

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Doctor of Philosophy

EFFECTS OF WATER SALINITY, LIMB AUTOTOMY AND REPRODUCTIVE HORMONES ON MOULTING AND MATURATION PERFORMANCE OF FEMALE ORANGE MUD CRAB, *Scylla olivacea* (HERBST, 1796)

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The objectives of this study were (1) to determine the body weight (BW) and carapace width (CW) increment, confirmation of ovary maturation Stage 1 of newly moulted crabs, and the effect of water salinity on the survival of *Scylla olivacea* in-captivity, (2) to identify the effects of water salinity and limb autotomy on moulting performances, maturation stages, and glucose concentration, and (3) to study the effects of reproductive hormones on maturation stages and hormone levels of *S. olivacea*. *S. olivacea* were sampled from Setiu Wetlands, Terengganu, on the East Coast of Peninsular Malaysia (5°40'47.93"N 102°42'45.04"E) from July 2015 to July 2019. Results indicated that 135 crabs (newly moulted) dissected were confirmed in Stage 1 ovary (colouration and histology). There was positive correlation ($r = 0.263$, $p = 0.001$) between CW and BW

of moulting crabs with an average increment of 0.82 ± 0.27 cm and 8.39 ± 2.72 g, respectively. For the salinity tolerances, results indicated that *S. olivacea* could tolerate salinity ranges between 5.14 ppt to 36.67 ppt. The moulting performances of *S. olivacea* in 20 ppt was the best, followed by 30 ppt, and 10 ppt, both in non-ablated and ablated crabs. As for the maturation stages determination, overall results indicated that salinity affect maturation ($p < 0.05$) of *S. olivacea*, with 20 ppt produced the highest number of Stage 4 ovary based on colouration, GSI, and produce the largest oocyte (114.01 ± 81.81 μm) and hepato-tubule diameter (276.14 ± 61.16 μm) following 60-day treatments period. Meanwhile, the glucose level was not affected ($p > 0.05$) either in wild crabs (control) or salinity treated crabs. Hormones influence the ovary maturation of *S. olivacea*, with 17β -estradiol recorded as the best treatment, followed by 17α -hydroxyprogesterone, and progesterone. ELISA analysis indicated that those hormones were detected in hemolymph, ovary, and hepatopancreas. The hormone levels in hepatopancreas recorded as the highest (0.84 ± 0.05 pg/ml - 17β -estradiol; 0.86 ± 0.04 pg/ml - progesterone; 0.72 ± 0.03 pg/ml - 17α -hydroxyprogesterone) compared to the ovary and hemolymph, suggesting that the hepatopancreas may act as the main regulatory organ for reproductive hormone regulation in *S. olivacea*. Overall, both salinity and hormones does affect maturation stages of *S. olivacea*. Meanwhile, salinity may not directly affect moulting and only supports the effects of limb autotomy. Limb autotomy plays a role in modifying the hormone levels that regulate growth and moulting of *S. olivacea*.

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**KESAN SALINITI AIR, AUTOTOMI ANGGOTA DAN HORMON
PEMBIAKAN TERHADAP BERSALIN KULIT DAN PRESTASI
KEMATANGAN KETAM SEPIT MERAH BETINA, *Scylla olivacea* (HERBST,
1796)**

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Objektif kajian ini ialah (1) untuk menentukan kadar pertambahan berat badan dan lebar cengkerang, pengesahan ovari Peringkat 1 bagi ketam bersalin kulit, dan kesan saliniti air terhadap kelangsungan hidup *Scylla olivacea* dalam kurungan, (2) untuk mengenalpasti kesan saliniti air dan pemotongan kaki terhadap prestasi bersalin kulit, peringkat kematangan, dan kepekatan glukosa, dan (3) untuk mengkaji kesan hormon pembiakan terhadap peringkat kematangan dan paras hormon ketam *S. olivacea*. Sampel ketam *S. olivacea* ditangkap dari Tanah Bencah Setiu, Terengganu, di Pantai Timur Semenanjung Malaysia (5°40'47.93"N 102°42'45.04"E) dari Julai 2015 sehingga Julai 2019. Dapatan kajian mendapati 135 ketam (baru bersalin kulit) sememangnya berada

dalam ovary Peringkat 1 (warna dan histologi). Terdapat hubungan yang positif ($r = 0.263$, $p = 0.001$) diantara lebar cengkerang dan berat badan selepas ketam bersalin kulit, dengan purata pertambahan sebanyak 0.82 ± 0.27 cm dan 8.39 ± 2.72 g. Bagi eksperimen toleransi terhadap saliniti air berbeza, dapatan kajian mendapati *S. olivacea* dapat bertoleransi dalam lingkungan 5.14 ppt sehingga 36.67 ppt. Dapatan kajian mendapati prestasi bersalin kulit bagi ketam *S. olivacea* normal dan ketam autotomi dalam 20 ppt merupakan yang terbaik, diikuti oleh 30 ppt, dan 10 ppt. Bagi eksperimen penentuan kadar kematangan, secara keseluruhannya, saliniti didapati memberi kesan terhadap kematangan ($p < 0.05$) *S. olivacea*, dengan 20 ppt merekodkan kadar penghasilan ovary Peringkat 4 tertinggi melalui warna, GSI, saiz diameter oosit ($114.01 \pm 81.81 \mu\text{m}$) dan tubul-hepato ($276.14 \pm 61.16 \mu\text{m}$) terbesar direkodkan sepanjang 60 hari tempoh kajian. Sementara itu, tiada kesan signifikan ($p > 0.05$) direkodkan bagi kepekatan glukosa ketam kawalan dan juga ketam rawatan saliniti. Hormon mendorong perkembangan ovary *S. olivacea*, dengan 17β -estradiol direkodkan sebagai rawatan terbaik, diikuti oleh 17α -hydroxyprogesteron, dan progesteron. Analisis ELISA mendapati setiap hormon dikesan di dalam darah, ovary dan hepatopankreas. Kadar hormon dalam hepatopankreas mencatat kadar tertinggi (0.84 ± 0.05 pg/ml - 17β -estradiol; 0.86 ± 0.04 pg/ml - progesteron; 0.72 ± 0.03 pg/ml - 17α -hydroxyprogesteron) berbanding ovary dan darah, lantas memberi andaian bahawasanya hepatopankreas bertindak sebagai organ utama penghasilan hormon pembiakan bagi *S. olivacea*. Secara keseluruhannya, saliniti dan hormon mempengaruhi tahap kematangan *S. olivacea*. Sementara itu, saliniti mungkin tidak mempengaruhi persalinan kulit secara langsung dan hanya menyokong kesan pemotongan kaki. Pemotongan kaki berperanan dalam mengubah paras hormon yang mengatur perkembangan dan persalinan kulit ketam *S. olivacea*.