UNIVERSITI PUTRA MALAYSIA

REPRODUCTIVE BIOLOGY AND POPULATION DYNAMICS OF Crassostrea (Magallana) saidii WONG & SIGWART, 2021 IN THE ESTUARY OF SUNGAI MUAR, JOHOR, MALAYSIA

ARUN CHANDRA BARMAN

FP 2022 35

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By

ARUN CHANDRA BARMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

July 2022

DEDICATION

To my parents, beloved wife (Doli Rani Kar) and lovely daughters (Ahenjita Barman Alo & Arattrika Barman Tory), who always kept praying for me day and night to achieve my goal

To my family members

and

To all my friends who supported me all these years

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

REPRODUCTIVE BIOLOGY AND POPULATION DYNAMICS OF *Crassostrea* (*Magallana*) *saidii* WONG & SIGWART, 2021 IN THE ESTUARY OF SUNGAI MUAR, JOHOR, MALAYSIA

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Chairman : Nur Leena Wong Wai Sin, PhD
Faculty : Agriculture

Crassostrea (Magallana) saidii Wong & Sigwart, 2021 was recently described as a new oyster species to science. It is an endemic species with its only known distribution limited to Muar estuary, Malaysia, and has been actively harvested from its natural habitat by the locals since at least 1858. This latest taxonomic development warrants a closer look into the reproductive biology and population structure of *C.* (*M.*) *saidii*, which was thought to be a morph-variant of other oyster species in the same vicinity but has a widespread distribution in the country. Additionally, concern arises as the population size is believed to reduce, implicating local fisheries. However, the objectives of this research were to provide information on the reproductive biology and population dynamics of *C.* (*M.*) *saidii* from the estuary of Sungai Muar, Johor, Malaysia. The first study was conducted to evaluate the sex ratio and reproductive phenology of *C.* (*M.*) *saidii* through gonad histology and biometry. Samples of 30 individual oysters were taken monthly from November 2018 to January 2020 from the sole population at Sungai Muar estuary, Johor, Malaysia. Histology revealed that among 450 oysters analyzed, male, female, hermaphrodite, and undifferentiated individuals were at 35.33%, 58%, 1.56%, and 5.11%, respectively. Overall male and female sex ratio was 1:1.64, with female numbers significantly higher. However, the sex ratio was different when the oysters were divided into groups according to shell height (SH) in intervals of 10 mm. The proportion of males decreased with increasing shell height, although the shell height of males and females greatly overlapped. The sex reversal pattern of the oyster might go from male to female and again female to male, with an indicator of rhythmical hermaphroditism. In the current study, statistically significant differences in the size of oogenic cells (mature > maturing > degenerating > immature > oogonia) and the spermatogenic cells (spermatogonia > spermatocyte > spermatid > spermatozoa) were observed. Gametogenesis occurred around the research period with remarkable time-based variations. The oyster spawned over the study period, with peaks in April (2019) and November (2018 and 2019). Condition index (CI) ranged from 70.03 (October 2019) to 31.90 (April 2019), exhibiting four peaks when most of the oysters were maturing

stage and subsequently decreases in CIs indicating spawning. Three distinct peaks in maturity indices (MIs) of the oyster were noted during March, August, and October 2019, when the majority of oysters were mature and ready for spawning. The second study was carried out to correlate environmental variables with the gonadal maturity of *C.* (*M.*) *saidii*. Thirty oysters were taken monthly from November 2018 to January 2020 from the Sungai Muar estuary. The findings showed that there was no significant correlation between water quality parameters like temperature, salinity, pH and total suspended solids, whereas a significant positive correlation was identified between upstream rainfall and spawning gonad ($r^2 = 0.534$, $p < 0.05$). A multiple regression between spawning individuals and environmental variables (temperature, pH, salinity, total suspended solids, and rainfall) indicated that rainfall (beta = 0.535, $p < 0.05$) triggered the spawning of the oyster *C. (M.) saidii* in the estuary. The effects of salinity on the gonadal maturation of the oyster were observed in a controlled environment, conducted in the International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia (UPM), between December 2020 and February 2021 with a duration of 60 days. The oysters were conditioned using three salinities of 10, 15, and 20 ppt. The study revealed that all the salinities were suitable for the maturation of the gonad of the oysters after 30 days and 60 days of the observation. The condition index was not affected by the salinity concentrations over the study period. The species' size-weight relationship and population structure were assessed in 2019 to determine its population dynamics. Results indicated that the gradient of the size-weight equation ($b = 2.5422$) was significantly different ($t = 38.93$, $p < 0.01$) from the isometric value (3), indicating negative allometric growth of the *C.* (*M.*) *saidii* in the estuary. The asymptotic shell height (SH_{∞}) and growth coefficient (K) were observed at 15.23 cm and 0.69 per year, respectively. The maximum observed height and predicted extreme height was 14.50 cm and 14.72 cm, respectively. The calculated growth performance index (φ') was 2.204. The annual total mortality (Z) using the length-converted catch curve was calculated at 3.02, annual fishing mortality (F) at 1.27, and annual natural mortality (M) at 1.75. The recruitment pattern of the oyster showed continuous recruitment, with two peaks over the study period. The exploitation level (E) of the oyster was computed at 0.42, while the allowable limit of exploitation (E_{max}) was 0.782 for the highest yield. The current exploitation level ($E = 0.42$) indicates that the oyster stock was exploited at almost the optimum ($E = 0.5$) from the estuary. The understanding of the reproductive biology and population dynamics could provide information for optimal and accurate management of the natural population of oyster *C.* (*M.*) *saidii* in its sole habitat. The identification of maturation and spawning season of this species will allow their conservation through aquaculture approach while reducing commercial pressures from the natural stock.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIOLOGI REPRODUKTIF DAN DINAMIK PENDUDUK *Crassostrea* (*Magallana*) *saidii* WONG & SIGWART, 2021 DI MUARA SUNGAI MUAR, JOHOR, MALAYSIA

Oleh

ARUN CHANDRA BARMAN

Julai 2022

Pengerusi : Nur Leena Wong Wai Sin, PhD
Fakulti : Pertanian

Crassostrea (*Magallana*) *saidii* Wong & Sigwart, 2021 baru-baru ini telah diklasifikasikan sebagai spesies tiram baharu. Ia adalah spesies endemik dengan taburan yang terhad di muara Muar, Malaysia, dan telah dituai secara aktif dari habitat semula jadi oleh penduduk tempatan sejak tahun 1858. Perkembangan taksonomi terkini dapat menentukan dengan lebih tepat berkenaan biologi pembiakan dan struktur populasi *C.* (*M.*) *saidii*, yang dianggap sebagai varian-morf bagi spesies tiram lain di kawasan yang sama tetapi mempunyai taburan yang meluas di negara ini. Selain itu, saiz populasi yang dipercayai berkurangan membabitkan perikanan tempatan menambahkan kebimbangan akan spesies ini. Walau bagaimanapun, objektif penyelidikan ini adalah untuk memberi maklumat tentang biologi pembiakan dan dinamik populasi *C.* (*M.*) *saidii* dari muara Sungai Muar, Johor, Malaysia. Kajian pertama dijalankan untuk menilai nisbah jantina dan fenologi pembiakan *C.* (*M.*) *saidii* melalui kaedah histologi gonad dan biometri. Sebanyak 30 sampel tiram telah diambil setiap bulan dari November 2018 hingga Januari 2020 daripada populasinya di muara Sungai Muar, Johor, Malaysia. Kajian histologi mendedahkan bahawa antara 450 tiram yang dianalisis, jantan, betina, hermafrodit, dan individu yang tidak dibezakan adalah masing-masing pada 35.33%, 58%, 1.56%, dan 5.11%. Nisbah jantina jantan dan betina keseluruhan ialah 1:1.64, dengan bilangan betina jauh lebih tinggi. Walau bagaimanapun, nisbah jantina adalah berbeza apabila tiram dibahagikan kepada kumpulan mengikut ketinggian cangkerang (*SH*) dalam selang 10 mm. Perkadaran jantan berkurangan dengan peningkatan ketinggian cangkerang, walaupun ketinggian cangkerang jantan dan betina banyak bertindih. Corak pembalikan jantina tiram mungkin berubah dari jantan ke betina, dan sekali lagi betina ke jantan, dengan penunjuk hermafroditisme berirama. Dalam kajian ini, perbezaan ketara secara statistik dalam saiz sel oogenik (matang > matang > merosot > tidak matang > oogonia) dan sel spermatogenik (spermatogonia > spermatosit > spermatid > spermatozoa) telah diperhatikan. Gametogenesis berlaku sekitar tempoh penyelidikan dengan variasi berasaskan masa. Tiram bertelur sepanjang tempoh kajian, dengan puncaknya pada April (2019) dan November (2018 dan 2019). Indeks keadaan (*CI*) berjulat dari 70.03 (Oktober

2019) hingga 31.90 (April 2019), mempamerkan empat kemuncak apabila kebanyakan tiram berada dalam peringkat matang dan seterusnya nilai *CI* menurun yang menunjukkan pemijahan. Tiga kemuncak yang berbeza dalam indeks kematangan (*MI*) tiram telah dicatatkan pada bulan Mac, Ogos dan Oktober 2019, apabila majoriti tiram telah matang dan bersedia untuk bertelur. Eksperimen kedua kemudiannya dijalankan untuk mengaitkan pembolehubah persekitaran dengan kematangan gonad *C.* (*M.*) *saidii.* Sebanyak 30 ekor tiram diambil setiap bulan dari November 2018 hingga Januari 2020 dari muara Sungai Muar. Dapatan kajian menunjukkan bahawa tidak terdapat perhubungan yang signifikan antara parameter kualiti air seperti suhu, kemasinan, pH dan jumlah pepejal terampai, manakala perhubungan positif yang signifikan telah dikenalpasti antara hujan dan pemijahan gonad ($r^2 = 0.534$, $p < 0.05$). Regresi berganda antara individu pemijahan dan pembolehubah persekitaran (suhu, pH, kemasinan, jumlah pepejal terampai, dan hujan) menunjukkan bahawa hujan (beta = 0.535, $p < 0.05$) merangsang pemijahan tiram *C.* (*M.*) *saidii* dalam muara sungai. Kesan kemasinan ke atas pematangan gonad tiram diperhatikan dalam eksperimen terkawal, yang dijalankan di Institut Akuakultur dan Sains Akuatik Antarabangsa (I-AQUAS), Universiti Putra Malaysia (UPM), antara Disember 2020 dan Februari 2021 dalam tempoh 60 hari. Tiga tahap kemasinan 10, 15, dan 20 ppt telah ditetapkan untuk kajian ini. Kajian menunjukkan bahawa semua kemasinan adalah sesuai untuk pematangan gonad tiram selepas 30 hari dan 60 hari pemerhatian. Indeks keadaan tidak dipengaruhi oleh kepekatan kemasinan sepanjang tempoh kajian. Hubungan saiz-berat spesies dan struktur populasi telah dinilai pada 2019 untuk menentukan dinamik populasinya. Keputusan menunjukkan bahawa kecerunan persamaan saiz-berat ($b = 2.5422$) adalah berbeza secara ketara ($t = 38.93$, $p < 0.01$) daripada nilai isometrik (3), menunjukkan pertumbuhan alometri negatif bagi *C.* (*M.*) *saidii* dalam muara. Ketinggian cengkerang asimptotik ($SH\infty$) dan pekali pertumbuhan (*K*) diperhatikan pada 15.23 cm dan 0.69 setahun. Ketinggian maksimum yang diperhatikan dan ketinggian paling tinggi yang diramalkan ialah 14.50 cm dan 14.72 cm. Indeks prestasi pertumbuhan yang dikira (φ') ialah 2.204. Jumlah kematian tahunan (*Z*) menggunakan keluk tangkapan tukar panjang dikira pada 3.02, kematian penangkapan ikan tahunan (*F*) pada 1.27, dan kematian semula jadi tahunan (*M*) pada 1.75. Corak pengambilan tiram menunjukkan pengambilan berterusan, dengan dua kemuncak sepanjang tempoh kajian. Tahap eksploitasi (*E*) tiram dikira pada 0.42, manakala had eksploitasi yang dibenarkan (*Emaks*) ialah 0.782 untuk hasil tertinggi. Tahap eksploitasi semasa ($E = 0.42$) menunjukkan bahawa stok tiram telah dieksploitasi pada kadar yang optimum ($E = 0.5$) dari muara. Pemahaman tentang biologi pembiakan dan dinamik populasi boleh membantu untuk menyediakan pengurusan yang optimum dan tepat bagi stok tersedia tiram *C.* (*M.*) *saidii* di habitat tunggalnya. Pengenalpastian musim kematangan dan pemijahan spesies ini akan membolehkan pemuliharaan tiram ini melalui pendekatan akuakultur sambil mengurangkan tekanan komersial daripada stok semula jadinya.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Nur Leena Wong Wai Sin, the Chairman of the Supervisory Committee, for her invaluable supervision, productive discussion, constructive criticisms, and continuous encouragement throughout the study. I am much grateful and indebted to Assoc. Professor Dr. Murni Marlina binti Abd. Karim and Dr. Mohd Zafri bin Hassan, members of the supervisory committee, for their constructive suggestions and guidance in all phases of this research project. I express my deep respect to all of the lecturers and professors in the Department of Aquaculture for their valuable suggestions and constructive comments.

I am grateful to the laboratory staff of the Department of Aquaculture, Faculty of Agriculture

I am indebted to my parents, wife, daughter, brothers, and sisters for their support, deep love, and tolerance during the whole period of my study.

Thankfully acknowledge the assistance of the National Agricultural Technology Program- Phase 2 project (NATP-2), Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka-1215, Bangladesh, for financial support and facilitating my study and Universiti Putra Malaysia for providing research facilities under the Aquaculture Department, Faculty of Agriculture.

I certify that a Thesis Examination Committee has met on 6 July 2022 to conduct the final examination of Arun Chandra Barman on his thesis entitled "Reproductive biology and population dynamics of *Crassostrea* (*Magallana*) *saidii* Wong & Sigwart, 2021 in the estuary of Sungai Muar, Johor, Malaysia" in accordance with the universities and university college act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15th March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

Muta Harah Zakaria, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Aziz Arshad, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Annie Christianus, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

ZURIATI AHMAD ZUKARNAIN, PhD
Professor Ts. and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Nur Leena Wong Wai Sin, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Murni Marlina binti Abd. Karim, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Mohd Zafri bin Hassan, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 November 2022

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature:	
Name of Chairman of Supervisory Committee:	Dr. Nur Leena Wong Wai Sin
Signature:	
Name of Member of Supervisory Committee:	Associate Professor Dr. Murni Marlina binti Abd. Karim
Signature:	
Name of Member of Supervisory Committee:	Dr. Mohd Zafri bin Hassan

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LIST OF ABBREVIATIONS

CI	Condition index
cm	Centimeter
DoF	Department of Fisheries
E	Exploitation rate
ED	Early Development
ELEFAN	Electronic Length Frequency Analysis
F	Fishing mortality
FAO	Food and Agricultural Organization
FiSAT	FAO ICLARM Stock Assessment Tools
g	Gram
K	Growth coefficient of VBGF
Kg	Kilogram
Km	Kilometer
L	Liter
LD	Late Development
MI	Maturity index
mg/L	Milligram per liter
mm	Millimeter
NN	Nylon net
ppt	Parts per thousand
S.E	Standard error
Und	Undifferentiated
UPM	Universiti Putra Malaysia
µm	Micrometer

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Oysters are ecologically keystone species in marine ecosystems by providing ecological services as filter feeders, clarifiers and reef-builders, and for the last reason, they are known as bioengineers (Smaal et al., 2019). They are listed as commercially important species within the invertebrates in many regions of the world (Piedra et al., 2014; Posada et al., 2014). However, in Malaysia, two oyster species are mainly used for commercial purposes, namely *Crassostrea iredalei* and *Crassostrea belcheri* (Suzana et al., 2011). Fishers in Malaysia usually collect spats from the wild to culture oysters on a commercial basis, but currently, the supply of seeds of the oyster has declined (Fakhrina et al., 2018) due to siltation, acidification, disease, overfishing, and lack of proper knowledge on management (Tan et al., 2014).

In Malaysia, marine science has received much attention in recent years, but marine molluscs studies are still overseen by many researchers (Wong & Arshad, 2011). However, some studies on the culture of oysters in Malaysia have been conducted, supported by the International Development and Research Canada (IDRC) (Tan et al., 2014). The larval development of local oyster species *C. belcheri* and *C. iredalei* larvae has been established in the country, considering substrates, storage temperature, and salinity (Devakie & Ali, 2002). The chemical interaction on larval settlement of the oysters has also been studied (Teh et al., 2012). Moreover, the relationship between environmental parameters on the development and survival of broodstock oysters was also documented (Hawkins et al., 1998; Izwandy, 2006). Genetic diversities in two cultured oyster species, *C. iredalei* and *C. belcheri* were assessed (Suzana et al., 2011). The use of genetics approaches of diploid and triploid (Rahim et al., 2008) and tetraploid induction in oysters, *C. iredalei* and *C. belcheri* also has been studied (Tan et al., 2017). Besides, gonad maturation, larval development, and settlement of the slipper cupped oyster *C. iredalei* was recorded at Sungai Terus, Batu Lintang, Kedah (Wahab, 2017). A systematics description of the Muar oyster *Crassostrea (Magallana) saidii* was reported in the estuary of Sungai Muar, Johor, Malaysia (Sigwart et al., 2021).

The oyster *C.* (*M.*) *saidii* has been marketed and consumed in Malaysia for over 160 years as an undescribed species. Recently, this oyster was named by Sigwart et al. (2021) according to a resident of Muar, Md Saidi Bin Mohamed, who has been actively promoting research and conservation for the sustainability of this species. The Muar oyster *C.* (*M.*) *saidii*, locally known as *Tiram Putih* (white oyster), co-occurring with the worldwide established species *C. belcheri* (*Tiram kapak*, Axe oyster). The oyster is distributed from the river mouth of Muar River, Johor, Malaysia to approximately 6 km upstream. The oyster *C.* (*M.*) *saidii* is morphologically and genetically distinct, limited to Sungai Muar, Malaysia, the only known population in the scientific record (Sigwart et al., 2021). Because of its creamy white meat and excellent quality, the species is

known as the white oyster among fisherfolk, and locals, and is more popular with oyster fans than the *C. belcheri* (Axe Oyster) species, which can also be found in the estuary of Muar (Sigwart et al., 2021). The oyster fishery in Muar estuary was first reported in 1858 by a British Captain who described Muar oyster as a delicacy reserved for the royals and high officials (Macpherson, 1858). It was then sold at the Singapore market, which is about 200 km away (Hanitsch, 1908), but later Muar oyster fishery was reported as unsustainable in 1929, probably due to the decline in the oyster population (Dover, 1929). Muar oysters have been farmed through bottom ranching by returning empty oyster shells to certain points of the Sungai Muar estuary, and it is still practiced to this day.

The oyster *C.* (*M.*) *saidii*, however, due to urbanization, is on under potential threat, and the production of this species is declining in the estuary (Sigwart et al., 2021). This declining led to the research on the *C.* (*M.*) *saidii*, especially in reproductive biology and population dynamics

1.2 Statement of problems

The oyster *Crassostrea* (*Magallana*) *saidii* is an important fishery in the estuary of Sungai Muar, Johor and the small population is threatened by urbanization, pollution, and habitat destruction (Sigwart et al., 2021). Moreover, in Malaysia, wild seeds are the mainly source for the farmers to culture this oyster. Again, there is no study conducted on the artificial conditioning of *C.* (*M.*) *saidii* which can facilitate its introduction in aquaculture. Although, it is important to know the reproductive biology and population structure to sustain its natural stock, there is no information on the biological characterization and *ex-situ* conditioning of the Muar Oyster *C.* (*M.*) *saidii*. Therefore, it is essential to find out the spawning environment, artificial conditioning, and population structure of the oyster in the respective area to facilitate its conservation and effective seed production for aquaculture. Furthermore, the population dynamics and stock assessment will provide the base to avoid overfishing and for the sustainable management of this species. Therefore, the current research will focus on the gonad development, correlation between environmental factors and gonadal maturation, impacts of salinity on the maturation of gonads in *ex-situ* conditions, and the population parameters of the *C.* (*M.*) *saidii* in the estuary of Sungai Muar, Johor, Malaysia.

1.3 Significance of the study

The information on gonad development could be useful for assessing the possible breeding behavior of *C.* (*M.*) *saidii*. Moreover, a thorough understanding of these reproductive cycles, condition index, and maturity index will allow farmers to determine the optimal moments for harvesting, intending to obtain oysters with a higher meat weight before spawning and seed collection. The knowledge of the relationship between the environmental variables and gonadal development are essential for the planning of breeding and seed collection for *C.* (*M.*) *saidii*. Besides, the information on the impacts of different salinities on gonad maturation in *ex-situ* conditions will provide information

in extending the seed production season of *C.* (*M.*) *saidii* for commercial purposes. The commercial importance of oysters in Malaysia is increasing gradually with the growing demand for oysters for human consumption. The research studies focusing on its population parameters will be of considerable necessity for future economic valorization and sustainable management of the species in Malaysian waters.

The present study investigated the biology and the population dynamics of the edible oyster *C.* (*M.*) *saidii* to define the biological characterization of the oyster population in Sungai Muar estuary. Data from the present study provide essential information for the sustainable management and proper utilization of the oyster *C.* (*M.*) *saidii* natural stocks, and possibly for the similar oyster stocks of the world.

1.4 Objectives

The objectives of the research were as follows:

1. To identify sex ratio and phenology of the oyster *C.* (*M.*) *saidii* through gonad histology and biometry
2. To determine the correlation between environmental variables and the gonadal maturity of the oyster *C.* (*M.*) *saidii* at Sungai Muar, Johor
3. To observe the impacts of salinity on the gonadal development of the oyster *C.* (*M.*) *saidii* in *ex-situ* condition.
4. To assess the length-weight relationship and population parameters of the oyster *C.* (*M.*) *saidii* at Sungai Muar.

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