

EFFECTS OF HEAVY METALS ON EGGS AND LARVAE OF  
HORSESHOE CRAB *Tachypleus gigas* (Muller 1785)

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**EFFECTS OF HEAVY METALS ON EGGS AND LARVAE OF  
HORSESHOE CRAB *Tachypleus gigas* (MÜLLER 1785)**

**BRYAN RAVEEN NELSON S/O NELSON BERNETT**

**Thesis Submitted in Fulfilment of the Requirement for the Degree  
of Master of Science in the Institute of Tropical Aquaculture  
Universiti Malaysia Terengganu**

**May 2012**

## DEDICATION

I would like to dedicate this thesis to my parents and all those who had been involved in making this study a success. I also would like to dedicate it to my family and all my friends for their unending support, motivation and inspiration.

[Bryan Raveen]

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Master of Science.

**EFFECTS OF HEAVY METALS ON EGGS AND LARVAE OF HORSESHOE CRAB *Tachypleus gigas* (MÜLLER 1785)**

**Bryan Raveen Nelson**

**May 2012**

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Horseshoe crabs inhabit coastal waters of Peninsular Malaysia, with nesting activity observed at Pantai Balok, Pahang. Pantai Balok is exposed to a variety of human threats such as boating and leisure activities which contribute to metal contamination. Hatching success of horseshoe crab eggs could be affected because eggs were left to mature buried in sediments during mating periods. The aim of this study was to predict hatching success and survival of *Tachypleus gigas* (Müller, 1785) eggs and larvae in the presence of metals. Horseshoe crab prints indicate nests in sediments and plastic scoops were used to unearth the eggs. 15 days sub-acute bioassay on eggs and larvae was conducted using CdCl<sub>2</sub>, and Pb(NO<sub>3</sub>)<sub>2</sub> at concentrations of 1000, 2000

and 5000 mg/l; 34 days chronic bioassay was conducted on eggs using 25, 50 and 1000 mg/l of CdCl<sub>2</sub>, and Pb(NO<sub>3</sub>)<sub>2</sub> and 4 days sub-acute bioassay was conducted on larvae using CdCl<sub>2</sub>, CuSO<sub>4</sub>, MgSO<sub>4</sub>, Pb(NO<sub>3</sub>)<sub>2</sub>, and ZnSO<sub>4</sub> in a series of 4 bioassays, each with higher intensity of exposure and the final in varying salinities, 15‰ and 28‰. Egg and larvae weight was obtained before and after the sub-acute bioassay and on days 5, 14, 21 and 34 of the chronic bioassay. Haematoxylin & Eosin (H&E) combined with Toluidine-0 and Sudan IV was used for specimen preparation. Advanced Research Microscope (ARM) and Scanning Electron Microscope (SEM) were used to observe deformities. Heavy metal accumulation was observed for egg and larvae, but types of metal differed with concentration of exposure. Egg shell accumulated Cd<sup>2+</sup> and Cu<sup>2+</sup> at high intensities compared to egg yolk. Development of eggs was delayed compared to the control and Cd<sup>2+</sup> more intense than Pb<sup>2+</sup> exposure. LC<sub>50</sub> was not established because of high survival rate and larvae gill lamellae thickening were observed with elevated metal exposure. High survival rate was observed for the 4 days sub-acute bioassay, but mortality increased with higher metal exposure intensities and lower salinity, mainly for Cd<sup>2+</sup> and Pb<sup>2+</sup> with higher mortality in Cd<sup>2+</sup> exposures compared to Pb<sup>2+</sup>. Cd<sup>2+</sup> delayed development and growth whereas Pb<sup>2+</sup> slowed development. Segment defect deformities in developing embryos varied in Cd<sup>2+</sup> and Pb<sup>2+</sup> exposures.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai keperluan untuk ijazah Master Sains.

**KESAN LOGAM BERAT KE ATAS TELUR DAN LARVA  
BELANGKAS *Tachypleus gigas* (MÜLLER 1785)**

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Belangkas diperhatikan mendiami perairan pantai Semenanjung Malaysia dan bertelur di Pantai Balok, Pahang. Pantai Balok terdedah kepada pelbagai ancaman manusia seperti penggunaan bot dan aktiviti masa lapang yang menyumbang kepada pencemaran logam. Semasa musim pengawanan, penetasan telur belangkas mungkin terjejas kerana dibiarkan matang terkubur dalam sedimen. Tujuan kajian ini adalah untuk meramal penetasan dan kadar hidup telur dan larva *Tachypleus gigas* (Müller, 1785) dalam kehadiran logam. Bentuk menyerupai belangkas pada sedimen menunjukkan lokasi sarang dan senduk plastik digunakan untuk mengeluarkan telur. Pendedahan sub-akut 15 hari pada telur dan larva telah dijalankan dengan menggunakan  $CdCl_2$  dan  $Pb(NO_3)_2$  pada kepekatan 1000, 2000 dan 5000 mg/l; ujikaji

kronik 34 hari dijalankan menggunakan 25, 50 dan 1000 mg/l  $\text{CdCl}_2$ , dan  $\text{Pb}(\text{NO}_3)_2$  dan ujikaji sub-akut 4 hari dijalankan menggunakan  $\text{CdCl}_2$ ,  $\text{CuSO}_4$ ,  $\text{MgSO}_4$ ,  $\text{Pb}(\text{NO}_3)_2$  dan  $\text{ZnSO}_4$  dalam siri 4 bioassay, setiap dengan peningkatan intensiti pendedahan dan yang terakhir dalam kemasinan 15‰ dan 28‰. Berat telur dan larva diperolehi sebelum dan selepas ujikaji sub-akut dan pada hari-hari 5, 14, 21 dan 34 untuk ujikaji kronik. Spesimen kajian disediakan menggunakan Haematoxylin & Eosin (H&E), Toluidine-0 dan Sudan IV. Pemerhatian menggunakan (Mikroskop Kajian Canggih) ARM dan (Mikroskop Elektron Pengimbas) SEM dijalankan untuk mengenalpasti kecacatan. Telur dan larva diperhatikan menggumpalkan logam, tetapi jenis berbeza dengan kepekatan pendedahan. Kulit telur lebih menggumpul  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  berbanding dengan kuning telur. Pembantutan perkembangan meninggi berbanding dengan kawalan dan pendedahan kepada  $\text{Cd}^{2+}$  lebih membantut berbanding dengan  $\text{Pb}^{2+}$ .  $\text{LC}_{50}$  tidak tercapai kerana kadar hidup tinggi tetapi lamela insang larva menebal dengan peningkatan logam pendedahan. Kadar hidup adalah tinggi untuk ujikaji sub-akut 4 hari, tetapi kematian meningkat dengan keamatan logam pendedahan dan pengurangan kemasinan, dimana  $\text{Cd}^{2+}$  menunjukkan kesan meninggi berbanding dengan  $\text{Pb}^{2+}$ . Perkembangan dilambatkan dalam pendedahan kepada  $\text{Cd}^{2+}$  dan diperlahankan dalam  $\text{Pb}^{2+}$ . Kecacatan segmen semasa perkembangan embrio berbeza untuk pendedahan kepada  $\text{Cd}^{2+}$  dan  $\text{Pb}^{2+}$ .