SOME ASPECTS ON THE BIOLOGY OF *MESOCYCLOPS ASPERICONIS* (COPEPODA, CYCLOPOIDA) AND ITS EFFICIENCY IN THE CONTROL OF VECTOR MOSQUITO LARVAE.

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BEBERAPA ASPEK BIOLOGI *MESOCYCLOPS ASPERICONIS* (COPEPODA, CYCLOPOIDA) DAN KEBOLEHANNYA DALAM KAWALAN LARVA NYAMUK VEKTOR

ABSTRAK

Suatu kajian mengenai taksonomi dan biologi *Mesocyclops aspericonis* (Copepoda: Cyclopida) dan efikasinya sebagai agen kawalan nyamuk vektor telah dijalankan.

Mesocylops aspericonis yang telah disampel daripada takungan air di sawah padi telah menunjukkan perkembangan dalam kedua-dua media kultur yang disediakan untuk kajian ini, iaitu media air tawar yang disediakan bersama beberapa bahan kimia dan media yang disediakan bersama daun-daun kering. Apabila kopepod ini dimasukkan dalam kedua-dua media dalam bilangan 20, 15 dan 10, media kultur air tawar merupakan media yang telah menghasilkan populasi yang tinggi berbanding dengan media kultur daun-daun. Walau bagaimanapun, media kultur daun-daun telah menunjukkan penghasilan kopepod yang lebih signifikan apabila 5 ekor dan seekor kopepod yang gravid dimasukkan.

Kajian juga telah dijalankan dalam dua keadaan iaitu di dalam makmal dan juga di lapangan. *Mesocyclops aspericonis* telah membuktikan kebolehan dan kecenderungannya sebagai pemangsa terhadap larva nyamuk instar pertama dan kedua empat spesies nyamuk vekltor iaitu *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus* dan *Anopheles sp.* dalam kajian ini. Kopepod ini telah diperhatikan menyerang dan memakan larva-larva nyamuk sebagai makanan mereka.

Kopepod ini juga telah mengurangkan populasi larva instar ketiga dan keempat *Aedes albopictus* setelah sebulan di dalam kajian lapangan yang menggunakan tayar-tayar lama sebagai bekas pembiakannya.

Dalam kajian tentang mangsa yang lebih digemari oleh spesies kopepod ini, didapati bahawa secara amnya, kopepod ini menyerang kesemua empat spesies *nyamuk yang dikaji. Walau bagaimanapun, Mesocyclops aspericonis lebih menggemari Aedes aegypti* berbanding *Anopheles sp.* tetapi lebih menggemari *Culex quinquefasciatus* dan *Aedes albopictus* apabila digandingkan bersama *Aedes aegypti.* Pemangsa ini lebih menggemari *Aedes albopictus* berbanding *Culex quinquefasciatus* tetapi lebih menggemari *Anopheles sp.* berbanding dengan *Aedes albopictus.* Dalam kombinasi *Culex quinquefasciatus* dan *Anopheles sp.*, kopepod ini lebih menggemari *Culex quinquefasciatus.*

SOME ASPECTS ON THE BIOLOGY OF *MESOCYCLOPS ASPERICONIS* (COPEPODA: CYCLOPOIDA) AND ITS EFFICIENCY IN THE CONTROL OF VECTOR MOSQUITO LARVAE.

ABSTRACT

Mesocyclops aspericonis from the family Cyclopoida is a freshwater free-living copepod and was studied in this research. This cyclopoid is an efficient biological agent of vector mosquito larvae. The biology and its efficiency as a biological agent of mosquito larvae of this cyclopoid were examined.

Through out this study, only one species was obtained, the *Mesocyclops aspericonis* (Daday, 1906). The behaviour and the biology of this species were observed through out the study.

The production of *Mesocylops aspericonis* that was obtained from the sampling freshwater of paddy fields showed significant in both culture media that was prepared. When they were placed in groups of 20, 15 and 10 in each media, the freshwater media was the appropriate media in the production of the cyclopoids. Leaf foliage media was the best media in the culture when they were grouped in 5 and when only 1 gravid cyclopoid was placed.

The copepods were confirmed as efficient biological agents of vector mosquitoes in this study. They were tested in both laboratory and field conditions. They were proved to be vicious predators of first and second instars of all the four vector mosquito species; *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus* and *Anopheles sp.* that were tested. This tiny organism was seen maiming and eating the larvae as its food source. The *Mesocyclops aspericonis* suppressed the amount of third and fourth instars of the *Aedes albopictus* larvae in the field trial using tires as the artificial breeding containers, after a month's evaluation.

Finally, in the prey preference test, the cylopoids preyed on all the four species efficiently. *Mesocylops aspericonis* preferred *Aedes aegypti* compared to *Anopheles sp.* but preferred *Culex quinquefasciatus* and *Aedes albopictus* in the combinations with *Aedes aegypti*. The predator prefers *Aedes albopictus* compared to *Culex quinquefasciatus* but it prefers *Anopheles sp.* compared to *Aedes albopictus*. Lastly, in the combination of *Culex quinquefasciatus* and *Anopheles sp.*, it preferred *Culex quinquefasciatus*.

CHAPTER 1

General Introduction

GENERAL INTRODUCTION

Mesocylops aspericonis (Daday, 1906) (Copepoda, Cyclopoida) is a very unique and important micro-organism which is known for its vital role in the control of vector mosquito larvae. Loss of lives due to mosquito borne diseases such as malaria and dengue vectored by these small yet dangerous mosquitoes have been increasing day by day all over the world.

Mosquitoes especially *Culex* and *Aedes* have existed for 26-38 million years and they have adapted well to many environmental changes. Therefore, it's impossible to create a mosquito free environment but its definitely possible to achieve a mosquito safe environment.

Cyclopoids along with their other family members such as *Acanthocyclops vernalis*, *Macrocyclops albidus*, *Mesocyclops edax* and many more are very important potential predators' of mosquito larvae. They are very efficient bio-control agents. Their perfect adaptation in all adverse situations makes them easy organisms to be maintained in both field and laboratory conditions.

The successful widespread use of biological control agents against mosquitoes requires a much better understanding of the ecology of predator-prey and pathogen-host relationships (Service, 1983). The opportunistic characteristics of many species are their ability to exploit temporary habitats and great dispersal potential. Mosquitoes typically exploit many aquatic habitats. Often a biological control agent has a much narrower range of environmental activity than the target mosquito has. Thus, in many situations a number of different biological control agents and appropriate methods will be necessary to control vector mosquitoes across its range of exploitable breeding sources.

Biological control agents such as these copepods should be considered a set of tools that a mosquito control program can use when it is economically practical. When combined with conventional chemicals such as the *Bacillus thuringiensis* var. *israelensis* and physical control procedures, bio-control agents can provide short and occasionally a long term control.

One advantage of bio-control agents is host specificity. The cyclopoid copepods mainly prey on mosquito larvae and other tiny microorganisms. This factor affords minimal disturbance to non-target species and to the environment. Ironically, it is this specificity that deters commercialization and application of these bio-controls, in addition to the generally narrow market for industry, increased outlays of capital and the training required for personnel for mosquito control programs. However, in the future societal changes such as environmental awareness are likely to increase the interest in the use of these agents. Thus, increased knowledge of alternative control strategies such as these bio-control agents is.

Besides the cyclopoids, several species of fish are used for the biological control of mosquitoes and these species together form the major successes in the field. Unfortunately, their usefulness is limited to more permanent bodies of water and even under these situations, their impact on the target species has been only partially successful. The *Gambusia affinis* is the best known fish for mosquito control. *Romanomermis culicivorax* is the pathogenic nematode for mosquitoes. This nematode is active against a wide range of mosquito species, has been mass produced and has been utilized in a number of field trials. This species was commercially produced and sold under the name, 'Skeeter Doom'. However, the eggs showed reduced viability in transport and therefore the product is no longer sold (Service, 1983).

The ability of certain cyclopoid copepods to destroy larval mosquitoes was noted in 1938 by Hurlbut. These tiny microcrustaceans were seen preying on newly hatched larvae. Field predations in Rongaroa (French Polynesia) later demonstrated that Mesocyclops can be used in larvicidal interventions against *Aedes aegypti* and *Aedes polynesiensis*

(Riviere, et al. 1987b). These crab hole applications reduced the abundance of adult *Aedes polynesiensis* by nearly 76%. In Colombia, the abundance of copepods in natural settings inversely correlates with the abundance of larval anopheline mosquitoes (Marten, 1989).

The container-breeding mosquito, *Aedes aegypti*, is the major global vector of dengue viruses, causing around 50 million infections annually. Brian Kay and Vu Sinh Nam from The Queensland Institute of Medical Research, Australia have developed a mosquito control strategy from 1998-2003, incorporating four elements: (1) a combined vertical and horizontal approach that depends on community understanding; (2) prioritised control according to the larval productivity of major habitat types; (3) use of predacious copepods of the genus *Mesocyclops* as a biological control agent; (4) community activities of health volunteers, schools, and the public.

Mesocyclops managed to eliminate Aedes from 32 of 37 community. As a result, no dengue cases have been detected in any commune since 2002. These findings suggested that this strategy is sustainable in Vietnam and applicable in all breeding sites of vector mosquitos.

This method is low in cost as *Mesocyclops* are available locally, have a high predacious capacity, are easy to be inoculated and released, and can survive for a long time. *Mesocyclops* are especially appropriate for large containers like cement cisterns, wells, steel tanks and clay pots (of big size). In combination with the community recycling it, *Mesocyclops* is an easy and inexpensive method of vector mosquito control that should be effective in this country.

Hence, this dissertation aims to explain the taxonomy, biology and the efficiency of this specific cyclopoid, *Mesocylops aspericonis* in the control of vector mosquito larvae in the field and laboratory conditions. Thus, four important objectives are outlined in this investigation.

1. To determine the species and to study the biology of predacious copepods as potential bio-control agents in Malaysia.
2. To determine the most efficient media for the culture of *Mesocyclops aspericonis.*
3. To evaluate the efficiency of *Mesocyclops aspericonis* as a biological control agent against vector mosquitoes in the laboratory and field predations.
4. To determine the species of mosquito larvae most preferred by the *Mesocyclops aspericonis.*

Information obtained from this study can be used in developing and implementing a successful biological control for vector mosquito larvae in Malaysia.

CHAPTER 2

Literature Review

2.1 Biology of copepods

2.1.1 Distribution of copepods

The name copepod (Edwards, 1840) is derived from the Greek word 'oar' and 'podus' which means 'hope foot'. This name refers to their broad and paddle like swimming legs. Copepods are aquatic crustaceans and they are also known as minute relatives of the crabs and shrimps. These petite creatures are abundant in most marine habitats. There are nearly 14,000 species identified that successfully colonised all aquatic regimes from freshwater to marine and hyper-saline inland waters and all temperature regimes from polar waters up to hot springs. They also have an enormous vertical range existing from depths of 9995-10002 metres in the Philippine Trench (Wolff, 1981) to an altitude of 5540 metres up in the Himalayan mountains (Loffler, 1968).

Their habitats vary from marine plankton, freshwater plankton, marine sediments, plant associates, hidden habitats (discarded tyres), subterranean habitats, deep sea and also among animal associates. Copepods are particularly abundant in forest litter even at high altitudes. They often colonise water tanks, farms and buildings providing water is available. Based on studies, flourishing populations of freshwater copepods were discovered inhabiting the roof of the National History Museum in London (Reid, 1986).

The sheer abundance of copepods in marine plankton is inexpressible. Sir Alister Hardy (1970) estimated that the copepods are the most numerous animals in the world, even outnumbering the insects which have more species.

Copepods are also abundant in freshwater planktonic communities. Members of the families Cyclopidae: Cyclopoida, Canthocamptida, Harpacticoida, Diaptomidae and Calanoida are particularly successful in all kinds of freshwater habitats varying from the saline lakes in the Antarctic Vestfold Hills (Burton & Hamond, 1981) to the high altitude lakes on the southern slopes of the Himalayan mountains (Loffler, 1968). Harpaticoid copepods are usually benthonic and rarely found in the plankton habitat. Species such as *Acanthocyclops robustus, Diacyclops thomasi, Mesocyclops edax*, *Tropocyclops prasinus mexicanus, Cyclops strenuus*, and *Cyclops scutifer* can be abundant in the offshore waters of large lakes, seemingly without any orientation to the bottom (Loffler, 1968). Species such as *Mesocyclops americanus* and *Megacyclops fuscus* may spend more time in the water column, whereas species such as *Microcyclops rubellus* and *Ectocyclops phaleratus* spend more time attached to sediments or plant surfaces (Burton & Hammond, 1981).

Copepods also live in marine sediments, inhabiting the microscopic spaces between the sediment particles. In this community they are naturally second in abundance only to nematodes. These copepods tend to become more abundant as the particle size of the sediment increases and in coarse sands they often outnumber the nematodes (Hicks & Coull, 1983). They are found in all sediment types from mud to sand and at all depths from the inter-tidal zone to the deepest oceanic ooze. The density changes with sediment type and with depth.

Other habitats exploited by free living copepods are damp terrestrial situations. Reid (1986) surveyed many of these cryptic habitats. In the wet organic soil in tropical South America, she discovered densities ranging from 1,000 to 178,000 per square metre. Copepods are particularly abundant in forest litter, even at high altitude. Sphagnum bogs and terrestrial mosses are also preferential habitats for copepods. They often colonise water tanks in farm and other buildings and are frequently taken in drinking water. Copepods have been reported from even more bizarre habitats, such as the pools between the leaves of bromeliads in tropical rainforests. *Phyllognathopus viguieri* (Maupas) is commonly found in the liquid retained at the bases of leaves of pineapples in Botanic Gardens (Lowndes, 1931) and in supermarkets in U.S.A. The cyclopoid *Cryptocyclops anninae* was first collected from water contained in empty coconut shells (Lowndes, 1928). Yeatman (1983) also surveyed extraordinary microhabitats in some South Pacific Islands and reported copepods from taro leaf axils, tree holes, crab burrows and discarded car tyres. They even occur in hot springs, where they are active at water temperatures between 38 and 58°C (Itô & Burton, 1980).

2.1.2 Basic morphology of copepods

Copepods are very ancient arthropods. They poorly fossilised, and thus it is rare to discover such traces of their remains in sediments which could have facilitated the study of their morphological, physiological, and ecological evolution (Frey, 1964). Copepods are from the class, Crustacea and they are microcrustaceans according to the Bowman and Abele classification, 1971. The subclass of copepods is Copepoda (Edwards, 1840) and consists of over 7500 free-living and parasitic species.

Fossil records verify that copepods contain the primary pelagic radiation and also display several independent parasitic evolutions. The fossil records of copepods are sparse thus limited. Only a few free living copepod fossils are known (Palmer, 1969). Harding (1956) described a harpacticoid copepod, *Enhydrosoma gariene*, from southern England. Though, the single male specimen was shriveled rather than fossilised and he was able to re-hydrate it prior to study. The first true fossils found were the harpacticoids and cyclopoids reported by Palmer (1960, 1969). One of these forms was identified as a *Cletocamptus Schmankewitsch* species, the others were classified only to ordinal level. The most spectacular fossil copepod is undoubtedly *Kabatarina pattersoni* a fish parasite from the Lower Cretaceous period (Cressey & Patterson, 1973; Cressey & Boxshall, 1989). The copepods are preserved as solid objects and are in excellent condition, complete with appendages bearing spines, setae and surface ornamentation This discovery considerably extends the known fossil record of the Copepoda.

Copepods are typically very small creatures. The marine planktonic forms, total body length is usually between 0.5 to 5.0mm. The benthic copepods from the Harpaticoidae family fall within the range 0.2 to 2.5mm (Manton, 1977). The adult body of a free living copepod could be divided into a wide anterior end, the prosome (cephalosome and metasome), narrow posterior end and finally the urosome (Plate 2.1). The cephalosome consists of 5 cephalic somites and the first thoracic somite bears the maxilipeds.

With the exemption of the antennules, all copepods appendages are basically biramous where each consists of a basal protopod and two terminal rami, an internal endopod and an external exopod. The protopod maybe divided into a coxa and basis and may have lateral exites or mesial endites protrusion (Manton, 1977; McLaughlin, 1980). Exites or epipods as they are called usually develop as the principal respiratory structures and may develop into highly specialized gills. They normally aid in directing water flow beneath carapace.

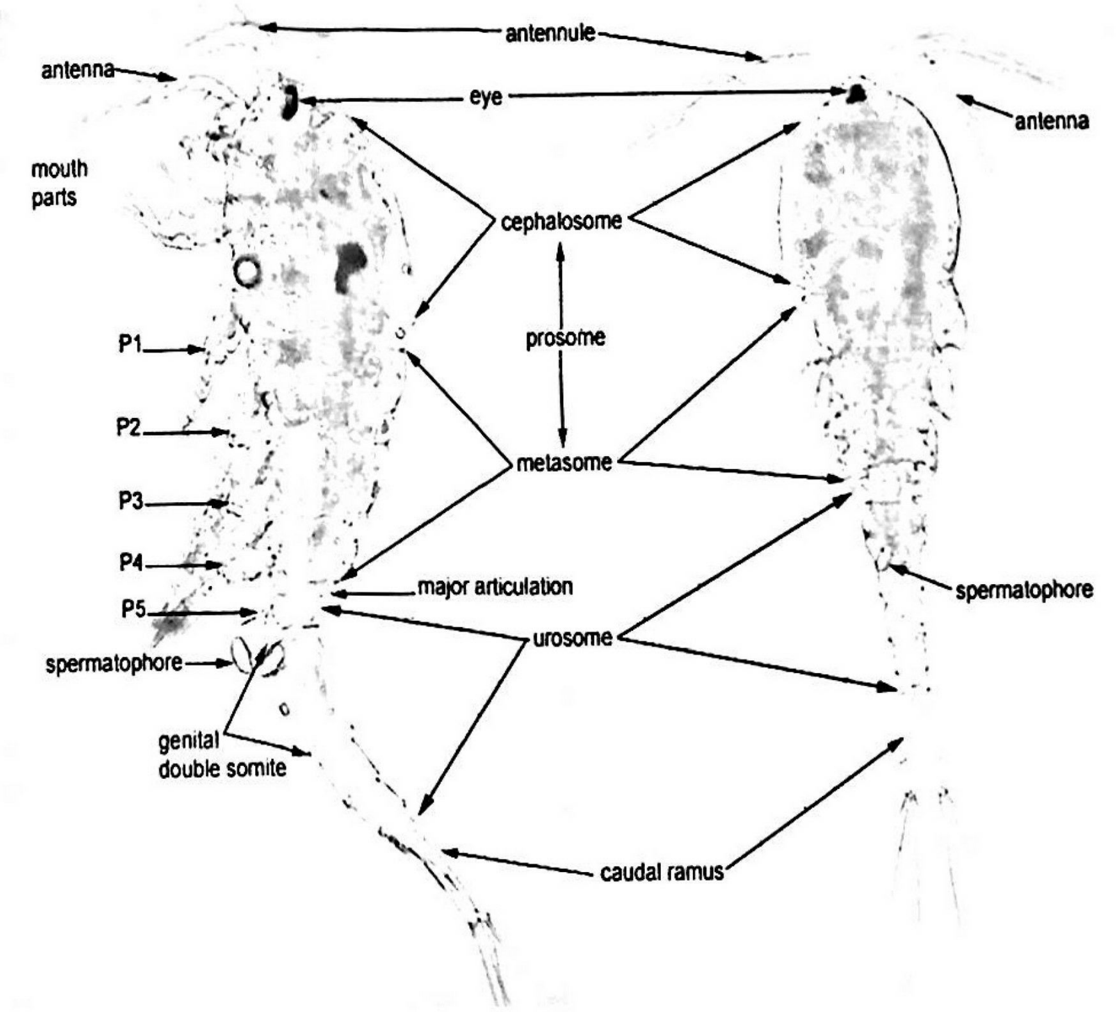

Plate 2.1: Basic morphology of the Copepod (Cyclopoid) copepod
P1: First pair legs; P2 : Second pair legs; P3: Third pair legs; P4: Fourth pair legs; P5: Fifth pair legs **(Adapted from the www.worldof copepods.com)**

Endites at the mouth play an important role in the movement of food and are variously developed and adapted for these specific purpose. The adaptations include teeth and denticles, spines and spinules that bristle and simple along with pinnate, plumose, pectinate and serrate setae. Basically, endites form as outgrowths of the mesial border of the protopod (coxa or basis) even though enditic processes are occasionally developed from the border of the endopodal ischium (Moore and McCormick, 1969).

Endopods are well developed and consists of several divisions. A variety of modifications have distorted the endopods into sensory, feeding, burrowing and swimming limbs (Moore and McCormick, 1969). These modifications have been made possible by the hardening of the exoskeleton to give rigidity and support to the appendages where the segments have become joined by flexible membranes. The freedom of movement is provided by the joints. They also proved that exopods play vital roles as the swimming limb except when it is modified for feeding or water movement.

The copepod abdomen usually terminates in a telson or a terminal lobe. Debate has flared sporadically as to whether or not the telson can reasonably be called a body somite (Sharov, 1966; Bowman, 1971; Schminke, 1976). Embryological research has verified that the region of growth lies between the telson and the prior body somite. The telson usually carries paired appendage like structures, the caudal rami or also called as caudal furca. The anus usually opens onto the telson either terminally or ventrally (Anderson, 1973).

Sharov (1966) has proposed that the telson when it has a caudal rami and a terminal anus, is not actually a telson but rather a terminal body somite. Bowman (1971) concurs and using the suggestion thus reclassified the structures related to the terminal lobe or segment. If the anus is terminal, the lobe would be the last anal somite and its appendage like structures will not be caudal rami but uropods. Schminke (1976) opposed strongly with this interpretation and presented evidence to attest the position of the anus as being variable during its development. However, until the Sharov-Bowman proposition can be more substantiated the appendage like structures linked to the terminal segment is maintained as caudal rami or caudal furca.

The antennules are the only embryonic pre-oral appendages. The antennules are well developed and are also multi articulate. One or both the antennules may be modified for grasping in the males for the mating purposes. Each antennule consists of two segments, the distal segment being enlarged. Each segment is equipped with one or more spines with a short flagellum usually present (Anderson, 1973).

The antennae functions in both feeding and locomotion. The male antenna is specialized for gripping the female's carapace during copulation (Boxshall, 1990). Antennae may be adapted for a number of other functions as well in both sexes such as burrowing, climbing, propulsion and floatation.

Mandibles compared to any other copepod appendages have always been the topic of structural, functional and phylogenetic investigations (Cannon & Manton, 1927; Manton, 1982b, 1964, 1973a, 1977; Snodgrass, 1951). Maturity of the mandibles has been shown to be an outcome from the enlargement of the proximal endite of each of the first pair of adult appendages. The shape and form of the mandibles is dependant upon the types of food and modes of feeding.

Manton (1977) describes the primordial mandible as a proportioned grinding molar process capable of rolling in either direction. Throughout the advancement, the symmetry has been lost and the molar process has progressed from a simple grinding surface through a cusped surface to one that is toothed. Supplementary specification has come through the development of the incisor process which has provided the basic biting aptitude.

Anderson (1973) has presented embryological evidence to suggest that the maxillae become associated with the cephalon relatively late in development. Maxillae and maxillulae are somewhat similar in morphological structure and functions mainly as feeding appendages and differs from the thoracic appendages. Therefore, the maxillae are treated as cephalic appendages. Both pairs of appendages normally consist of a one or two segmented protopod with well developed endites. One or both rami regularly are reduced or absent. The reduced endopods are referred to as palps. The maxillulae are usually biramous in copepod and usually small and may be prehensile. The maxillae are uniramous and the endopods are provided with dense setae that form filtering screens or scoop nets.

Maxillipeds are two or three cephalic somites combining with the cephalon, and these appendages are adapted for feeding and food handling. One or two uniramous pairs of maxillipeds are usually present in copepods (Anderson, 1973).

Thoracic appendages are relatively unspecialized appendages, each consisting of an undivided protopod with several endites and epipod and two rami. The exopod is two segmented along with the endopod usually with five or six segments. Four or five pairs of thoracic appendages are usually present in copepods. Exopods are variously equipped and ornamented with spines, spinules and setae. Sars (1903) has described the first exopods of many harpaticoids (copepods) are customised as grasping structures. The last pair is well developed and vastly modified in calanoid males, but absent in cyclopoid males as copulatory structures, proficient at grasping and transferring spermatophores (Manton, 1977).

2.1.3 Copepod reproduction

After attaining maturity, copepods begin to produce gamets and then prepare themselves for the mating process. Males generally mature rapidly but their life spans are shorter than the females (Boxshall 1990). Female copepods are able to produce viable embryos within few days of maturation. A single adult female is able to produce a new clutch of eggs every few days, thus several hundred eggs through out her life span.

Copulation is initiated when the male grasps the female's third or fourth leg or urosome with its modified antennules (Plate 2.2). After achieving a good hold, the male rapidly strokes the seminal receptacle of the female with its legs. In this position, the male and female genital segments lie opposite one another and the male attaches his paired spermatophores to the sides of the female's seminal receptacle. Cyclopoid spermatophores range in shape from short and cylindrical to kidney-shaped (Boxshall 1990). The contents of the spermatophores are then transferred by the male into the female and eventually the hollow shells are shed. The entire mating process may take five to twenty minutes. One male can fertilize several females. Some males may guarantee themselves a mate by clasping a juvenile female until she molts into an adult, thereby ensuring his chance at being first on the scene. This is known as precopulatory mate guarding (Boxshall 1990). Females carry two egg sacs, which may contain from 2-100 eggs, laterally on their urosomes. Under normal conditions, eggs will hatch within a few days of being extruded.

Females may produce up to 12-13 clutches of eggs per year, laying a total of 800-1,000 eggs. Female cyclopoids can store sperm for extended periods of time, enabling them to produce fertile clutches of eggs in the absence of males (Smyly, 1970, Whitehouse and Lewis, 1973, Hicks and Coull, 1983). It has been postulated that some species produce resting eggs, but it is not known whether these eggs are able to withstand drying or freezing, or whether they are merely providing a temporary buffer against unfavorable conditions in permanent bodies of water (Lowndes 1930, Robertson 2000)

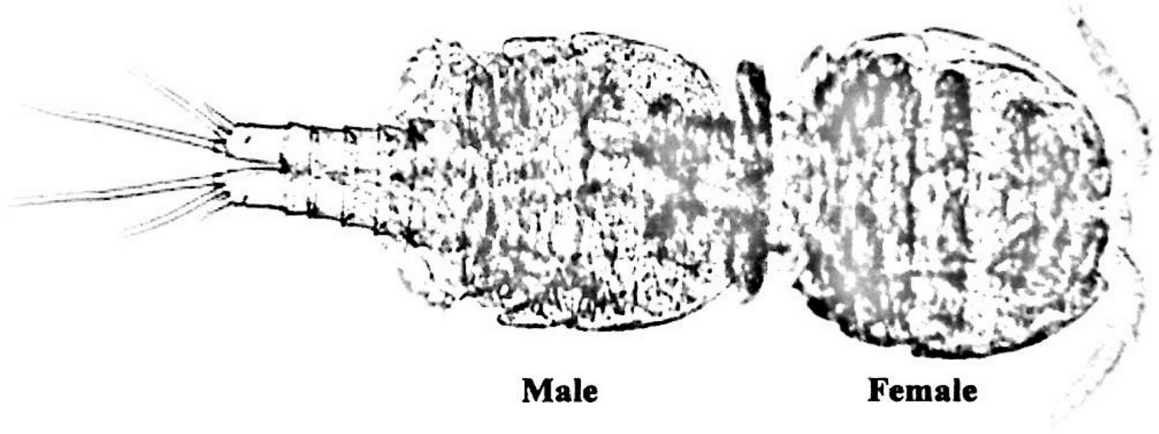

Plate 2.2 Mating process of *Mesocyclops aspericonis*
(Adapted from the www.world of copepods.com)

2.1.4 Life cycle and growth of copepods

The life cycle of copepods begins from eggs, five stages of the nauplii, the intermediate juvenile stage and five or six stages of copepodite, the advanced juvenile stage (Figure 2.1). Copepods follow two essential developmental patterns each having ecological significance; (a) development with a larva hatching at the end of embryonic development and (b) complete development within the egg which hatches to juvenile stages (Sastry, 1970).

Development from a newly oviposited egg all the way through the larva until the juvenile phase is an never-ending process. Egg development is sustained on the yolk within the egg while attached to the female parent (Anderson, 1973). The larval phase is sustained by the vigor derived from feeding but influenced by availability and the quality of food present.

Studies on pelagic larvae of Crustacea have been conducted from plankton samples collected in the field (Lebour, 1928; Gurney, 1942) and from laboratory cultivation under controlled conditions (Costlow and Bookhout, 1960a; Rice and Williamson, 1970; Sastry, 1970, 1975). Field studies have been concerned with the depiction of early life cycle stages of species and the descriptions of distribution and profusion of larvae in geographical area. The development of techniques for cultivation of larvae through all phases to the juvenile has permitted descriptions of the larvae of a species (Costlow and Bookhout, 1959; Rice and Provenzano, 1964; Sastry, 1977), thus made probable a variety of studies on the biochemistry, physiology and the behaviour of the larvae.

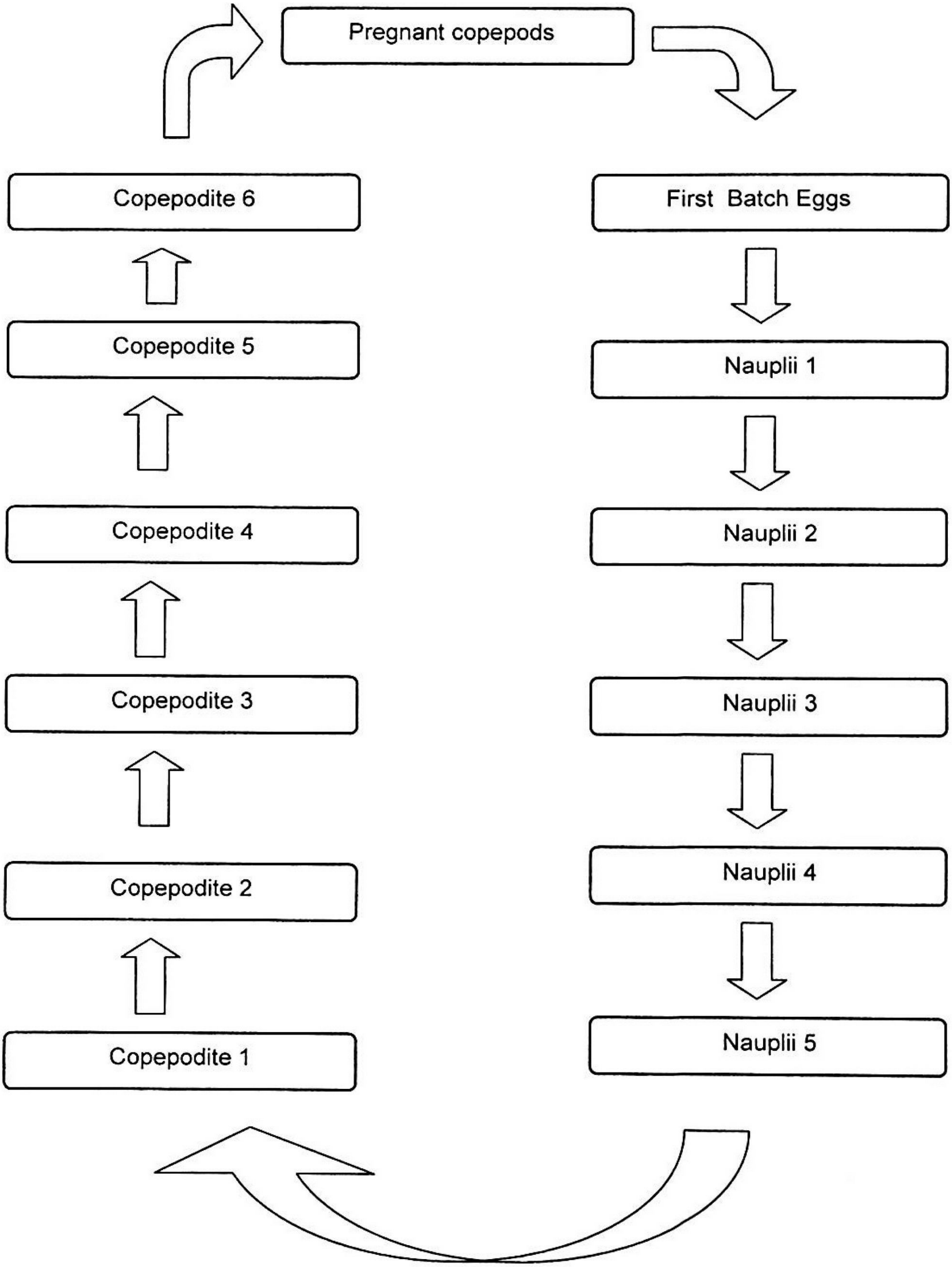

Figure 1.1 The life cycle of copepods

The newly oviposited egg of a copepod is a self-reliant structure with all the required material for synthetic processes associated with embryogenesis and morphogenesis and all of the compounds required for oxidative metabolism and energy production. The egg contains nutrient reserves in the form of proteinaceous yolk and lipid vesicles scattered all over the cytoplasm. The yolk also contains carotenoid pigments giving a characteristic coloration of eggs (Herring and Morris, 1975). The rate of egg development (incubation period) to a larva is ecologically imperative to the timing of larval discharge into the pelagic environment and may be influenced by both endogenous factors and interacting environmental factors.

Egg development can be divided into different stages based on the developmental events or the percentage of yolk volume utilized though it is a continuing process requiring raw resources and free energy provided by the nutrient reserves in the oocyte during oogenesis (Sastry, 1970). The rate of development of egg is dependant to genetic factors, composition of nutrient reserves, the speed of substrate utilization for maintenance and tissue production and size of eggs. Changes in the DNA content that can be related to cell propagation and RNA content to protein synthesis also takes place during egg development (Achituv and Barnes, 1976).

The rate of egg development can also be affected by temperature (Patel and Crisp, 1960a; McLaren, 1965, 1966; Wear, 1974) and also salinity (Crisp and Costlow, 1963). Many studies on the effects of temperature have shown that the rate of development increases linearly within the viable temperature range and as the temperature approaches the upper limit the rate will first decrease and then becomes even before the death of the embryo with a further increase in temperature (Patel and Crisp, 1960a,b; Bernard, 1971; Wear, 1974).

Egg size may also manipulate the rate of development but such a relationship does not seem to apply to vaguely related species (McLaren, 1965, Wear, 1974). Among closely allied species of copepods (McLaren, 1965) the development rate is conversely related to the egg size. McLaren (1966) found that development time of copepod eggs increases curve-linearly with diameter among smaller eggs and linearly among larger eggs.

The viability and development of eggs are affected by various environmental factors such as temperature, salinity, photoperiod and dissolved gases and chemicals in freshwater (Sastry, 1970). Normal development of larva takes place within a defined range of environmental conditions. Effects of temperature have shown that the rate of development increases linearly within the viable temperature range (Patel and Crisp, 1960a&b; Bernard, 1971; Wear,1974). At lower temperature, rate of development gradually slows down or stops.

The hatching mechanisms of copepods include osmotic uptake of water and bursting of the egg membrane. The first identified crustacean larvae was the newly hatched form of the copepod Cyclops dated 1699 (Gurney, 1942). Similar forms were then given names as nauplius and copepodites.

Cyclopoid copepods begin life as fertilised eggs. A few hours or days after copulation, one or two egg sacs are formed by the female thus, attached on each side of the females first urosomal segment. There is no special copulatory organ for an internal fertilization. These eggs sacs may contain from 2–100 eggs ranging in size from 60-100 um, depending on species and time of season. Under normal conditions, eggs will hatch within a few days of being extruded (Achituv and Barnes, 1976).

Most species of copepods produce more than one clutch of eggs, however, the number and size of the clutches does vary considerably among species. The duration of development from egg to adult is governed primarily by temperature. A complete cycle may take three to four weeks at optimal temperatures and up to several months at low temperatures. In addition, growth rates vary among species (Manton, 1977). As a result, a species may have one to several generations per year in a given body of water. These generations will not necessarily be in synchrony with other co-occurring species. Number of eggs per brood tends to become lesser with their increasing age (Manton, 1977).

The larval stage of the copepods begins with the naupliar stage. They are as small as 20 µm. There has been ambiguity as to the accurate number of naupliar instars in the life cycle of free living copepods. There is still no agreement as to whether there are five or six stages. The first in depth record of this problem is that of Oberg (1906), who gave six naupliar instars for the marine copepod, *Oithona similes* which he illustrated in his early drawings. Six naupliar instars in freshwater cyclopids were further recorded by Walter (1922), Ziegelmayer (1925), Ewers (1929), Borutskii (1949), Dukina (1956), Elgmork (1965) and Eppacher (1968).

Each naupliar stage is separated by a molt. The first stages have only three pairs of appendages that are responsible for locomotion and feeding. The older stages show buds of further mouth appendages and swimming legs. Increasing number of body segments and appendages becomes functional. The final naupliar stage molts into the first copepodite. Each molt is accompanied by important morphological changes (Elgmork, 1965). The emerging copepodite stage resembles the adult more or less. Finally, after the fifth molt adulthood is reached and the fresh copepod is all set for reproduction.

During the copepodite stages one to four, discrete body segmentation appears, appendages are added, and older appendages develop further. In the first copepodite stage (CI), the body consists of five segments, the antennules are usually 6-segmented and there are three pairs of swimming legs (Eppacher, 1968). The first two pairs of legs are well-developed while the third is simple. Also in CI, the paired caudal rami are well-developed and bear lateral, dorsal, and all four apical setae. By the time the copepodite has reached the fifth stage, the body consists of 9 segments consisting at least of 11 antennular segments, and the fifth pair of legs is formed (Elgmork, 1965). During this period, the male at the fifth copepodite level will add one urosomite by the division of the anal somite, whereas the female will add one urosomite by the division of the anal somite, but the genital and succeeding somites will combine. This result in the adult females still having 9 somites, while the adult males have 10 somites.

Under unfavorable conditions, cyclopoids can produce a non-encysted resting stage during the copepodite phase, usually in CIV or CV. Densities of diapaused individuals within the sediments can reach several million per square meter (Bowman, 1971). This resting stage is generally characterized by a dark color (in preserved specimens), an empty gut, antennae that are stretched closely along the body, relatively large oil globules, and an abdomen held at approximately right angle to the cephalothorax.

The various cyclopoid species may stay in the resting stage for differing lengths of time ranging from 6-28 weeks. The occurrence and timing of diapause (resting stage under extreme conditions) are known to vary among species as well as between populations of a single species living in different habitats (Santer 1998). In some cases, copepodites can withstand freezing, or can survive years of aridness

(Wyngaard et al. 1991). In addition to improving a copepod's chance of survival under adverse conditions in its home body of water, these resting stages provide a mechanism for the transfer of species from one continent or locality to another by human agencies (Reid and Pinto-Coelho 1994) or passive transport on waterfowl (Reid and Reed 1994).

2.1.5 Feeding habits of copepods

Energy is such a fundamental requirement and is not surprising that there is a continuing interest in the dietary preferences and requirements of these copepods. In many species, dietary preferences change with the stage of the life cycle. In observing the diet of natural and cultured laboratory populations of a dominant copepod in Lake Kinneret, Israel, Gophen (1977) found that the nauplii and early copepodid stages were herbivorous whereas later stages were carnivorous and selectively preyed on certain species.

Feeding is not only necessary for growth and maintenance of the organism but it is also important for reproduction. To illustrate this point, Lampert (1978) demonstrated that egg production in a selected species was restricted if the food concentration was less than 0.2mg/liter, but at 0.7mg/liter, egg production showed an increase.

Feeding efficiency of freshwater copepods may be influenced by a number of environmental factors. Studies proved that the foraging rate of these copepods decreased when the oxygen concentration dropped below 3mg/liter (Kring and O'Brien, 1976). They emphasized that the food intake is also influenced by temperature but not always in a predictable manner.

Cyclopoid copepods do not create currents to aid their feeding but instead grasp their food directly with their first maxillae and their maxillipeds (Fryer, 1957. These appendages help in the foraging of food. Cyclopoids detect their prey with the help of the mechanoreceptors on their first antennae. The nauplii stages use their mandibles and second antennae to capture their food (Monakov, 1976).

2.1.6 Adaptations of copepods

Cyclopoids use their swimming legs, antennules and urosome to swim through the water. Their progress is marked by a hopping motion, followed by a sinking phase (Snodgrass, 1951). Surface benthic forms probably use a combination of swimming and crawling to navigate the top 1-2 cm of the sediments. Some cyclopoids swim dorsal-side-up while others swim upside-down. Some cyclopoid copepods make diel migrations that involve swimming downward 10 m or more into the deeper, darker strata at dawn, with a reverse upward migration at dusk (Williamson and Reid 2001).

Copepodites search for food not only while swimming, but also while clinging to substrates, upon which they graze. They may take a wide variety of food, depending on the copepod species and the life stage (Fryer 1957). Food items range in size from unicellular algae to larval fish. Carnivorous cyclopoids use their maxillules as the primary grasping appendages, aided by the maxillae and maxillipeds. The movement of these three pairs of appendages is directed toward the mouth region, where the mandibles tear up larger prey and stuff pieces into the esophagus (Fryer 1957, Williamson 1986).