.4), similar to the stages defined for the maturity index. These stages are described below.

5.3.4.1 Oogenesis

Recovery stage ovaries were marked by initiation of oogenesis with abundant pre-vitellogenic oocytes ($\lambda = 16.10$ $\mu$m diameter, $se = 0.90, n = 30$) lining the germinal epithelium (Fig. 5.3A). Ovaries in the recovery stage had thick gonad walls that gradually decreased in size, reaching a minimal size during the mature stage. Onset of vitellogenesis distinguished growing ovaries from those in recovery stage. Oocytes began to develop progressively into early- ($\lambda = 36.80$ $\mu$m diameter, $se = 1.20, n = 30$), mid- ($\lambda = 56.50$ $\mu$m diameter, $se = 0.90, n = 30$) and late- ($\lambda = 80.40$ $\mu$m diameter, $se = 1.10, n = 30$) vitellogenic oocytes (Fig. 5.3B – D). Each oocyte developed within a follicle and became increasingly eosinophilic as growth proceeded. Mature stage ovaries were packed with fully-grown oocytes with a prominent germinal vesicle (Fig. 5.3C, D). The ovary wall reached minimal thickness also during the mature stage. Partly-spawned ovaries were characterised by loosely packed oocytes and phagocytes in the lumen. (Fig. 5.3E). Gametogenesis was re-initiated in many partly-spawned specimens along the germinal epithelium. It is not clear if the eggs in these partly-spawned ovaries would be subsequently released or resorbed. Spent ovaries had a shrunken and wrinkled appearance and contained a variable number of relict oocytes (Fig. 5.3F).

5.3.4.2 Spermatogenesis

Recovering testes of *A. mauritiana* were characterised by the presence of spermatogonia and developing spermatocytes along the germinal epithelium (Fig. 5.4A). The spermatocytes developed in short columns that extended into the lumen. Growing testes developed numerous infolds of the germinal layer with columns of spermatocytes (Fig. 5.4B). In early mature testes, spermatocytes were still evident (Fig. 5.4C) but were absent
Figure 5.3.
Oogenesis in *A. mauritiana*. A Recovery stage: ovary with abundant pre-vitellogenic oocytes (*arrows*). B Growing stage: active vitellogensis with oocytes growing from early (*eo*) to later (*lo*) stages of vitellogenesis. C and D Mature stage: mature oocytes developed within follicles (*f*). E Partly-spawned stage: the tubules are reduced in size and contain loosely packed oocytes. F Spent stage: ovary tubules shrunken and wrinkled but may contain relict oocytes. (*Scale bar in* A, B = 107 μm; in C = 140 μm; in D = 67 μm; in E, F = 400 μm).
in fully mature testes (Fig. 5.4D). Mature testes were fully packed with spermatozoa. Partly-spawned testes still contained sperm and exhibited a renewal of spermatogenesis with spermatocytes present along the germinal epithelium (Fig. 5.4E). The testis wall increased in thickness and the lumen became spacious and contained debris and phagocytes. Spent testes were shrunken and wrinkled and in some specimens could not be identified as testes unless relict spermatozoa were present (Fig. 5.4F).

5.3.5 Cycle of Gametogenesis

The combined 1996 and 1997 data from Aruligo showed gametogenesis occurring in parallel in females and males (Fig. 5.5). Histological examination of indeterminate gonads revealed they were at the spent or recovery stage of gametogenesis. The frequency of females at each gametogenic stage is shown in Fig. 5.5A. The recovery stage was present in all months except October. Females with gonads in the growing stage were mostly encountered from June to August. Mature females were collected predominantly in October and partly-spawned individuals were sampled from September through to December, suggesting that spawning or release of gametes took place from September to December. Individuals with spent gonads were present in all monthly samples except in July, June and December. The highest percent of spent females was recorded in March. Males in the recovery stage were also present in all monthly samples except November and December (Fig. 5.5B). Males with growing gonads were sampled in increasing numbers from July through to September and mature males were sampled mostly from October through to December. Partly-spawned males were collected mostly in November. Males with spent gonads were present in all monthly samples except in September and November. *A. mauritiana* from Epannga in 1998 showed a similar gametogenetic pattern (data not shown), with 80% of females and males having mature gonads from October to December.

5.3.6 Environmental Factors

During the four years of this study, mean monthly seawater temperature varied by 3 °C, from 27.28 to 30.19 °C (Fig. 5.6A). Initiation of gametogenesis in March, coincided with broadly decreasing temperature and the time when days became shorter than nights. Spawning coincided with increased water temperature and day length (Fig. 5.6A,B), and the beginning of the wet season.
Figure 5.4.
Spermatogenesis in *A. mauritiana.* A Recovering testis: spermatocytes (*arrow*) lined the germinal epithelium. B Growing testis: infolds of growing epithelium lined with columns of developing spermatocytes (*sc*). C Early mature testis: increasing predominance of spermatozoa (*sz*) in the lumen although spermatocytes (*sc*) still persist. D Late mature testis: absence of spermatocytes, lumen completely filled with spermatozoa (*sz*). E Partly-spawned testis: lumen spacious in parts with both unspawned spermatozoa (*sz*) and newly formed spermatocytes (*arrow*) present. F Spent testis: tubules shrunken and wrinkled but containing relict spermatozoa. (*Scale bar in A, B, C, D = 107 μm; in E, F = 140 μm.*)
5.4 Discussion

The data on gonad index, tubule morphology and gonad histology indicates that *A. mauritiana* has an annual reproductive cycle in the Solomon Islands, spawning during summer. An annual reproductive cycle with a summer spawning season has also been recorded for this species in New Caledonia and in Guam, and has been documented for other tropical and temperate aspidochirotids (Smiley *et al.*, 1991; Conand, 1993a; Hopper *et al.*, 1998).

Spawning in *A. mauritiana* in the Solomon Islands occurs between October and January as revealed by histology and the gonad index cycle. However, spawning trials indicated that the breeding season lasted only two months with peak reproductive activity occurring between mid November to mid December (Battaglene *et al.*, in press). In New Caledonia, *A. mauritiana* spawns between December and January while in Guam the species has a mid-year summer breeding season but mature individuals are found throughout the year (Conand, 1993a; Hopper *et al.*, 1998). In the Solomon Islands, mature individuals were mostly found during the breeding season. Spawning in *A. mauritiana* is partial with mature and spawned tubules evident in dissected specimens during the breeding season. Furthermore, histology revealed that not all gametes were released from spawned tubules and that re-initiation of gametogenesis occurred in these tubules. Partial-spawning has been documented for *Holothuria fuscogilva* (Chapter 3) and has also been observed in other holothurians (Sewell, 1992; Hamel *et al.*, 1993). As indicated for *H. fuscogilva*, it is not known if whether unspawned tubules will eventually release their gametes (Chapter 3).

Gametogenesis in *A. mauritiana* is an annual event and occurred in synchrony in both sexes. Initiation of gametogenesis occurs in March/April coincided with a period when days are shorter than night and so appears to be entrained by short day length as has been suggested for *H. fuscogilva* (Chapter 3) and has been demonstrated for sea urchins (Pearse *et al.*, 1986). Gonad maturation is reached mostly by October as sea temperature and day length increases.
Figure 5.5.
Gametogenic cycle of (A) female and (B) male *Actinopyga mauritiana* based on histological examination of specimens collected at Aruligo in 1996 and 1997.
Chapter 5. Reproduction of Actinopyga mauritiana

Spawning in *A. mauritiana* in Solomon Islands coincided the warmest annual temperatures as is the case for this species in New Caledonia and Guam (Conand, 1993a; Hopper *et al.*, 1998). Induction of spawning by thermal stimulus supports the suggestion that temperature may be a factor that cues gamete release in the field (Battaglene *et al.*, in press). On the reef crest, which is regularly exposed during low tide, *A. mauritiana* would experience temperatures several degrees above that of ambient seawater and this might be sufficient to induce spawning. These observations support the suggestion that gametogenesis and spawning in *A. mauritiana* might be triggered by threshold levels of temperature and photoperiod (Hopper *et al.*, 1998).

After spawning, gonad tubules were resorbed. My results suggest that total resorption of the gonads is common in *A. mauritiana* as also observed in *H. fuscogilva*. Re-initiation of gametogenesis in partly-spawned gonads is similar to that reported for several *Holothuria* species (Engstrom, 1980; Chapter 3). As noted for *H. fuscogilva*, the fate of these gametes is not known because there are no traces of them in subsequent spent stage gonads (Chapter 3).

The results presented here showed that the changes in tubule size correlates closely with their gametogenic development, suggesting that changes in the tubule morphology can be used to identify the reproductive stage of individuals. The ability to assess the condition of broodstock during the reproductive season using a simple biopsy technique based on tubule morphology is of great benefit to sea cucumber culture (Yanigasawa, 1998; Chapter 3).

In contrast to other intertidal aspidochirotes that commonly propagate by fission, reproduction in *A. mauritiana* has sexual reproduction and has high fecundity (Conand, 1993a; Hopper *et al.*, 1998). Asexual reproduction by reef-flat aspidochirotes is suggested to have evolved in response to a decreased capacity for production in this stressful habitat, which is characterised by high risk of insolation and high temperatures (Lawrence & Herrera, 2000). Although the reef-crest zone occupied by *A. mauritiana* would appear to be stressful, asexual reproduction has not been selected for and the high fecundity sexual life history of *A. mauritiana* suggests that the capacity for production in this habitat is higher than that on the nearby reef flat.
Figure 5.6.

(A) Mean monthly variations in temperature collected in 1995 to 1997 and (B) annual day length in 1997.
I have demonstrated that *A. mauritiana* has an annual reproductive cycle with a summer spawning period extending from October to January. Although the four-month spawning period of *A. mauritiana* limits gamete availability for aquaculture, this species has other features that should make it suitable for aquaculture programmes including restocking and stock enhancement. The predictable pattern of change in gonad tubule morphology facilitates the use of the biopsy technique to assess the reproductive stage of increasingly scarce broodstock in the field. In addition, spawning was induced readily in this species using thermal stimulation and its high fecundity provides ample fertile eggs for hatchery production (Chapter 7). Finally, the intertidal distribution of *A. mauritiana* and small home range should make it relatively easy to monitor changes in populations following releases of juveniles and, ultimately, to harvest the stocked animals.
CHAPTER 6

Assessment of the ‘Tubule Recruitment Model’ for ovary development in *Holothuria fuscogilva*, *H. scabra* and *Actinopyga mauritiana*

*Status:*
The applicability of the Tubule Recruitment Model (TRM) of holothurian ovary development was assessed in three tropical aspidochirotes, *H. fuscogilva*, *H. scabra* and *A. mauritiana*. The TRM predicts that ovary tubules exist in distinct multiple cohorts, with gametogenesis being synchronous within each tubule cohort but asynchronous across cohorts. In the three species examined here, the ovaries comprised a single cohort of tubules with gametogenesis synchronous across the entire gonad. The TRM also predicts sequential recruitment of tubules, with new tubules appearing at the anterior end of the gonad while those at the posterior end being most mature. Progressive tubule recruitment did not occur in the three species. In contrast, the tubules were arranged radially around the gonad basis and developed uniformly to maturity. Another feature of the TRM is synchronous development of oocytes within ovary tubules. In the three species examined here however, overlapping generations of oocytes were present in each tubule. Finally, the TRM predicts that tubules take 3 years to become mature. In the species investigated here however, the ovary tubules matured in less than a year. These findings show that the TRM does not apply to oogenesis in *H. fuscogilva*, *H. scabra* and *A. mauritiana* and add to the growing weight of evidence that suggests that the TRM is an exception, not the rule for oogenesis in Holothuroidea.
6.1 Introduction

Holothurian gonads comprise of one or two tufts of elongate tubules which develop as a single cohort or as distinct multiple cohorts that differ in age (Smiley et al., 1991). In species where gonad development is uniform, the gametogenic state of tubules is similar across the entire gonad and all the tubules have uniform appearance. In species where the gonad has multiple cohorts of tubules, gametogenesis is synchronous within cohorts but asynchronous across cohorts (Smiley & Cloney, 1985). Typically, the small-sized tubules are in an early gametogenic stage and the largest tubules are mature. In these gonads the tubules are recruited progressively from anterior to the posterior across the gonad basis. Such progressive evolution of tubules has been described in detail for Stichopus californicus and provided the basis for the ‘tubule recruitment model’ (TRM) for ovarian development in holothurians (Smiley & Cloney, 1985; Smiley, 1988; 1994; Smiley et al., 1991).

In a given annual reproductive cycle, the gonad of S. californicus is organized into three distinct cohorts (primary, secondary and fecund) of tubules. The primary tubules are attached to the anterior section of the gonad basis and contain previtellogenic oocytes. In the central region of the gonad the secondary tubules contain vitellogenic oocytes. The fecund tubules are attached to the posterior end of the gonad basis and contain only late vitellogenic oocytes. After spawning, these tubules are resorbed. In S. californicus it takes at least two years for recruiting tubules to become mature. Primary tubules appear in Year N and progressively develop into secondary tubules in Year N+1. Secondary tubules become the fecund tubules in Year N + 2 (Smiley & Cloney, 1985). It was suggested that this pattern of oocyte and ovary development might apply broadly across the Class Holothuroidea (Smiley & Cloney 1985).

Assessing the applicability of the TRM for the Holothuroidea, Sewell et al., (1997) reported that ovary development in many species of the orders Dendrochirotida, Apodida and Mopadida does not conform to the model. Most holothuroids in these orders appear to possess ovaries with all their tubules at a similar stage of development. Although the TRM was based on an aspidochirote species, only the ovaries of Holothuria forskali (Order
Aspidochirotida) have subsequently been found to conform to the predictions of the model (Tuwo & Conand, 1992). Like *S. californicus*, *H. forskali* is a temperate aspidochirote. This Chapter describes gonad tubule growth in *H. fuscogilva*, *H. scabra* and *Actinopyga mauritiana*, in Solomon Islands and assesses the pattern of tubule growth with respect to the TRM. The TRM is based on ovary growth and so the Chapter focuses on ovary tubules. The reproductive cycles of the three species are presented in Chapters 3, 4 and 5.

### 6.2 Materials and Methods

Sites, methods of collection of each species, methods of gonad processing and histology are provided in Chapter 2.

### 6.3 Results

#### 6.3.1 Gonad Morphology

**6.3.1.1 Tubule organisation**

The gonads of *H. fuscogilva*, *H. scabra* and *A. mauritiana* consisted of a single tuft of tubules arising from the gonad basis, as exemplified by the gonad arrangement in *A. mauritiana* (Fig. 6.1A). No distinct cohorts of tubules were encountered. The tubules were arranged radially around the gonad basis with the gonad basis taking a central location (Fig. 6.1A). A single gonoduct runs through the gonad base to the external gonopore.

**6.3.1.2 Tubule growth**

After the summer spawning period (August – December) of *H. fuscogilva* (Chapter 3) and *A. mauritiana* (Chapter 5) new tubules appeared in March or April. These tubules subsequently developed and reached maturity in August (*H. fuscogilva*) or October (*A. mauritiana*). In *H. scabra*, (Chapter 4) gonad development was asynchronous and spawning appeared to be continuous. In *H. scabra* gonad development differed among individuals but the state of the tubules was similar across individual gonads. In all three species the ovary tubules developed as a single cohort (Fig. 6.1A).

Gonad growth in all three species involved increase in the size (length and diameter) of the tubules and in the number of tubule branches. Branching of tubules increased the volume
Figure 6.1.
Gonad morphology in tropical apidochirotid holothurians. A. *A. mauritiana*. Mature ovaries consisted of a single tuft of tubules arising from the gonad basis (arrow). Note the radial attachment of tubules around the gonad basis. B. Tubule bifurcation (1 & 2) results in branches of variable lengths. C. Bifurcated tubule in *A. mauritiana* illustrating uniform gametogenic development within branches. D. Mature ovary tubule in *H. fuscogilva* packed with mature oocytes evident through the transparent tubule wall. E. Spent tubules in *A. mauritiana*. Note the reduced size of tubules. F. A portion of the spent tubule in E enlarged to show loosely arranged unspawned oocytes. *(Scale bar in A, B, E = 300 μm; in C = 90μm; in D = 53 μm; in F = 50 μm.)*
Chapter 6. Tubule morphology in tropical aspidochirotes

of the gonad. The tubules branched by bifurcation and this occurred once or twice along the length of the tubule (Fig. 6.1B). The gametogenic stage along the length of the tubules was similar (Fig. 6.1C). In *H. fuscogilva*, some short tubules were observed but the gametogenic stage in these short tubules was similar to the rest of the gonad. Gravid ovaries in all three species occupied more than half of the coelomic cavity, extending distally to posterior region of the coelomic cavity. They generally had wet weight ranging from 50 – 100 g. Gravid ovaries were usually packed with mature oocytes that were easily seen through the transparent ovary wall (Fig. 6.1D).

Spawning in all three species was not synchronous across the ovary. In the partly-spawned state, the ovaries contained both spawned and unspawned tubules. Examination of squash preparations of spawned and unspawned tubules revealed the presence of phagocytes and degenerating oocytes indicating that many of the unspawned eggs were being resorbed. In spent ovaries, all tubules had a wrinkled appearance, were reduced in size and contained unspawned eggs (Fig. 6.1E, F). In both *H. fuscogilva* and *A. mauritiana*, individuals lacking gonads were encountered. For these species it appears that total gonad resorption occurs in some post-spawned individuals each year. Individuals lacking gonads were not encountered in *H. scabra*.

6.3.1.3 Gametogenesis

Ovary histology indicated oogenesis in *H. fuscogilva* and *A. mauritiana* was synchronous with a new cohort of tubules appearing in March or April. At anytime the tubules were at a similar state of oogenesis. In *H. scabra*, gametogenesis was asynchronous with individuals having gonads at different stages of maturity through the year. Within each gonad however, the tubules were at a similar gametogenic state. The process of ovary development was similar in all three species with previtellogenic oocytes appearing along the germinal epithelium in recovery stage ovaries. As ovaries developed, vitellogenic activity was seen in growing ovaries with previtellogenic, early-, mid-, and late vitellogenic oocytes distributed through the tubules. Upon reaching the mature stage, fully grown oocytes dominated the tubules. In all three species, previtellogenic oocytes were present along the germinal epithelium throughout ovary development.
6.4 Discussion

The features of the TRM and the ovary development in *H. fuscogilva*, *H. scabra* and *A. mauritiana* are compared in Table 6.1. In all three species, the gonad consisted of a single cohort of tubules while multiple cohorts characterise the TRM. A distinctive pattern of small (primary), medium (secondary) and large (fecund) tubules as seen in *S. californicus*, the species which the TRM is based, was not observed in the species examined here. In *S. californicus*, the tubule cohorts attach to the gonad basis with a distinct pattern: primary tubules at the anterior end, secondary tubules in the mid-region and fecund tubules located posteriorly. This distinctive organisation allows the tubules to be recruited progressively. Such recruitment did not occur in *H. fuscogilva*, *H. scabra* and *A. mauritiana*. In these species, the tubules were arranged radially around the gonad basis and grew uniformly to maturity. Absence of progressive recruitment of tubules has been also reported other tropical aspidochirotes elsewhere in the Indo-Pacific (Conand, 1993a; Hopper et al., 1998; Ong Che & Gomez, 1985; Sewell et al., 1997; Reichenbach, 1999; Tuwo, 1999).

Absence of total tubule resorption is another feature of the TRM. In *S. californicus* (Table 6.1) new tubules were always recruited. Only the fecund tubules are resorbed while previously secondary tubules become the new fecund tubules. By contrast, total gonad resorption occurred occasionally in *H. fuscogilva* and *A. mauritiana*, as evident by the collection of adults lacking gonads (Chapters 3 & 5). For *H. scabra*, total gonad resorption may not occur. Adults without gonads were not encountered (Chapter 4).

Another prediction of the TRM is that gametes within tubule cohorts are at the same stage of development and only a single generation of oocytes occurs in each tubule at any one time (Smiley 1988). This prediction did not hold for *H. fuscogilva*, *H. scabra* and *A. mauritiana*. Instead, gametes were at the same stage of development across all gonad tubules. Furthermore, tubules had overlapping generations of oocytes suggesting that gametogenesis is continuous or prolonged within each tubule (Table 6.1). The presence of oocytes at different stages of development was particularly conspicuous in partly-spawned ovaries.
Table 6.1. Description of gonad tubule morphology in three tropical holothurians compared to the tubule recruitment model (TRM).

<table>
<thead>
<tr>
<th>TRM features</th>
<th><em>Holothuria fuscogilva</em></th>
<th><em>Holothuria scabra</em></th>
<th><em>Actinopyga mauritiana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Distinct tubule cohorts</td>
<td>single cohort</td>
<td>single cohort</td>
<td>single cohort</td>
</tr>
<tr>
<td>Progressive tubule recruitment</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>No total tubule resorption</td>
<td>occasional tubule resorption</td>
<td>no tubule resorption</td>
<td>occasional tubule resorption</td>
</tr>
<tr>
<td>No gametogenic renewal in post-spawning tubules</td>
<td>gametogenic renewal</td>
<td>gametogenic renewal</td>
<td>gametogenic renewal</td>
</tr>
<tr>
<td>Single generation of oocytes within tubules</td>
<td>overlapping generations of oocytes</td>
<td>overlapping generations of oocytes</td>
<td>overlapping generations of oocytes</td>
</tr>
<tr>
<td>Oocytes need more than one year to mature</td>
<td>oocytes matured in less than a year</td>
<td>oocytes may mature in less than a year?</td>
<td>oocytes matured in less than a year</td>
</tr>
</tbody>
</table>
In *S. californicus* it takes 3 years for the primary tubules to become the fecund ones, a feature of the TRM. This developmental time is considerably longer than that for the tubules of *H. fuscogilva*, *H. scabra* and *A. mauritiana* where tubules matured in less than a year. In *H. fuscogilva* and *A. mauritiana*, a new cohort of tubules appeared in March/April and was mature by August and September, respectively (Chapters 3 & 5). The rate of gonad growth in *H. scabra* was not determined as this species has asynchronous reproduction (Chapter 4).

Although not the focus of this TRM-based comparison, it is important to note that the pattern of testis development in *H. fuscogilva*, *H. scabra* and *A. mauritiana* as a single cohort of tubules is similar to that noted for the ovaries.

Ovary development in *H. fuscogilva*, *H. scabra* and *A. mauritiana* elsewhere in the Indo-Pacific is similar to that described here (Conand, 1993a; Hopper *et al.*, 1998; Ong Che & Gomez, 1985; Reichenbach, 1999; Tuwo, 1999). Assessment of ovary development in *H. atra*, *H. floridana*, *H. mexicana*, *H. nobilis*, *A. echinites*, *S. variegatus*, and *Thelenota ananas*, revealed that their ovaries do not develop according to the predictions of the model (Pearse 1968; Engstrom 1980; Conand 1981, 1982, 1993a,b; Sewell *et al.*, 1997). The increasing numbers of tropical aspidochirote species with gonad development that do not conform to the TRM suggests that this model is not the rule for holothuroid oogenesis (Frick *et al.*, 1996; Sewell *et al.*, 1997).
CHAPTER 7

Development of *Holothuria scabra*, *H. fuscogilva*, and *Actinopyga mauritiana* in Hatchery Culture

*Status:*


55
Abstract

Development of the tropical sea cucumbers *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana* was investigated under hatchery conditions. *H. scabra* was reared through the feeding auricularia, the nonfeeding doliolaria and the pentactula larval stages, typical of aspidochirote holothurians. Development took 14 – 17 d at 26 – 28 °C. *H. fuscogilva* was reared to the auricularia stage while *A. mauritiana* was reared to the doliolaria stage. In all three species, the early auricularia stage was reached 40 – 70 h post-fertilisation and *H. scabra* and *A. mauritiana* reached the late auricularia 14 – 16 d followed by transition to doliolaria over one day, marked by formation of transverse ciliated bands. In *H. scabra*, metamorphosis of the doliolaria to pentactula on days 13 – 15 was marked by development of five primary tentacles and a single ventroposterior podium. Newly-settled pentactula of *H. scabra* used their tentacles to feed on the biological film growing on settlement plates. Elongation of the pentactula and the development of a second ventroposterior podium, marked the transition to the juvenile stage on days 14 – 17 in *H. scabra*. Hyaline spheres were a conspicuous feature of the late auricularia of *H. scabra* and appear to be an indicator of larval competence. The lack of development of these spheres in the larvae of *H. fuscogilva* larvae and their poor growth in *A. mauritiana* was taken as evidence that the diet used might not be sufficient to support complete development in these species. Determination of food conditions that promote hyaline sphere development may be essential for successful culture of these species.
Chapter 7. Hatchery rearing of three tropical aspidochirotes

7.1 Introduction

Although the bêche-de-mer fishery for tropical sea cucumbers has promoted significant interest in the reproductive and fisheries biology of these organisms, little information exists on their larval biology. Descriptions of the development of aspidochirotid holothurians are largely restricted to temperate Stichopus species (Smiley, 1986; Arakawa, 1990; Archer, 1996). Amongst tropical species, only the development of Actinopyga echinites has been described in detail (Chen & Chian, 1990). For other tropical aspidochirotes, aspects of the development of H. scabra, H. mexicana, H. atra and H. nobilis have been reported (Mortensen, 1937; Lacalli, 1988; James et al., 1994; Ramofafia et al., 1995; Martinez & Richmond, 1998; Morgan, 2000b; Hamel et al., 2001).

Most aspidochirote holothurians (Holothuriidae, Stichopodidae) have planktotrophic (indirect) development where the gastrula develops into a planktonic (feeding) auricularia before developing into a nonfeeding lecithotrophic doliolaria (Smiley et al., 1991). The auricularia larva is unique to holothurians and is characterised by a single continuous ciliary band that functions in swimming and feeding (Strathmann, 1971).

Modes of development in the Holothuroidea, as for most marine invertebrates are correlated with egg size (Vance, 1973; Sewell & Young, 1997). Planktotrophic developing holothurians produce small oligolecithal eggs (<180 μm diameter) in large quantities while lecithotrophic developers produce fewer larger (>180 μm in diameter) macrolecithal eggs (Sewell & Young, 1997). In holothurians, a bimodal egg size distribution reflecting planktotrophic and lecithotrophic development has been reported (Sewell & Young, 1997).

The application of aquaculture for the purpose of stock restoration and enhancement of overfished commercial tropical aspidochirote species (Battaglene & Bell, 1999), necessitates the study of the larval biology of target species. Particular emphasis has been placed in determining the factors influencing larval growth and survival (Ramofafia et al., 1995; Battaglene, 1999; Morgan, 2000b). Of about 20 commercially exploited species only a few holothurians have been investigated for aquaculture and reared to settlement including the tropical species Holothuria scabra and H. fuscogilva, and the temperate
species *Stichopus japonicus* and *S. mollis* (James et al., 1988; YSFRI, 1991; Archer, 1996; Battaglene & Seymour, 1998; Battaglene, 1999; Yanagisawa, 1998; Morgan, 2001).

This chapter reports on the work undertaken between 1995 to 1998 involving mass culture of *H. scabra*, *H. fuscogilva* and *A. mauritiana*. Chronology of development, growth and survival data are presented for each species. Factors influencing the culture of these species are discussed. This study extends the knowledge on the developmental biology of these important commercial holothurian species, particularly knowledge pertaining to their larval culture.

### 7.2 Materials and Methods

**7.2.1 Collection of Samples**

*Holothuria scabra* was collected from Vona Vona Lagoon (Fig. 2.1) and transported to the laboratory at the CAC as described in Chapters 2. *H. fuscogilva* was obtained from Tulagi, Gela Island (Fig. 2.1, Chapter 2) and were brought to the CAC in fish boxes as described in Chapter 3. *A. mauritiana* were collected from Savo Island and brought to the CAC as described for *H. fuscogilva*. Collection sites were described in Chapters 3, 4, 5 for *H. fuscogilva*, *H. scabra* and *A. mauritiana* respectively. Hatchery culture of *H. scabra* was investigated in September 1996, 1998 and 1999 while that of *H. fuscogilva* was conducted in August 1995, August and September 1998. Culture of *A. mauritiana* was carried out in November 1996, October 1997 and November 1998.

**7.2.2 Induction of Spawning**

The specimens arrived at the laboratory between 1500 and 1900 hours and were placed immediately in an aerated 2000 l tank where they were held overnight. They were monitored for spawning activity because the stress associated with transportation was usually sufficient to stimulate the release of gametes. Samples that did not spawn overnight were induced to spawn on the next day following the protocol outlined in Table 7.1. Individuals exhibiting spawning behaviour were transferred to 60 l boxes (one specimen per box) containing filtered (5 μm) seawater where they were held until they...
Table 7.1. Protocol used to induce *Holothuria fuscogilva* to spawn.

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 am</td>
<td>Tank cleaned and filled with fresh filtered (5 μm) seawater to a volume sufficient to cover the dorsal surface of the largest specimen. Aeration and water systems were shut off. Tank water was allowed to increase 3 °C above ambient and this was maintained until 1 pm.</td>
</tr>
<tr>
<td>9 am</td>
<td>Animals were introduced into the tank.</td>
</tr>
<tr>
<td>11 am</td>
<td>Dried algae Algamac-2000 (100 g) added to the tank. Aeration system was opened to provide uniform mixing of the algae. The animals were monitored for spawning activity.</td>
</tr>
<tr>
<td>1 pm</td>
<td>Tank volume was doubled to return water temperature to ambient.</td>
</tr>
</tbody>
</table>
Chapter 7. Hatchery rearing of three tropical aspidochirotes

released gametes. The first animal that spawned was always a male and was left in the tank to stimulate spawning in other animals.

7.2.3 Collection, Fertilisation and Stocking of Eggs
In the hatchery, spawned eggs were siphoned through a 53 μm sieve into 18 l culture containers. Egg density, counted in four to six 1 ml aliquots taken from a uniform suspension, ranged from 30 to 70 eggs ml⁻¹. These data were also used to estimate female egg production by dividing the total number of eggs by the number of females that spawned. The eggs were fertilised with sperm at a density of around 10⁴ sperm ml⁻¹ and then stocked into three to six 250 or 500 l rearing tanks (Table 7.2) at a density of 1 to 9 eggs ml⁻¹. A single air stone positioned at the bottom of each culture tank provided sufficient aeration and ensured gentle but total water circulation.

7.2.4 Rearing of Larvae
The three species were reared in the hatchery following the protocol presented in Table 7.3. The water in each tank was changed every second day. This was done by draining the tanks through a 53 μm sieve to retain the larvae. The larvae were then transferred into 18 l aerated containers and were returned to tanks filled with fresh filtered (1 μm) sea water. After the first water change, the early auricularia larvae were stocked at a density of 0.1 - 3 larvae ml⁻¹. Density decreased through the rearing period due to larval mortality. Feeding commenced on day 2, when the auricularia had a functional gut. The larvae were fed with an equal mixture (by dry weight) of 2 or 3 species of microalgae (Table 7.2). Feeding commenced at an initial density of 2.0×10⁴ cells ml⁻¹ and was gradually increased to 4.0×10⁴ cells ml⁻¹ over the rearing period (Table 7.3). On the days that water was not changed, algae were added to each tank to maintain algal density. A commercially available dried algae, Schizochytrium sp. (Algamac-2000, Bio-Marine, Hawthorne, CA), was also supplied (5.0 gr l⁻¹) to each tank during metamorphosis and settlement (Table 7.3).

Seven days after fertilisation, 6 to 12 sets of fibreglass settlement plates were conditioned in an outdoor tank supplied with filtered running seawater to promote growth of a biological film and diatoms (Battaglene & Seymour, 1998). Each set of plates consisted of 4 to 6 wavy PVC plates measuring 850 × 240 mm, stacked with a 40 mm gap. When the late auricularia stage was reached, the plates were placed in the rearing tanks. For the 250
Table 7.2. Summary of trials conducted between 1995 and 1998 to culture *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Trial</th>
<th>Number spawned (Female:Male)</th>
<th>No. of tanks used</th>
<th>Tank Vol (l)</th>
<th>Algal feed combination</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. scabra</em></td>
<td>1</td>
<td>2:9</td>
<td>4</td>
<td>500</td>
<td>Tetraselmis chuii, Chaetoceros muelleri, C. calcitrans</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2:4</td>
<td>4</td>
<td>500</td>
<td>Rhodomonas salina, C. muelleri, C. calcitrans</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1:2</td>
<td>3</td>
<td>250</td>
<td>R. salina, C. muelleri, C. calcitrans</td>
</tr>
<tr>
<td><em>H. fuscogilva</em></td>
<td>1</td>
<td>1:4</td>
<td>5</td>
<td>500</td>
<td>R. salina, C. muelleri, C. calcitrans</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:4</td>
<td>6</td>
<td>250</td>
<td>R. salina, C. muelleri, C. calcitrans</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3:3</td>
<td>3</td>
<td>250</td>
<td>R. salina, C. muelleri, C. calcitrans</td>
</tr>
<tr>
<td><em>A. mauritiana</em></td>
<td>1</td>
<td>3:1</td>
<td>4</td>
<td>500</td>
<td>Isoerysis galbana, C. muelleri</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4:5</td>
<td>3</td>
<td>500</td>
<td>R. salina, C. muelleri, C. calcitrans</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11:5</td>
<td>3</td>
<td>500</td>
<td>R. salina, C. muelleri, C. calcitrans</td>
</tr>
</tbody>
</table>
Table 7.3. General protocol used to rear *H. scabra*, *H. fuscogilva* and *A. mauritiana* in the hatchery.

<table>
<thead>
<tr>
<th>Age (day)</th>
<th>Water exchange</th>
<th>Microalgae</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>static</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>static</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>sieve</td>
<td>mixed*</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>static</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>sieve</td>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>static</td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>6</td>
<td>sieve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>static</td>
<td></td>
<td>2.6</td>
</tr>
<tr>
<td>8</td>
<td>sieve</td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>9</td>
<td>static</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>10</td>
<td>flow-through**</td>
<td></td>
<td>3.2</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td></td>
<td>3.4</td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td></td>
<td>3.8</td>
</tr>
<tr>
<td>14</td>
<td>&quot;</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>15</td>
<td>&quot;</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>16</td>
<td>&quot;</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>17</td>
<td>&quot;</td>
<td></td>
<td>4.0</td>
</tr>
</tbody>
</table>

*Refer to Table 7.2 for details of diet

**The tanks were put through flow-through system after larvae were sieved and when they had reached the auricularia stage.
Chapter 7. Hatchery rearing of three tropical aspidochirotes

1 tanks, one set of plates was used while 2 or 3 sets of settlement plates were placed in the 500 l tanks. With introduction of settlement plates, tanks were put onto a flow-through system at a filtered seawater flow rate of 200 ml min\(^{-1}\). Continuous water exchange was maintained until the juvenile stage was reached and the tanks were emptied and the juveniles detached, counted and transferred to outdoor nursery systems (Battaglene et al., 1999).

7.2.5 Collection and Analysis of Data
The embryos were photographed every 15 – 30 mins for the first 24 hours of development and then hourly until the early auricularia stage. Thereafter, daily observations were made. Growth and survival data were obtained every second day after larvae were sieved by taking four 1 ml replicate samples. Larval length \((n = 5 - 10 \text{ tank}\(^{-1}\) stage\(^{-1}\)) was measured using an ocular micrometer or scale profile projector.

7.3 Results

7.3.1 Induction of spawning
Spawning of *H. scabra* and *A. mauritiana* usually occurred soon after introduction to the holding tank. On one occasion one female and eight male *H. scabra* spawned on the day after capture. Spawning in *H. fuscogilva* was achieved only after the specimens were induced by increased temperature and with the addition of dried algae. Three trials were undertaken for each species (Table 7.2). Pre-spawning behaviour in all 3 species included rolling, twisting, raising of the anterior end and side to side swaying movements.

Individual female *H. scabra* spawned a mean of \(4.63 \times 10^6\) eggs \((se = 1.2 \times 10^6, n = 5)\) while *H. fuscogilva* spawned a mean of \(2.68 \times 10^6\) eggs \((se = 1.51 \times 10^6, n = 5)\). Female *A. mauritiana* spawned a mean of \(6.00 \times 10^6\) eggs \((se = 6.83 \times 10^5, n = 7)\). The mean egg diameters of *H. scabra*, *H. fuscogilva* and *A. mauritiana* were 156.37 \(\mu m\) \((se = 2.92, n = 30)\), 151.71 \(\mu m\), \((se = 5.24, n = 45)\) and 109.85 \(\mu m\), \(se = 0.99, n = 45)\), respectively. Spawned eggs were uniform in appearance and primary oocytes were never observed during spawning.
7.3.2 Embryogenesis and Larval Development
Development of the three species was typical for planktotrophic aspidochirotes. *H. scabra* was reared to the juvenile stage in all tanks in each of the 3 trials. In contrast, *H. fuscogilva* and *A. mauritiana* only reached the late auricularia or the doliolaria stages.

7.3.2.1 *H. scabra*
In the 3 trials, development to the juvenile stage of *H. scabra* was completed in 14 - 17 d (Table 7.4). Larval growth in culture is shown in Fig. 7.1 and the developmental stages are shown in Figs. 7.2 and 7.3. After eggs were fertilised (Fig. 7.2A), expulsion of polar bodies occurred 10 - 15 mins later (Fig. 7.2B). First cleavage was radial and holoblastic and occurred within the first hour of development (Fig. 7.2C). The second and the third divisions were meridional and equatorial respectively. Beginning with the fourth cleavage, meridional and equatorial divisions alternated. Cleavage was synchronous up to the 32-cell stage. Thereafter, cleavage was asynchronous. The early blastula stage (Fig. 7.2D) was reached at 5 h and by 7 h the embryos were blastula with a conspicuous ciliary cover. Coeloblastulae (9 h) (Fig. 7.2E) rotated continuously within their fertilisation envelope propelled by their cilia. The vegetal plate then began to invaginate, ingressing into the blastocoel (Fig. 7.2E). Hatching occurred at 12 - 14 h and the gastrula elongated along the animal-vegetal axis (Fig. 7.2F, G). The archenteron extended inward and gave rise to mesenchyme cells at its tip, which moved into the blastocoel (Fig. 7.2H). Gastrulation was complete by 20 h and the embryos started to develop into auricularia larvae with differentiation of the digestive tract.

When the archenteron reached the midway point, its anterior end turned towards an ectodermal invagination on the mid-ventral surface of the larvae, which marked the position of the stomodeum. The anterior end of the archenteron then fused with the stomodeal invagination to become the esophagus. The stomodeum opened forming the mouth. The posterior region of the archenteron enlarged and differentiated into the stomach. The blastopore became the anus, and gradually shifted upwards along the ventral surface. The larvae were now early auricularia with a complete gut. The pre-oral lobe formed a shelf over the oral region of the early auricularia (Fig. 7.2I). Development of the hydropore and hydroporic canal also began. The single looped ciliated band also formed. By 30 h, the early auricularia had a barrel-like shape (Fig. 7.2I) and had a mean length of 318 μm ($se = 0.09, n = 10$).
Table 7.4. Chronology of development of *H. scabra*, *H. fuscogilva* and *A. mauritiana* at 26–28 °C. Larvae were considered to have reached a new stage when at least 50% of the culture had attained the indicated stage.

<table>
<thead>
<tr>
<th>Larval stage</th>
<th>Species</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>H. scabra</em></td>
<td><em>H. fuscogilva</em></td>
<td><em>A. mauritiana</em></td>
</tr>
<tr>
<td>Fertilization</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>First cleavage</td>
<td>1 h</td>
<td>1 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Third cleavage</td>
<td>3 h</td>
<td>3 h</td>
<td>3 h</td>
</tr>
<tr>
<td>Early blastula</td>
<td>5 h</td>
<td>5 h</td>
<td>5 h</td>
</tr>
<tr>
<td>Ciliated blastula</td>
<td>7 h</td>
<td>7 h</td>
<td>8 h</td>
</tr>
<tr>
<td>Coeloblastula</td>
<td>9 h</td>
<td>15-16 h</td>
<td>-</td>
</tr>
<tr>
<td>Hatched gastrula</td>
<td>12-14 h</td>
<td>18-20 h</td>
<td>12-15 h</td>
</tr>
<tr>
<td>Early auricularia</td>
<td>40-70 h</td>
<td>40-70 h</td>
<td>40-70 h</td>
</tr>
<tr>
<td>Mid auricularia</td>
<td>5-7 d</td>
<td>5-7 d</td>
<td>5-10 d</td>
</tr>
<tr>
<td>Late auricularia</td>
<td>9-12 d</td>
<td>12-16 d</td>
<td>12-22 d</td>
</tr>
<tr>
<td>Doliolaria</td>
<td>12-13 d</td>
<td>-</td>
<td>16-29 d</td>
</tr>
<tr>
<td>Pentactula</td>
<td>13-15 d</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Juvenile</td>
<td>14-17</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- data not available
Figure 7.1.

Growth of *H. scabra* in 3 trials. (A) Length of larvae was measured at the early auricularia (EA), late auricularia (LA), doliolaria (D) and pentactula (P) stages. Egg size at fertilisation (F) is also shown. For each stage, 5 larvae were measured in each tank. Trial 1 (●), 4 tanks; Trial 2 (○), 4 tanks; Trial 3 (▲), 3 tanks. PF = post-fertilization. *(Vertical bars are standard error of mean).*
Growth of *H. scabra* larvae was similar in all trials (Fig. 7.1). By 3 d post-fertilisation, the early auricularia had increased in body length ($\bar{x} = 474.29$ μm; $se = 9.63$, $n = 55$) and complexity. The pre-oral and the post-oral lobes were more pronounced and one or two pairs of lateral processes began to extend from the side of the body (Fig. 7.3A,B). Internally, the left and right somatocoels were evident. By day 5, the larvae were mid-auricularia (Fig. 7.3B) and by day 9 were late auricularia (Fig. 7.3C). Late auricularia had four pairs of processes, unequal in length. Hyaline spheres developed by days 8 – 9 at the tip of these processes and continued to grow to the doliolaria stage (Fig. 7.3C). In the late auricularia the spheres were 50 – 70 μm in diameter. A single ossicle was located at the tip of the posterior process and appears as a dark spot just below the hyaline sphere in Figure 7.3C. Development of somatocoels and the hydrocoel also continued. The mean length of the late auricularia was 853.16 μm ($se = 17.30$, $n = 55$), with considerable variation among individuals. The largest specimens measured 1 mm in length.

Development to the non-feeding doliolaria stage was asynchronous within and among tanks in each trial and was marked by a great reduction in larval length (Fig. 7.1). The transition period from the auricularia to doliolaria took 10 – 12 h (Fig. 7.3D) and involved total resorption of the lateral processes and fragmentation of the ciliated band which became rearranged into five transverse bands. The transverse ciliated bands formed where the lateral processes had been located. By this stage, the preoral and postoral had disappeared, although the portion of the ciliated band associated with these lobes contributed to formation of the transverse ciliated bands. The newly formed doliolaria was still transparent and hyaline spheres reached their maximum size of 60 – 80 μm (Fig. 7.3D). Transition to the doliolaria larvae resulted in a marked reduction in larval length (Fig. 7.1). Doliolaria had a mean length of 427.64 μm ($se = 17.66$, $n = 55$) and were barrel-like in shape (Fig. 7.3E). Fully developed doliolaria were demersal and began to occupy the lower part of the rearing tanks whilst continuing to swim with their ciliated bands. Although most (~ 70 %) larvae developed to doliolaria, some remained as late auricularia stage in all tanks and appeared to be arrested at this stage. These larvae included larvae (~5%) with large hyaline spheres (> 50 μm in diameter), larvae (10%) with poorly developed hyaline spheres (< 30 μm in diameter) and those lacking hyaline spheres (15%).

62
Figure 7.2.
Metamorphosis to the pentactula stage occurred on days 13 - 15. Pentactula larvae possessed five well-developed primary tentacles and a single ventroposterior podium which they used to attach to the settlement plates or onto the walls or bases of the tanks. They had a mean length of 411.20 µm (se = 10.28, n = 55) (Fig. 7.3F). Pentactula used their newly formed buccal tentacles to feed on the biological film growing on the settlement plates. The transverse ciliated bands were no longer evident. The body of the pentactulae continued to elongate along the anterior-posterior axis as they developed into juveniles. Some pentactulae, however, developed a second podum before elongation. Development of the juvenile endoskeleton was evident shortly after assumption of benthic existence. The juvenile stage was reached on days 15 - 17. Newly metamorphosed juveniles were 1 mm in length.

7.3.2.2 *H. fuscogilva* and *A. mauritiana*

The development of *H. fuscogilva* and *A. mauritiana* was very similar to that of *H. scabra* (Table 7.4) and their growth pattern to the late auricularia stage is shown in Fig. 7.4. Early auricularia stage was reached by day 3 with mean lengths of 427.00 µm (se = 7.45, n = 30) and 306.14 µm (se = 5.46, n = 30) recorded for *H. fuscogilva* and *A. mauritiana*, respectively. By day 5 larvae of both species were mid auricularia (Fig. 7.5) and by day 10 - 12, were late auricularia. The late auricularia larvae of *H. fuscogilva* had a mean length of 714 µm (se = 12.55, n = 30) and that of *A. mauritiana* had a mean length of 703.60 µm (se = 29.50, n = 30). For *H. fuscogilva*, total mortality occurred in all trials during the late auricularia stage, on days 10 and 16. In the 3 trials with *A. mauritiana* (Table 7.4), larvae in 2 trials died on days 16 and 24 at the doliolaria stage while the larvae in the third trial only reached late auricularia stage (15 d). Hyaline spheres did not develop in any *H. fuscogilva* larvae. In *A. mauritiana*, the hyaline spheres had diameter of 20 - 40 µm.

7.3.3 Larval Survival of *H. scabra*

Only 50% of the *H. scabra* embryos hatched and developed to the early auricularia stage. In the 3 trials, survival decreased through development with < 7% successfully settled (Table 7.5). The numbers of juvenile produced in the three trials ranged from 16222 to 93266 (Table 7.5).
Figure 7.4.
Growth (mean length) of *H. fuscogilva* (○) and *A. mauritiana* (●) from fertilisation (F) through early auricularia (EA) to late auricularia (LA). For each stage, 5 larvae were measured in each tank. ○, 14 tanks, ●, 10 tanks. (Vertical bars standard error of mean).
Figure 7.5.

Chapter 7. Hatchery rearing of three tropical aspirochirotes

7.4 Discussion

_H. scabra_ and _A. mauritiana_ spawned spontaneously in the laboratory after transport as reported for other sea cucumber species (YSFRI, 1991; Reichenbach, 1999). In contrast, successful spawning of _H. fuscogilva_ required an increase in temperature together with introduction of a powered algae as an inducing agent. A recent study demonstrated that this algae ( _Schizochtrium_ sp) can also be used to facilitate spawning in _H. scabra_ and _A. mauritiana_ (Battaglene et al., in press). The mechanism by which the algae stimulates gamete release is not known. The spontaneous spawning observed in _H. scabra_ and _A. mauritiana_ and the use of the powered algae as an inducing agent did not cause shedding of primary oocytes as often the case with sea stars and sea urchins when induced with KCl (Byrne, per. comm). The quality of eggs produced in each of the three species appeared to be uniform.

Development of _H. scabra_, _H. fuscogilva_, and _A. mauritiana_ is typical of planktotrophic aspirochirotes (Smiley et al., 1991). The relatively short larval developmental period for _H. scabra_ and possibly also for _H. fuscogilva_ and _A. mauritiana_, indicates that their development is actaeplanic (short larval cycle) (Levin & Bridges, 1995). A short larval cycle has also been reported for one tropical species, _A. echinites_ and three temperate species of _Stichopus_ (Smiley, 1986; Arakawa, 1990; Chen & Chian, 1990; Archer, 1996).

Embryonic development in the species studied here was similar to that reported for _Stichopus californicus_, _A. echinites_ and _S. mollis_ (Smiley, 1986; Chen & Chian, 1990; Archer, 1996). As typical of holothurians, hatching occurred at the gastrula stage (Smiley et al., 1991; Archer, 1996). The feeding auricularia stage was reached by day 3 and the larvae reached the late auricularia stage by days 10 – 12, similar to that described for _A. echinites_ (Chen & Chian, 1990). The late auricularia of all three species had mean lengths ranging from 700 to 900 μm similar to that reported for _A. echinites_, _S. californicus_ and _S. mollis_ (Chen & Chian, 1990; Smiley et al., 1991; Archer, 1996). The maximum length of the auricularia of _H. scabra_ however, is strongly influenced by food quality and quantity (Morgan, 2001; Ramofafia, _pers obs._).

Development of hyaline spheres at the tip of the lateral processes is a characteristic feature of the late auricularia stage of aspirochirotes. These spheres are suggested to function as a
### Table 7.5

Survival (%) of *H. scabra* in culture (3 trials) from mid auricularia to settlement. Larvae were stocked at 0.1 – 3 larvae ml⁻¹ in each tank at the early auricularia on day 2. Data are expressed as $X \pm se (n)$

<table>
<thead>
<tr>
<th>Trial</th>
<th>Mid auricularia</th>
<th>Late auricularia</th>
<th>Juvenile</th>
<th>Number settled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.00 ± 10.34 (4)</td>
<td>23.63 ± 10.27 (3)</td>
<td>0.61 ± 0.29 (3)</td>
<td>18000</td>
</tr>
<tr>
<td>2</td>
<td>63.60 ± 20.65 (4)</td>
<td>23.62 ± 11.42 (4)</td>
<td>2.94 ± 2.10 (4)</td>
<td>93266</td>
</tr>
<tr>
<td>3</td>
<td>40.26 ± 6.88 (3)</td>
<td>13.40 ± 6.30 (3)</td>
<td>7.95 ± 3.13 (3)</td>
<td>16222</td>
</tr>
</tbody>
</table>
nutritive store and their presence and size is regarded to be an indicator of larval competence (Chen et al., 1991; Smiley et al., 1991; Battaglene, 1999). They are also thought to play a role in larval buoyancy (Dautov & Kashenko, 1995). A potential nutritive role for hyaline spheres is supported by histochemical data showing they contain lipids (Chen et al., 1991). Larvae of *H. scabra* lacking hyaline spheres arrested at the auricularia stage, perhaps due to inadequate nutritive reserves. In contrast, Smiley et al. (1991) and Dautov (1997) reported that temperate species *S. californicus* and *S. japonicus* developed through metamorphosis regardless of the presence or absence of hyaline spheres in the larvae. Further research is required to determine the nature and function of the hyaline spheres in sea cucumbers.

The transition between the planktotrophic auricularia and lecithotrophic doliolaria stages was short in *H. scabra* with the rearrangement of the ciliated band to five transverse ciliated bands at positions previously occupied by lateral processes. This is similar to that reported for several aspidochirote auriculariae (Smiley, 1986; Archer, 1996; Lacalli, 2000). Development of the pentactula stage took 12 h in *H. scabra* as characteristic of aspidochirotids (Smiley et al., 1991). The pentactula of *H. scabra* first attached to the substratum by means of their ventroposterior podium, similar to that reported for *S. californicus* (Smiley et al., 1991). In the field, pentactulae of *H. scabra* settle on sea grass (Mercier et al., 2000). Feeding and locomotion were carried out by the newly functional buccal tentacles.

The larval cycle of *H. scabra* is short and it is likely that the larval cycles of *H. fuscogilva* and *A. mauritiana* are similar in duration. In the field, these species may have a short planktonic period, thereby reducing the period during which they are vulnerable to predation, and may also enhance the potential of locating suitable settlement substrata (Rumrill, 1990). However, the prolonged development of some *H. scabra* larvae with prominent hyaline spheres, albeit a low proportion of the total, shows that response to settlement cues is variable and that delayed metamorphosis is possible (Pechenik et al., 1998). This phenomenon has been common in batches of planktotrophic echinoderm larvae reared in the laboratory and may indicate selection for cohorts of early and late settling larvae (Byrne, *pers com.*). In the wild, where naturally available food is well below the density of food used in laboratory cultures (Lucas, 1982), metamorphosis is likely to be a function of nutritive history, larval competence and response to specific
chemical cues (Pechenik, 1999). Results from larval cultures however, indicate that if the larvae of the three species raised here are in a poor nutritive condition, they may not be capable of completing metamorphosis.

The present studies show that *H. scabra* can be reared successfully to the juvenile stage. The failure of *H. fuscogilva* and *A. mauritiana* to complete development indicates that the culture conditions may not have been appropriate. Development of hyaline spheres as seen in *H. scabra* was never observed in *H. fuscogilva* and less frequently in *A. mauritiana*. If hyaline spheres have nutritive function, then inadequate nutrition may have contributed to larval mortality in the two species. Determination of food conditions that promote hyaline sphere development may be essential for successful culture of *H. fuscogilva* and *A. mauritiana*. It is likely that the optimal diet may be specific for each species. For example, *H. scabra* raised on the mixed algal diet used in these studies developed hyaline spheres while in *H. fuscogilva* and *A. mauritiana* these spheres were lacking or poorly developed. The recent successful production of juvenile *H. fuscogilva* in Kiribati was achieved using *Chaetoceros muelleri* and antibiotics (Battaglene, 1999). It is not known if the larvae in this study developed hyaline spheres. Susceptibility to infection may be an important consideration for culture of *H. fuscogilva* and *A. mauritiana* (Battaglene, 1999). Antibiotics are commonly used in tropical hatcheries for giant clams and pearls oyster to control bacterial proliferation, despite the cost and potential problems in long-term bacterial drug resistance.

Hatching, metamorphosis and settlement are crucial stages in the development and hatchery culture of sea cucumbers. Hatching appears to be a vulnerable stage in the development of *H. scabra* with only 50 % surviving to the early auricularia stage. Mortality during hatching can be reduced by stocking embryos at lower densities (Yanagisawa, 1998; Battaglene, 1999). The egg density used here (1 to 9 eggs ml⁻¹) may have been too high. By the early auricularia stage the larvae were stocked at a density of 0.1 – 3 larvae ml⁻¹. In a recent study, density of 0.5 larvae ml⁻¹ was determined as optimal for cultured *H. scabra* with an initial egg density of 2 eggs ml⁻¹ (Battaglene, 1999). Despite the low survival rate of *H. scabra* larvae in the present study, large numbers of juveniles were produced resulting in sufficient numbers for experimental reseeding trials (Dance *et al.*, in press).
Survival of sea cucumber larvae during metamorphosis and settlement is a function of competence, nutritive reserves and settlement conditions. Larvae need to metamorphose and respond to appropriate settlement cues. Settlement substrates like vinyl chloride, polycarbonate jagged plates coated with benthic diatoms are used to settle *S. japonicus* in Japan, while less sophisticated substrates including tiles and stones are used to settle the species in China (YSFRI, 1991; Ito, 1995; Yanagisawa, 1998). It appears that competent larvae can settle on a wide range of conditioned substrata. Nevertheless, careful husbandry to reduce growth of bacteria and other micro-organisms in rearing systems is also essential. The introduction of the settlement plates into the cultures of *H. scabra* in this study, along with potentially harmful micro-organisms might have contributed to the high mortality of settling pentactulae. The influence of settlement plates on larval survival needs further investigation.

The successful culture of *H. scabra* in this study and elsewhere in the Indo-Pacific (James *et al.*, 1988; Battaglene, 1999; Morgan, 2001; Pitt, 2001) indicates that this species is amenable to hatchery culture. By contrast, other species, including *H. fuscogilva* and *A. mauritiana* have proven more difficult. The contrasting coastal mangrove habitats of *H. scabra* with their fluctuating environmental conditions, and the more stable lagoonal and reef habitats of *H. fuscogilva* and *A. mauritiana* suggests that the *H. scabra* adults and larvae may be, comparatively, more physiologically tolerant. The larvae of *H. scabra* appear robust for hatchery culture. The eggs of *H. scabra* are among the largest recorded for aspidochirotes (Sewell & Young, 1997), indicating that the embryos start development comparatively well provisioned. On the other hand, the eggs of *H. fuscogilva*, are of similar size, while those of *A. mauritiana* are among the smallest for holothuroids. For successful culture of *H. fuscogilva* and *A. mauritiana*, particular attention to reduce damage during culture may be required. Procedures involving less frequent water exchange and care when handling larvae during sieving need to be considered.

In summary, the present chapter described the development of *H. scabra*, *H. fuscogilva* and *A. mauritiana* under hatchery conditions. Of the three species, only *H. scabra* settled successfully, while *H. fuscogilva* and *A. mauritiana* did not progress beyond the larval stage. The results show that the three species did not respond equally to rearing conditions. Successful culture of *H. scabra* was correlated with hyaline sphere development. Further research is required to optimise larval rearing conditions for *H. scabra*, *H. fuscogilva* and
A. mauritiana and may involve use of diets that promote hyaline sphere development and attention to physiological requirements of the larvae.
8.1 Research focus

Increased demand for bêche-de-mer in the international markets has prompted unsustainable exploitation of commercial aspidochirotes by fishers in the Indo-Pacific region, resulting in depletion and overfishing stocks wild stocks (Conand & Byrne, 1993; Conand, 1997). In Solomon Islands, *Holothuria fuscogilva*, *H. scabra* and *Actinopyga mauritiana* are among 23 commercial aspidochirotes that are heavily fished, particularly in the last twenty years. The research detailed in this thesis was undertaken with the aim to document the reproduction and development of these three species. The results were used to assess their potential for aquaculture. The species were chosen for this research because they have a high commercial value as bêche-de-mer species and were the most heavily fished species in Solomon Islands.

8.2 Reproduction of *H. fuscogilva*, *H. scabra* and *A. mauritiana*

8.2.1 Reproduction

Research over a 4-year period for *H. fuscogilva* and *A. mauritiana* revealed that they had annual reproduction, characterised by synchronous and seasonal gametogenesis in Solomon Islands. These data add to the growing evidence indicating that seasonal reproduction is characteristic of most tropical holothurians (Smiley et al., 1991; Conand, 1993a, b). In contrast, reproduction of *H. scabra* over 3 years of investigation was continuous. Gonad development in this species was characterised by asynchronous gametogenesis and year round availability of mature gametes. Seasonal reproduction is reported for this species at higher latitudes (Table 4.1). *H. scabra* appears to be one of the few aspidochirotes that supports the long-standing paradigm that spawning of tropical marine invertebrates becomes seasonal with increasing latitudes (Thorson, 1950). In Solomon Islands this species appears to be an opportunistic spawner with spawning influenced by factors associated with local habitats and lunar periodicity (Chapter 4).
Histological examination revealed that the gonad development in all three species proceeded through the Recovery, Growing, Mature, Partly-spawned and Spent stages, characteristic of holothurian gametogenesis (Smiley et al., 1991). Gametogenesis in *H. fuscogilva* and *A. mauritiana* was synchronous and seasonal and its initiation was suggested to be influenced by day length, as reported for other echinoderms (Pearse et al., 1986; Byrne et al., 1998; Walker & Lesser, 1998). The aseasonal gonad development of *H. scabra* did not correlate with predictable environmental factors and is suggested to reflect the opportunistic spawning strategy of this species. The stages of gonad development based on histology were calibrated with stages based on macroscopic examination of the tubules. Thus, gonad condition in the three species could be determined by direct examination of gonad tubules collected by the gonad biopsy technique similar to that reported in previous studies.

### 8.2.2 Spawning

#### 8.2.2.1 Proximal cues of spawning

*H. fuscogilva* and *A. mauritiana* spawned in spring/summer and *H. scabra* had a period of enhanced spawning activity in summer. Spawning in summer has been reported for other tropical holothurians and correlates with increased water temperature and day length. Spawning also coincided with the end of the dry season, before the onset of the monsoon season in December. It is suggested that spawning at this time favours larval development due to the presence of enhanced levels of phytoplanktonic food following nutrient-rich terrestrial run-off (Birkeland, 1982).

#### 8.2.2.2 Artificial induction of spawning

Spawning occurred in all three species when induced, with males releasing their gametes first. *H. scabra* and *A. mauritiana* spontaneously released gametes in response to the stress associated with collection and transportation to the laboratory. Thermal shock was also a successful spawning induction agent in these two species. In contrast, *H. fuscogilva* did not spawn spontaneously after collection. For this species, thermal shock and addition of dried algae were required to induce spawning.
8.3 Development of *H. fuscogilva*, *H. scabra* and *A. mauritiana* in hatchery culture

*H. scabra* developed through the feeding auricularia larvae, the lecithotrophic doliolaria and pentactula larval stages, typical of aspidochirote development. Its larval cycle was short, with settlement attained within 17 days at 26 to 28 °C. *H. fuscogilva* and *A. mauritiana* did not develop beyond the auricularia stage.

*H. scabra* was raised successfully in artificial culture using a mixed microalgal diet. This diet supported growth of hyaline spheres, structures which contain lipids (Chen *et al.*, 1991) and which were useful indicators of larval competence. *H. scabra* lacking hyaline spheres did not complete development. Although overall survival of *H. scabra* was low, thousands of juveniles were produced for grow-out experiments. Future research is required to optimize culture conditions to increase survival rates of *H. scabra* larvae and settling juveniles.

*H. fuscogilva* and *A. mauritiana* were not raised to settlement but a short larval cycle is also likely for these species. The failure of these species to complete development under the same rearing conditions as *H. scabra* highlighted the difference among holothurian species with respect to nutritional and physiological requirements of larvae in artificial culture. The nutritional and physiological larval requirements of the development stages of *H. fuscogilva* and *A. mauritiana* for aquaculture require additional research.

8.4 Conclusions and future research

- A 3-month spawning period was identified for *H. fuscogilva* and *A. mauritiana*. During this period, broodstock can be collected from the wild for breeding purposes. Due to the year-round spawning of *H. scabra*, this species can be collected and induced to spawn at any suitable time. However, the enhanced spring/summer spawning period identified for this species indicates that this period would be optimal for spawning induction. A closure of the sea cucumber fishery during the spring/summer spawning period should be considered as a management tool.
Chapter 8. General Discussion

- In all three species, histology revealed that gamete release did not necessarily correlate with GI peaks or decline in the GI. This demonstrates that changes in GI alone cannot be used to estimate the timing and duration of gamete release. Future reproductive studies of tropical holothurians must utilize histology as a necessary first step in determination of spawning periodicity.

- Documentation of the macroscopic appearance of the gonad tubules through the year revealed that the tubule biopsy technique could be used to determine the reproductive condition of the three species. As a result gonad condition can be assessed without sacrificing valuable broodstock. Mature female and male *H. fuscogilva* and *A. mauritiana* can be identified readily during the summer spawning period by using the biopsy technique. Due to the continuous pattern of reproduction in *H. scabra*, the biopsy method will be important in selective harvesting of mature specimens. Use of the biopsy technique in the field is recommended to avoid sacrificing specimens from already depleted populations.

- Thermal shock and the use of dried algae facilitated gamete release in captivity. All three species were highly fecund providing millions of gametes for artificial culture. This feature along with the established method for induction of spawning indicates that all three species have potential for aquaculture.

- At this stage, *H. scabra* appears to be the only tropical commercial aspidochirote holothurian that can be reliably cultured to the juvenile stage. Future research of the culture of this species is required to optimize larval survival rates. Conditions for the artificial culture of *H. fuscogilva* and *A. mauritiana* remain to be determined. To evaluate such conditions, collaboration among research institutions for the purpose of information sharing is strongly recommended. For instance, information gathered from the recent successful settlement of *H. fuscogilva* in Kiribati (Battaglene, 1999) should be accessible to interested parties working to restore and enhance populations of this overfished species.

- The data presented in this thesis is a comprehensive investigation of reproduction and development in the three tropical sea cucumbers and is the first detailed study
undertaken on marine invertebrates in Solomon Islands. Given the commercial importance of the three species investigated here and the need for their sustainable management of wild stocks, the work presented here will benefit fisheries agencies and aquaculture projects throughout the Indo-Pacific region.
References


References


References


