

**BIOLOGY AND CULTURE OF A TROPICAL HARPACTICOID COPEPOD,
NITOCRA AFFINIS CALIFORNICA LANG**

By

HAZEL MONICA MATIAS-PERALTA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Science**

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Dedication

For my husband Eduardo and my son Zeckiel.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Masters of Science

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Chairman: Fatimah Md. Yusoff, Ph.D.

Faculty : Science and Environmental Studies

The complete post-embryonic development of *Nitocra affinis* was studied on specimens cultured under normal laboratory conditions (30°C and 12:12hours L:D cycle). Egg sac is produced 1-2 days (0.95 ± 0.08 days) following a successful insemination. Female produced an average of 7.0 ± 0.0 broods, minimum of 6.0 ± 0.0 and a maximum of 8.0 ± 0.0 . The average number of eggs per sac was 18.3 ± 0.25 (ranges from 12-25 eggs per sac). The egg sacs were carried for 18-29 hours or 22.8 ± 2.0 hours on average. Development of nauplii (stages NI through NVI) is completed within 4-5 days (4.66 ± 0.06 days) while copepodid development (stages CI through CVI) occurred within 5-7 days (6.21 ± 0.15 days). The total generation time (egg to adult) was 9-12 days (10.91 ± 0.18 days). Body length of nauplii ranged from 65.4 ± 0.6 μm (NI) to 214.1 ± 2.8 μm (NVI) while copepodid ranged from 235.2 ± 2.9 μm (CI) to 389.6 ± 7.2 μm (CVI or adult).

0Usin0g different food items (three different algal species; *Chaetoceros calcitrans*, *Nannochloropsis oculata* and *Tetraselmis tetrathele* and their combinations; and three artificial food; baker's yeast, shrimp feed and rice bran) and feeding densities (10^4 , 10^5 , 10^6 and 10^7 cells/ml for algal food and 0.02, 0.04 and 0.06 mg/ml for artificial food), the growth and development of *N. affinis* were studied. In both single and combined algal food, the highest ($p < 0.05$) egg and copepod production/female was achieved using the density 10^6 cells/ml whereas 0.06 mg/ml was the best ($p < 0.05$) feeding for artificial diet. The highest ($p < 0.05$) fecundity was achieved with copepod fed with mixed algal diet with an average of 18.3 ± 0.3 eggs/sac. The highest ($p < 0.05$) offspring production was achieved with *N. affinis* fed *C. calcitrans* (with mean total production reaching a maximum of 132.0 ± 3.8 offspring/female), followed by those fed with mixed (124.8 ± 1.7 offspring/female) and combination of *C. calcitrans*:*T. tetrathele* (113.0 ± 1.3 offspring/female). Likewise the highest ($p < 0.05$) survival from nauplii to adult (97-98 %) and highest ($p < 0.05$) maximum specific growth rate ($K = 0.43 \pm 0.0$) was achieved by copepod fed algal food *C. calcitrans*, and all its combinations. The shortest ($p < 0.05$) time for egg maturation and time interval between egg sac was found in the *T. tetrathele* fed copepod. Likewise the shortest ($p < 0.05$) development time from nauplii to copepodid and copepodid to adult resulted from feeding with *T. tetrathele*.

The effects of some environmental parameters (salinity, temperature, and light conditions) on the reproduction and development of harpacticoid copepod, *N. affinis* under controlled laboratory conditions were determined. Seven different salinity levels (5-35 ppt) four different temperatures (20-35 °C) three light intensities (25-130 $\mu\text{mol}/\text{m}^2/\text{s}$) and three different photoperiods were employed in this study. The highest ($p < 0.05$) reproduction and fastest development time were achieved by copepods reared under 30-35 ppt salinity and lowest light intensity (25 $\mu\text{mol}/\text{m}^2/\text{s}$). The optimum temperature for the maximum reproduction was 30°C while development time was shortest ($p < 0.05$) and maximum length achieved by adult copepods was longest ($p < 0.05$) under 30°C and 35 °C compared to other temperature levels. Continuous light (24h:0h LD) inhibited the egg production while continuous darkness (1h:23h LD) and 12h:12h LD significantly favoured the overall reproductive activity of the female. Photoperiods 1h:23h and 12h:12h LD yielded highest total ($p < 0.05$) offspring/female coupled with highest ($p < 0.05$) survival percentage and longest ($p < 0.05$) total body length of copepods.

A study to describe a relatively simple and reliable small scale mass production and the nutritional composition of *N. affinis* was undertaken. The result confirmed the feasibility of maintaining *N. affinis* on a small scale for a long period. Within six weeks (42 days) operations a minimum harvest of 87.2×10^3 copepods/2L and maximum of 89.0×10^3 copepods/2L was obtained. Using several numbers

of basins for mass production it was feasible to harvest 30×10^3 copepods/2L daily after two weeks of inoculation. *Nitocra affinis* has a great potential as live food because they contain high protein (39-52%), lipid (13-23%) and carbohydrates (8-11%). It also contains high level of n-3 HUFA (up to 44%) and n-6 HUFA (up to 14%). In addition it contains high amount of DHA (up to 19%) and EPA (up to 25%). Moreover, levels and ratios of fatty acids closely match both the requirements of marine finfish larvae and of marine shrimp.

Survival and specific growth rate of *Penaeus monodon* larvae (postlarvae stage 1-15) fed with different live feeds (*Artemia* nauplii and *N. affinis*) and artificial diet were evaluated. The highest ($p < 0.05$) survival was achieved by shrimp larvae fed with *N. affinis* (60.6%), followed by those fed with artificial diet (43.0%) and a combination of *Artemia* nauplii and *N. affinis* (4.1%). The larvae fed with *Artemia* nauplii suffered high mortality probably due to the abrupt change from algal diet to a diet of relatively large *Artemia* nauplii. Likewise, highest ($p < 0.05$) specific growth rate was achieved by shrimp fed with *N. affinis* followed by shrimp fed artificial diet.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

BIOLOGI DAN KULTUR KOPEPOD HARPACTIKOID TROPIKA, *NITOCRA AFFINIS CALIFORNICA* LANG

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Perkembangan post-embriionik bagi *Nitocra affinis* di nyatakan dan diillustrasikan dengan lengkap berdasarkan spesimen yang dikultur di bawah keadaan laboratorii yang normal. Pundi telur dihasilkan 1-2 hari (0.95 ± 0.08 hari) diikuti dengan pernian yang berjaya. Betina menghasilkan purata penetasan 7.0 ± 0.0 , dengan kadar minimum 6.0 ± 0.0 dan kadar maksimum 8.0 ± 0.0 . Purata nombor kadar telur per pundi adalah 18.3 ± 0.25 (dengan julat 12-25 telur untuk setiap pundi). Pundi telur telah dikaji selama 18-29 jam atau 22.8 ± 2.0 jam secara purata. Pertumbuhan nauplii (tahap NI hingga NVI) selesai diantara 4-5 hari (4.66 ± 0.06 hari) manakala pertumbuhan kopepodid (tahap CI hingga CVI) muncul diantara 5-7 hari (6.21 ± 0.15 hari). Jumlah masa pertumbuhan (telur ke dewasa) adalah 9-12 hari (10.91 ± 0.18 hari). Ukuran panjang badan nauplii

berjulat dari $65.4 \pm 0.6 \mu\text{m}$ (NI) ke $214.1 \pm 2.8 \mu\text{m}$ (NVI) manakala kopepodid berjulat dari $235.2 \pm 2.9 \mu\text{m}$ (CI) ke $389.6 \pm 7.2 \mu\text{m}$ (CVI atau dewasa).

Menggunakan makanan yang berlainan (3 jenis spesies alga yang berbeza; *Chaetoceros calcitrans*, *Nannochloropsis oculata* dan *Tetraselmis tetraethele* dan kombinasinya; tiga makanan tiruan, yis baker, makanan udang dan bran beras) dengan kepekatan pemakanan (10^4 , 10^5 , 10^6 , 10^7 sel/ml untuk makanan alga dan 0.02, 0.04 dan 0.06 mg/ml untuk makanan tiruan), pertumbuhan dan perkembangan *N. affinis* dikaji. Di dalam kedua-dua, samada satu atau makanan alga yang telah dicampur, telur dan penghasilan kopepod/betina yang tertinggi telah diperolehi ($p < 0.05$) dengan menggunakan kepekatan 10^6 sel/ml dan 0.06 mg/ml merupakan yang terbaik ($p < 0.05$) untuk pemakanan bagi diet tiruan. Kesuburan yang tertinggi ($p < 0.05$) diperolehi dengan kopepod yang telah diberi makan diet alga yang dicampur dengan purata 18.3 ± 0.3 telur/pundi. Ini diikuti dengan kopepod yang telah diberi makan *C. calcitrans* dan yis dengan purata 16.7 ± 0.5 dan 15.1 ± 0.8 telur/pundi. Penghasilan anak yang tertinggi ($p < 0.05$) diperolehi pada *N. affinis* yang diberi makan *C. calcitrans* dengan total min penghasilan mencapai maksimum 132.0 ± 3.8 anak/betina, diikuti 124.8 ± 1.7 dan 113.0 ± 1.3 anak/betina, untuk campuran *C. calcitrans* dan *C. calcitrans:T. tetraethele*. Begitu juga dengan kebolehan untuk terus hidup yang tertinggi ($p < 0.05$) dari nauplii hingga dewasa (97-98%) dan kadar pertumbuhan spesifik maksimum ($K = 0.43 \pm 0.0$) yang tertinggi ($p < 0.05$) adalah diperolehi melalui kopepod yang telah diberi makan alga *C. calcitrans* dan kombinasinya. Masa penghasilan telur matang dan selang masa di antara pundi telur yang terpendek

($p < 0.05$) telah dijumpai pada kopepod yang telah diberi makan dengan *T. tetrathele*. Begitu juga dengan perkembangan masa yang terpendek ($p < 0.05$) dari nauplii kepada kopepodid dan dari kopepodid ke dewasa adalah terhasil dari pemakanan *T. tetrathele*.

Kesan beberapa parameter persekitaran (saliniti, suhu dan kesan cahaya) pada pembiakan dan perkembangan kopepod harpactikoid, *N. affinis* di bawah keadaan laboratorii yang terkawal telah diperolehi. Tujuh tahap saliniti yang berbeza seperti (5-35 ppt) 4 dari suhu yang berbeza (20°C - 35°C) 3 tahap kesan cahaya yang berbeza (25 - $130 \mu\text{mol}/\text{m}^2/\text{s}$) dan 3 tahap jangkamasa cahaya telah dijalankan di dalam kajian ini. Kadar pembiakan yang tertinggi ($p < 0.05$) dan masa pertumbuhan dan perkembangan tercepat dari segi saiz telah diperolehi pada kopepod yang diberi saliniti 30-35 ppt. Suhu yang paling optima yang diperlukan untuk pembiakan yang maksimum adalah 30°C manakala dibawah suhu 30°C dan 35°C , masa perkembangan kopepod adalah yang terpendek ($p < 0.05$) dan kepanjangan maksimum telah diperolehi pada kopepod yang dewasa ($p < 0.05$) dan kadar perkembangan *N. affinis* terendah ($p < 0.05$) adalah pada intensiti cahaya ($25 \mu\text{mol}/\text{m}^2/\text{s}$). Cahaya yang berterusan (24h:0h LD) merencat penghasilan telur manakala gelap yang berterusan (1h:23h LD) dan 12h:12h LD secara signifikannya sesuai untuk keseluruhan aktiviti pembiakan untuk betina. Jangkamasa cahaya 1h:23h LD menghasilkan jumlah tertinggi ($p < 0.05$) anak/betina dengan peratus kebolehhidupan yang tertinggi ($p < 0.05$)

dan jumlah kepanjangan badan yang terpanjang ($p < 0.05$) bagi copepod. Kajian ini mendapati walaupun *N. affinis* boleh menerima julat yang besar untuk keadaan persekitaran yang berbeza, pendedahan yang berterusan kepada keadaan yang subnormal akan memberi kesan pada pembiakan dan perkembangannya.

Satu kajian untuk menggambarkan satu sistem skala kecil dan ringkas bagi penghasilan *N. affinis* telah dilakukan. Keputusan mengesahkan keboleh laksanaan untuk mengkultur *N. affinis* pada skala yang kecil untuk jangkamasa yang lama. Dalam masa enam minggu (42 hari), operasi menuai yang minimum 87.2×10^3 copepods/2L dan maksimum 89.0×10^3 copepods/2L telah diperolehi. *Nitocra affinis* berpotensi sebagai makanan hidup kerana ia mengandungi protein yang tinggi (39-52%), lipid (13-23%) dan karbohidrat (8-11%). Ia juga mempunyai kandungan n-3 HUFA yang tinggi (sehingga 44%) dan n-6 HUFA (sehingga 14%). Spesies tersebut juga tidak ketinggalan mempunyai kandungan DHA (sehingga 19%) dan EPA (sehingga 25%) yang tinggi. Sebagai tambahan, paras serta nisbah asid lemak hampir menyamai keperluan kedua-dua larva ikan marin dan serta udang air masin

Kebolehhidupan dan kadar pertumbuhan spesifik larva *Penaeus monodon* (tahap post larva 1-15) yang telah diberi makanan dengan pemakanan hidup yang

berbeza (*Artemia* dan *N. affinis*) dan diet tiruan telah dikaji. Kebolehidupan yang tertinggi ($p < 0.05$) telah diperolehi pada larva udang yang telah diberi makan *N. affinis* (60.6%) diikuti dengan udang yang telah diberi makan diet tiruan (43.0%) dan kombinasi *Artemia* dan *N. affinis* (4.1%). Larva yang telah diberi makan *Artemia* mengalami kematian yang tinggi mungkin kerana perubahan mendadak dan diet alga kepada diet yang sebahagian besar yang terdiri daripada *Artemia*. Begitu juga, kadar pertumbuhan spesifik yang tertinggi ($p < 0.05$) diperolehi oleh udang yang telah diberi makan dengan *N. affinis* diikuti dengan udang yang diberi makan diet tiruan.

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I certify that an Examination committee met on October 19, 2004 to conduct the final examination of Hazel Monica Matias-Peralta on her degree thesis entitled "Biology and Culture of a Tropical Harpacticoid Copepod, *Nitocra affinis californica* Lang" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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Date: 20th October 2004

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