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NESTING BIOLOGY OF THE HORSESHOE CRAB, *TACHYPLEUS GIGAS* (MÜLLER, 1785) IN RELATION TO CHANGING ENVIRONMENTAL SETTINGS AT PANTAI BALOK AND TANJUNG SELANGOR, PAHANG

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DEDICATION

I would like to dedicate this thesis to my mother, Ms. Daisy Ann, family members and all my friends for their constant unending support, motivation and inspiration.

(Bryan Raveen)

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Despite the presence of *Tachypleus gigas*, *T. tridentatus* and *Carcinoscorpius rotundicauda* in Malaysian waters for ages, the available scientific literature on their population dynamics, breeding biology and nesting behaviour still remains limited. This includes the documentation at their breeding grounds, Pantai Balok and Tanjung Selangor, present on east coast of Peninsular Malaysia. Long-term investigations were carried out at both places in two Phases, 1 (2009-2010) and 2 (2010-2011). However an additional, Phase-3 (2012-2013), was only included for Pantai Balok.

T. gigas conducted its annual spawning between March and November at Pantai Balok and between June and October at Tanjung Selangor. The crab's nesting was intense during full moon and Southwest (SW) monsoon periods. Interestingly, the construction and land reclamation activities (*e.g.* wave breaker, fish jetty) at Pantai Balok that altered its beach from medium to fine sand, made sediment sorting better and increased the sediment kurtosis, all of which favoured the *T. gigas'* egg laying. But comparatively, the opposite was found at Tanjung Selangor as sand mining, jetty construction, and road/bridge building deteriorated its beach from medium to coarse sand, worsened the sediment sorting and increased its kurtosis. Together, Cadmium

(Cd^{2+}), Lead (Pb^{2+}) and Selenium (Se^{2+}) in sediment and Sulphide (S^{2-}) in water are responsible for the decreased spawning activity/egg counts. In addition, *T. gigas* nest shifting (between Sites 1 and 3) was associated with seasonal sediment changes (sand types, sorting, kurtosis, etc.), water salinity and S^{2-} concentrations. The Stepwise (BEST) and Principle Component Analysis performed between the environmental and the nesting/non-nesting conditions (using PRIMER *v*.6) supported the results obtained.

Since Pantai Balok and Tanjung Selangor were contaminated, lab-based assays were performed on eggs of *T. gigas* through Cd^{2+} and Pb^{2+} exposures at 5, 10 and 20 mg l^{-1}. Embryonic weight measurements, development changes and glutathione were estimated whereas histology and Scanning Election Microscopy examinations were carried out at intervals of 1, 3, 7, 14, 21 and 34 days. After 34 days, all embryos of the control group hatched (100%) but, exposures to Cd^{2+} and Pb^{2+} only produced 46.7 and 71.1% hatching with 11.9 and 45.3% of larvae having segment defects. Embryos of the control and Pb^{2+} groups achieved maximal weights within 21 days (229±19 and 229±22 mg) unlike the Cd^{2+} group, after 34 days (125±45 mg).

Advanced larval development were shown by embryos from control (aortic structures) and Pb^{2+} (ocelli) (5 mg l^{-1}) exposure groups. Comparatively, formation of fatty structures, connective tissues and dermal structure formation were found at similar rate among embryos from Cd^{2+} (5 and 10 mg l^{-1}) and Pb^{2+} (10 and 20 mg l^{-1}) exposure groups. In addition, delayed development of fatty structures and tissue formation were only found among embryos from 20 mg l^{-1} Cd^{2+} exposure group after 34 days. In Cd^{2+} and Pb^{2+} exposures, genital operculum deformities extend to enlargement of the hypodermis and mesoderm at the exopod and endopod sections whereas cephalothoracic appendage deformities occurred at the tibia and tarsus after comparisons were made with embryos from the control group. The poor affinity of Pb^{2+} to Sulfhydryl group caused its toxic effects to set in, as compared to Cd^{2+}, a more reactive metal. Clearly, embryos exposed to Cd^{2+} faced delaying embryogenesis and hatching when compared to embryos exposed to Pb^{2+}. But, embryos exposed to Pb^{2+} faced increased risks of segment defects despite their higher hatching succession than the embryos from Cd^{2+} exposure groups. Considering the plight of *T. gigas* (adults and eggs), it is imperative to declare Pantai Balok and Tanjung Selangor as 'protected areas' with required protection either, by Fisheries Act 1985 (Act 317) or Wildlife Conservation Act 2010 (Act 716). This, inevitably will allow the recovery of the horseshoe crab nesting beach as well as population of breeding crabs at both nesting areas.

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BIOLOGI PERSARANGAN BELANGKAS, *TACHYPLEUS GIGAS* (MÜLLER, 1785) BERKAITAN PERUBAHAN PENETAPAN PERSEKITARAN DI PANTAI BALOK DAN TANJUNG SELANGOR, PAHANG

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Walaupun *Tachypleus gigas*, *T. tridentatus* dan *Carcinoscorpius rotundicauda* telah berkurun mendiami perairan Malaysia namun, dokumentasi saintifik berkenaan populasi dinamik, biologi pembakaan dan persarangan masih terhad. Ini termasuk kawasan persarangan mereka yang masih aktif di pantai timur Semenanjung Malaysia iaitu Pantai Balok dan Tanjung Selangor. Kajian jangka panjang dirangka sebagai Fasa 1 (2009-2010) and 2 (2010-2011) untuk kedua-dua tempat ini. Walaubagaimanapun, sebagai tambahan, hanya Fasa-3(2012-2013) berlangsung di Pantai Balok.

Setiap tahun, *T. gigas* bersarang di Pantai Balok antara Mac dan November dan untuk Tanjung Selangor ianya berlaku antara Jun dan Oktober. Aktiviti ini meningkat semasa bulan penuh dan pada monsoon Barat Daya (SW). Menariknya, keadaan Pantai Balok menjadi lebih baik untuk persarangan *T. gigas*, setelah aktiviti pembinaan (*cth.* pemecah ombak, jeti ikan) dan penambakan menukar pantainya daripada pasir sederhana kepada halus, pengasingan sedimen diperbaikkan dan kurtosis telah meningkat. Akantetapi sebaliknya dilihat di Tanjung Selangor, dimana perlombongan pasir, pembinaan jeti dan pembangunan jalan/jambatan memudaratkan pantainya dengan menukar pasirnya daripada sederhana kepada kasar, menurunkan

pengasingan dan meningkatkan kurtosis sedimennya. Bersama, Kadmium (Cd^{2+}), Plumbum (Pb^{2+}) dan Selenium (Se^{2+}) dalam sedimen dan Sulfida (S^{2-}) dalam air dikaitkan dengan penurunan aktiviti bertelur/jumlah telur. Selain itu, penukaran tempat bersarang *T. gigas* (antara Tapak 1 dan 3) berhubungkait dengan perubahan komposisi pasir mengikut musim (jenis pasir, pengasingan, kurtosis, dll.), saliniti air dan kepekatan S^{2-}. Analisa Langkahan (BEST) dan Komponen Utama (PCA) antara persekitaran dan persarangan/ketiadaan digunakan untuk mengesahkan keputusan yang diperoleh (PRIMER *v*.6).

Memandangkan Pantai Balok dan Tanjung Selangor tercemar dengan logam berat, ujian-makmal dijalankan dengan mendedahkan telur *T. gigas* kepada Cd^{2+} dan Pb^{2+} pada kepekatan 5, 10 dan 20 mg l^{-1}. Perubahan berat, perkembangan dan glutathione dianggarkan manakala histologi dan Mikroskop Imbasan Elektron digunakan untuk pemeriksaan embrio pada hari ke- 1, 3, 7, 14, 21 dan 34. Selepas 34 hari, semua embrio kawalan menetas (100%) tetapi untuk kumpulan Cd^{2+} dan Pb^{2+}, hanya 46.7 dan 71.1% penetasan dengan kecacatan segmen sebanyak 11.9 dan 45.3% diperoleh. Berat embrio mencapai maksima selepas 21 hari (229±19 dan 229±22 mg) bagi kumpulan kawalan dan Pb^{2+} berbanding dengan Cd^{2+} yang mencapai maksima selepas 34 hari (125±45 mg).

Kumpulan kawalan (struktur aorta) dan Pb^{2+} (ocelli) (5 mg l^{-1}) menunjukkan perkembangan embrio yang maju. Apabila dibandingkan, Cd^{2+} (5 dan 10 mg l^{-1}) dan Pb^{2+} (10 dan 20 mg l^{-1}) mempunyai tahap perkembangan sama iaitu penghasilan struktur berlemak, tisu penghubung dan pembentukan lapisan dermis. Selain itu, perkembangan struktur berlemak dan tisu dalaman embrio dilihat lewat selepas 34 hari bagi pendedahan kepada 20 mg Cd^{2+}. Embrio yang didedahkan kepada Cd^{2+} dan Pb^{2+} nyata mempunyai kecacatan operculum kemaluan berikutan bengkak pada hipodermis dan mesoderma di bahagian endopod dan eksopod manakala kecacatan anggota cephalothorasik berlaku di tibia dan tarsus selepas perbandingan dilakukan dengan embrio daripada kumpulan kawalan. Kelemahan Pb^{2+} terhadap kumpulan Sulfhidril menyebabkan berlakunya kesan toksik berbanding dengan Cd^{2+} yang lebih reaktif. Ianya jelas, Cd^{2+} telah melambatkan perkembangan embrio dan penetasan berbanding dengan embrio yang terdedah kepada Pb^{2+}. Tetapi, embrio yang terdedah kepada Pb^{2+} lebih berisiko mempunyai kecacatan segmen walaupun mereka mempunyai peluang yang lebih tinggi untuk menetas berbanding dengan embrio daripada dedahan Cd^{2+}. Seiring dengan kesusahan yang dialami *T. gigas* (dewasa dan telur), ianya penting jika Pantai Balok dan Tanjung Selangor dijadikan 'kawasan lindung' dengan pantauan samada daripada Akta Perikanan 1985 (Akta 317) atau Akta Pemuliharaan hidupan Liar 2010 (Akta 716). Ini dapat membantu pemuliharaan kawasan persarangan serta populasi belangkas di kedua-dua kawasan tersebut.