Biology of the amphipod *Hyale* sp. (Gammaridea, Hyalidae)

by

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端足目玻璃鈎蝦屬 *Hyale* sp. (鈎蝦亞目, 玻璃鈎蝦科) 的生物學研究

蔡國豪, 香港中文大學生物系

碩士畢業論文

摘要

端足目鈎蝦亞目被廣泛地應用於評估水質環境污染的活體測試中。 *Hyale* sp. (玻璃鈎蝦屬)是香港吐露港常見的種類之一，有關該屬種的習性還未見報導，因此，研究 *Hyale* sp. 的生物學特性是發展其成爲本地指標生物的必要基礎。

本研究的其中一個重點爲 *Hyale* sp. 的生長。 剛孵化的幼體即被蓄養於一個特定的環境下，並記錄其脫殼週期，以及量度脫殼後身體不同部份的長度，例如體長、頭部或觸角長度等，直至所跟進鈎蝦死亡爲止。 根據觀察所得，雄性和雌性的平均壽命分別爲 178 和 175 日，最長壽命爲 262 日，並有 11 至 21 次不等的脫殼記錄。 牠們的體長隨著年齡而增加，直至第十四齡，兩性體長穩定於 8 至 10 mm 之間，最大體型可達 11.38 mm。 除了體長外，其他形質，如頭部和觸角亦可顯示出 *Hyale* sp.的生長情況。 本研究亦發現不同的鹽度可以影響幼體的生長速度，蓄養於不同鹽度水體中的幼體, 其相對生長速度爲 20 > 30 > 40 >10‰.

Hyale sp. 一般於第五至第八齡達到性成熟階段，兩性於體長、觸角和腮足掌節等特徵上均具明顯的差異性。 從以上性徵與體長間相對成長的分析，顯示出 *Hyale* sp. 的生命史分別有兩個不同的成長階段: 幼體和成體。 最早的性成熟個體發現於第五齡。 每個成熟的雌性個體，於同一窩內平均生產 13 個幼體，而每窩產量是正比於體長。 雌性鈎蝦的繁殖能力差異很大，一生可以生產 2 至 17 窩，共 16 至 290 個幼體。

本研究還探討溫度、鹽度與及重金屬鎘對 *Hyale* sp. 的影響。 *Hyale* sp. 能夠生存於 6 至 30°C 之間，但不存活於低至 0°C 或高於 34°C 的溫度中。 其幼體的半致死溫度爲 3.2 和 31.7°C，而其成體的半致死溫度爲 4.2 和 31.5°C。 *Hyale* sp. 可於 10 至 30‰ 鹽度的水體生存，但未能存活在低於 2‰ 或高於 62‰ 的水體中。 幼體的半致死鹽度爲 2.4 和 55.5‰，而成體的半致死鹽度爲 1.6 和 56.9‰。 於重金屬鎘的活體測試中，幼體的 24h, 48h, 72h 及 96h 的半致死濃度分別是 8.91, 4.47, 3.00 及 1.38 mg L^{-1}。 成體的 24h, 48h, 72h 及 96h 的半致死濃度分別是 8.80, 5.83, 2.12 及 0.96 mg L^{-1}。 兩個不同階段的半致死濃度並沒有顯著的差異。

根據本研究結果，玻璃鉤蝦屬 *Hyale* sp. 是一種具有應用前景的指標生物。其基礎生物學正爲發展該種鉤蝦成爲環境污染指標，提供了重要的基礎資料。

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Abstract

Amphipod is considered to be one of the most suitable test organisms for environmental assessments. The amphipod *Hyale* sp. (Suborder Gammaridea, Superfamily Talitroidea, Family Hyalidae) is found in Tolo Harbor, Hong Kong. Its availability makes it a potential testing organism for local environmental bioassays. The study of its general biology, salinity and temperature tolerance, and sensitivity to the heavy metal cadmium provides information for the development of environmental bioassays using this local species.

Hyale sp. was reared individually under standardized laboratory conditions. The growth parameters of body length, head length, eye length, antennae length, propodus length of gnathopods and merus length of pereopod 7 were measured after each molt throughout its life span. The amphipod had 11 to 21 instars in its life cycle. No terminal ecdysis or final instar was found in the development. The mean life span of males and females were 178 and 175 days, respectively, while the maximum life recorded was 262 days in a male. The body length increment was a prominent growth indicator of the animal. The body length of the two sexes stabilized within the range of 8 to 10 mm after instar 14. The maximum body length reached 11 mm. The other parameters measured also effectively indicated growth. The parameters were also used to study the salinity effect on growth. The study revealed that the salinity exerted significant effects on the growth of juveniles. A descending order of the growth rate of juveniles was 20 > 30 > 40 > 10‰.

In the development of *Hyale* sp., sexual maturation started at the range from instar 5 to 8. Body length, antennae length and propodus length of gnathopods exhibited strong sexual dimorphism. Two growth phases were differentiated in the allometric relationship between the sexually dimorphic characters and body length. The transition from the sexually immature to mature phase generally occurred at instar 5. An average of 13 juveniles was generated in a single brood (range, 3–33) of females. The brood size was positively correlated to the body length. The total number of offspring generated throughout the life span of females varied from 16 to 290 juveniles in 2 to 17 broods (mean, 6 broods).

The sensitivity and tolerance of *Hyale* sp. to temperature, salinity and the heavy metal cadmium were determined in the study. *Hyale* sp. could tolerate a range of temperatures between 6 and 30°C. No amphipod survived when the temperature reached below 0°C or above 34°C. The values of low range 96h median lethal temperatures were 3.2°C in adults and 4.2°C in juveniles. The corresponding values of high range temperature in adults and juveniles were 31.7 and 31.5°C, respectively. The amphipod also tolerated to salinities ranged between 10 to 30‰. No survival was found in the salinities below 2‰ and above 62‰. The values of low range 96h median lethal salinity in adults and juveniles were 2.4 and 1.6‰, respectively. The corresponding values of high range salinity were 55.5‰ in adults and 56.9‰ in juveniles. In cadmium toxicity tests, the values of 24h, 48h, 72h and 96h LC_{50} were 8.91, 4.47, 3.00 and 1.38 mg L^{-1} in adults, and 8.80, 5.83, 2.12 and 0.96 mg L^{-1} in juveniles, respectively. There was no significant difference in LC_{50} values between adults and juveniles under all exposure periods. Yet the time-dependent toxicity of cadmium was significantly greater in juveniles than in adults after 24h exposure.

The amphipod *Hyale* sp. is a potential test organism to be used in environmental bioassays. The information on its general biology, tolerance to environmental stress and sensitivity to cadmium is established from this study. Based on the information, local environmental bioassays by using this amphipod species can be developed.

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Chapter 1 Introduction

1.1 Literature Review

1.1.1 Biology of Amphipods

Amphipoda (Kingdom Animalia, Phylum Arthropoda, Class Crustacea, Subclass Malacostraca, Superorder Peracarida) is a diverse order of Crustacea. Six thousand species belonging to more than one hundred families are recognized (Schmitz 1992). The order is divided into four subordinal taxa, the Gammaridea Latreille, 1803, Hyperiidea Latreille, 1831, Caprellidea Leach, 1814, and Ingolfiellidea Hansen, 1903. External morphology, habitat, feeding habits and reproductive features of Amphipoda are discussed in this review.

1.1.1.1 External Morphology

Amphipods share common features that group the animals into a distinct taxon. The cephalon is formed by the fusion of the head and the thoracomere 1. The cephalon is generally short with a pair of sessile compound eyes and six pairs of appendages: two pairs of antennae, one pair of mandibles, two pairs of maxillae and one pair of maxillipeds (Schram 1986). The pair of maxillipeds is originated from thoracopods 1, which are modified for food manipulation. The compact arrangement of feeding appendages on the cephalon forms a well-defined buccal mass. A rostrum may be present at the anterior end of the cephalon, but it is more commonly reduced or absent (Schram 1986). The absence of a carapace is a typical characteristic in all amphipods.

The specific characteristics of each subordinal taxon are reviewed below. Gammarideans (Figure 1.1A) are typically shrimp-like in appearance because of their laterally compressed body with bilaterally expanded coxal plates. The expanded structure of the coxae project ventrally to protect gills and brood pouch. The presence

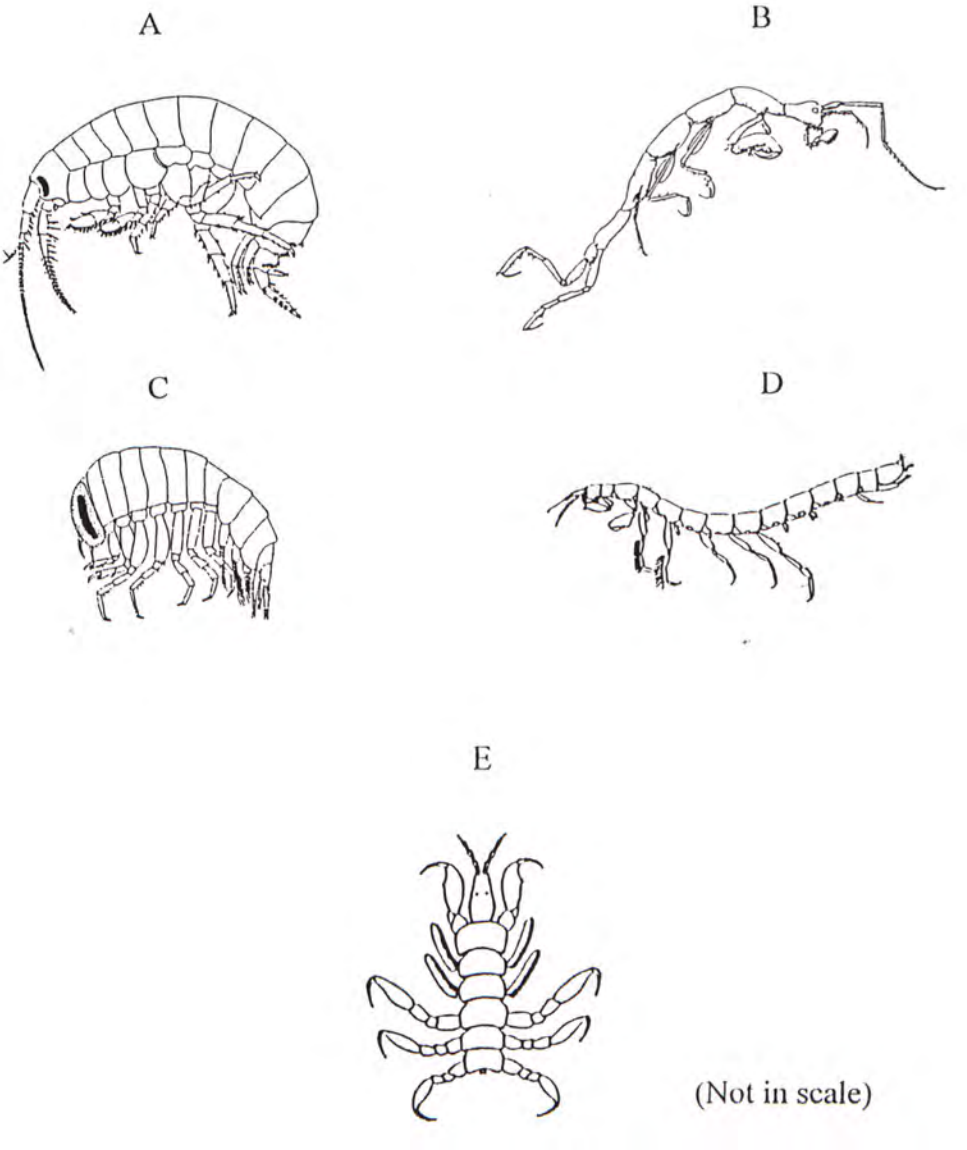


Figure 1.1 External structures of amphipods in lateral view. A, *Chaetogammarus marinus* (suborder, Gammaridea), female (from Hayward 1995); B, *Phtisica marina* (suborder, Caprellidea), male (from Hayward 1995); C, *Hyperia galba* (suborder, Hyperiidea), female (from Hayward 1995); D, *Ingolfiella leleupi* (suborder, Ingolfiellidea), female (from Dahl 1977); E, *Cyamus gracilic* (suborder Caprellidea) male (from Leung 1967).

of a palp in the maxilliped is characteristic in this suborder. Caprellidea (Figure 1.1B) is the most divergent suborder from the basic pattern of Amphipoda (Hayward 1995). Caprellideans are cylindrical and slender in body shape. Coxae and telson are vestigial. Abdominal segments are very reduced, with vestigial appendages. The cephalon is immovably fused with pereomere 1. Hyperiideans (Figure 1.1C) are plump, round amphipods with very large sessile and compound eyes covering a large part of the head. Coxae are small and often fused with the body. A palp is commonly absent in maxillipeds. Pleopods and uropods are usually well developed. Ingolfiellideans (Figure 1.1D) are typically elongated and cylindrical in body figure. The cephalon has a small pair of articulated ocular lobes instead of pigmented sessile eyes. The pleon and urosome are nearly equal in size. Pleopods are reduced in the pleon. The first 2 pairs of uropods are also reduced and the last pair is vestigial.

Most amphipods are small in body size. The average body length ranges from 5 to 15 mm. The smallest interstitial amphipods may be less than 0.1 mm long, but a large species *Alicella gigantica* may reach 14 cm in length (Schmitz 1992). In 1968, an undescribed benthic amphipod of the family Lysianassidae was discovered from a depth of 5300 meters of the Pacific Ocean and recorded to be 28 cm long (Ruppert & Barnes 1994).

A typical amphipod has seven 'free' thoracic segments (thoracomeres), each of which bears a pair of uniramous walking limbs (pereopods), and six abdominal segments including three pleomeres and uromeres. A pleomere bears a pair of multi-segmented biramous pleopods. Yet an uromere bears a pair of few-segmented biramous uropods. Each pair of the body appendages is formed as outgrowths of the body wall (Dennell 1960). Each appendage is made up of seven articulated podites, named from proximal to distal end as coxa, basis, ischium, merus, carpus, propodus and dactylus. The first two pairs of pereopods (gnathopods) are usually subchelate, except in hyperiideans (McLaughlin 1982). The subchelate gnathopods are usually larger than the other pereopods and show considerable sexual dimorphism. Gnathopods 2 are relatively larger and more complex in males than in females (Hayward 1995).

Pereopods 3-7 are relatively unspecialized appendages. The paired dactyls of pereopods 3 and 4 directed anteriorly, while the last three pairs pointed posteriorly (Schmitz 1992).

1.1.1.2 Habitat

Amphipods are widely distributed in the world. They can be found in all latitudes from both poles to the equator, from driftline to abyssal depths, from deep seafloor to home garden. Most amphipods are marine species, but they also inhabit lake, spring run, estuary and under moist leaf litter (McLaughlin 1980). The adaptation to the great diversity of habitats is facilitated by their body modifications. Different subordinal levels exhibit specific adaptations to different habitats.

Each subordinal taxon commonly adapts to its particular habitat. The gammarideans are primarily benthic and adapted for crawling, burrowing and tubicolous habits. Most freshwater gammarideans are common benthic inhabitants in algae and other vegetation in streams and ponds (Barnes 1994), such as *Gammarus lacustris*. Two examples of marine benthic gammarideans are *Ponyocrates arenarius* and *Leptocherius plumulosus*. *P. arenarius* is a sand-dwelling inhabitant (Beare & Moore 1996) and *L. plumulosus* is a silty-clay sediment-dweller (Schlekat *et al.* 1991). *L. plumulosus* builds U-shape tube in sediments. *Photis conchicola* is also a tube-builder but it does not build its tube in sediment but in an empty gastropod shell (Moore 1986). The small *Orchomene nanus* (Family Lysianassidae) inhabits specifically inside the carapace of dead crabs (Moore & Wong 1996).

Free-living caprellideans have long slender bodies and reduced abdomens. The cylindrical body configurations and grasping claws at the tips of their appendages are adapted for climbing and clinging on algae, hydroids and bryozoans (Hayward 1995). The caprellidean *Caprella danilevskii* is one of the dominant epifaunal species inhabiting the *Sargassum* zone along the rocky coast of Japan (Takeuchi & Hirano 1991). Hyperiideans are marine pelagic amphipods. Some species of the genera

Hyperia and *Rhabdosoma* have a domed and spherical cephalon. Their modified body configurations adapt for floating in pelagic habits (Ruppert & Barnes 1994). Ingolfiellideans are small amphipods. Their elongated and cylindrical body figures adapt for inhabiting in interstitial environments, such as ground water, caves and deep-sea habits (McLaughlin 1980).

The living habits of amphipods are highly correlated to their modes of movement, which are dependent upon the arrangement of their appendages. For example, clinging habit in macroalgal zone is related to pereopod orientation (Steele 1988). Most amphipods cannot walk effectively as the laterally compressed body often leans far over to one side. But they can swim effectively by pleopod movement and uropod propulsion in pelagic habitat. The specialty of amphipods is a tail-flip. A 2 cm-long *Talorchestia* can leap forward one meter on a sandy beach by means of a sudden backward stroke of the abdomen and telson (Ruppert & Barnes 1994). The semiterrestrial talitroids (Superfamily Talitroidea) walk under moist litter by flexion and extension of anterior pereopods, and aided by pushing action of urosomes (Steele 1988).

1.1.1.3 Feeding Habit

The differences in the niche of amphipods can be reflected by their diverse feeding habits. A wide range of feeding habits is found in amphipods, such as deposit-feeding, detritus feeding, filter feeding, carrion ingestion, epiphytic herbivory, broad spectra of omnivory or even ectoparasitic (Ladle 1974; Moore 1975). The benthic species are mainly scavengers, deposit-feeder or filter feeders. The seaweed inhabitants are mostly herbivores. For example, *Hyale hirtipalma* has a significant food preference on the red alga *Iridaea laminariodes* (Buschmann 1991). The cyamid (Family Cyamidae) is a group of caprellideans commensal on whales and they probably feed on debris accumulated on whale's skin (Ruppert & Barnes 1994).

Most amphipods are detritus feeders and scavengers. Some burrowing forms scrape detritus and diatoms from sand grains. The omnivorous amphipod, *Hyalella azteca* is a scavenger, grazer and deposit-feeder (Hargrave 1970). It feeds not only on algae and bacteria associated with the sediments and aquatic macrophytes (Hargrave 1970), but also on dead animal and plant materials (Cooper 1965). *Orchestia gammarellus* is basically a detritus feeder. Its hatchlings grow well when small pieces of fucoid alga with adult fecal pellets are provided as food sources (Morritt & Spicer 1996). Because *H. azteca* is a common prey of consumers in higher trophic level, it facilitates energy flow in aquatic ecosystems through the direct conversion of detrital energy into macro-consumer biomass (Cooper 1965; Wen 1992).

Some suspension feeders have evolved structural modifications to collect fine detritus. In the genus *Corophium*, the filter setae on the gnathopods are adapted to filter food materials from the feeding current created by the pleopods (Ruppert & Barnes 1994). Many caprellids feed on diatoms filtered on their antennae (Caine 1974). For example, the caprellid *Caprella danilevskii* was successfully reared in laboratory by providing the suspension of the diatom *Cylindrotheca closterium* as food material (Takeuchi & Hirano 1991).

Predation is a minor consideration in amphipods (Barnard 1971). Strictly predacious feeding is not common. Many of them only supplement their diet by predation. A study showed that *Gammarus fasciatus* grew significantly bigger in size when it was fed on filamentous algae (*Cladophora*) and animal matter (chironomids) than only fed on particulate organic matter (preconditioned sycamore leaves *Platanus occidentalis* (Delong *et al.* 1993). Also, parasitism is not prevalent among amphipods. The cyamid *Cyamus boopis* (Figure 1.1E), with the common name whale lice, is believed to be parasitic on humpback whale and sperm whale (McLaughlin 1980). But it is probably commensal on whales as it feeds on diatom and debris accumulated on the whale's skin (Ruppert & Barnes 1994). Hyperiideans are marine pelagic amphipods, usually being commensalistic or parasitic for at least part of their life cycle

on gelatinous marine plankters, such as salps and scyphozoans and colonial radiolarians (Ruppert & Barnes 1994; McLaughlin 1980).

1.1.1.4 Reproductive Biology

Amphipods are dioecious with distinct sexual dimorphism. The subchelate gnathopods 2, the eyes and the antennae are frequently larger or longer in males than in females (McLaughlin 1980). For example, the male *Lembos macromanus* has especially dense setae projecting anteriorly from article 5 of gnathopods 2 (Barnard 1971).

The gonads of amphipods are paired and tubular. The testes of gammarideans are paired, slender, and elongated organs lying ventrolaterally to the pericardial septum. The paired male gonopores open at the end of a pair of genital papillae on the sternum of the thoracomere 8 (Schmitz 1992). The ovaries of gammarideans are slender structures lying dorsolaterally to the midgut (Schmitz 1992). The female gonopores open on the coxae of thoracomere 6 (Ruppert & Barnes 1994).

Reproductive behaviors are induced by intrinsic and extrinsic factors. A complex pheromonal attraction of both sexes initiates courtship behaviors. Waterborne and contact pheromones induce mate location, pairing and copulation in *Gammarus palustris* and *Microdeutopus gryllotalpa* (Borowsky 1990). Iribarne *et al.* (1996) suggested that mating in *Eogammarus oclairi* can be driven by male-male competition and thus larger males are more favorable in sexual selection. The most important extrinsic factor in the control of reproduction of *Orchestia gammarellus* is temperature. Higher temperature (within the range 15–22°C) initiates courtship behavior more rapidly in *O. gammarellus* (Morritt & Stevenson 1993).

Precopulatory pairing is a characteristic sexual behavior in amphipods. The male uses gnathopods 2 to grasp the female beneath it and moves in pair. The male must accompany the female to ensure that copulation and fertilization occurs in a timely

manner because the female amphipod does not have any sperm storage organ (Borowsky 1990). Precopulatory pairing may last for several days. This precopulatory mate guarding strategy functionally isolates the female from other males during the guarding period (Dunham & Hurshman 1990). Actual sperm transfer is accomplished quickly. The male twists its abdomen around so that its uropods touch the female marsupium. As sperm are emitted, they are swept into the marsupium by the ventilating current (Ruppert & Barnes 1994).

The marsupium (brood pouch) is composed of a pair of spoon-shaped (Figure 1.2) or circular-shaped oostegites loosely overlapping together, the gaps between which are covered by fringing setae. The development of oostegites is induced by ovarian hormones. Charniaux-Cotton (1960) showed that oostegites were developed in an androgenectomized male after implantation of an ovary. Fertilized eggs are retained in the marsupium. Egg size is correlated to the structure of the marsupium. Species with narrow oostegites produce larger eggs than those with broad oostegites to prevent falling out of eggs (Steele & Steele 1991a). Other than the shape of oostegites, seasonal factor can also exert influences on the egg size. The egg size of *Pectenogammarus planicrurus* markedly varies with different season (Bell & Fish 1996; Moore & Wong 1996). The size of stage I (early) egg of *Gammarus insensibilis* developed in winter is 60% larger than in summer (Sheader 1996).

Incubation period of embryos may vary from one to three weeks. The interlocking brood setae in marsupium prevent the eggs from falling out within the incubation period (Ruppert & Barnes 1994). One annual brood of 15 to 50 eggs is common in most temperate freshwater species. But in marine species, the brood size may vary from 2 to 750 eggs in a clutch and more than one brood is produced per year (Ruppert & Barnes 1994). For example, the female *Caprella danilevskii* produces an average of 69 offspring within its life span (Takeuchi & Hirano 1991).

Larval development in almost all amphipods is epimorphic. A direct development commonly occurs in amphipods but except a few hyperiideans, which

A

B

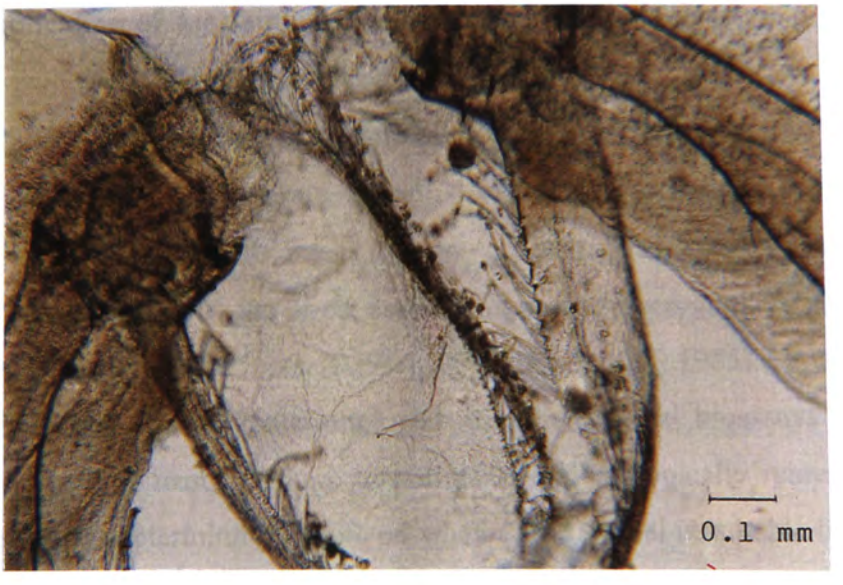


Figure 1.2 Oostegites of a female *Hyale* sp., anterior view. A, spoon-shaped oostegites forming a brood pouch; B, oostegite with curl-tipped fringing setae.

hatch as postlarvae with bud-like pleopods instead of juveniles with completely developed pleopods. All newly hatched juveniles have adult female like morphology but lack of secondary sexual characteristics (Barnard 1971; Borowsky 1990). The juveniles may not leave the marsupium immediately after hatching. They may retain in the marsupium for 1-8 days until the subsequent molt of mother (Schmitz 1992). Extensive parental care was observed in a soft-sediment dwelling amphipod *Leptocheirus pinguis* (Thiel 1997). Under the environment with constant food supply, females of *L. pinguis* retained its growing juveniles in its U-shaped burrow in muddy sediment until they reached a length of 5 mm (approximately one-third adult size) or sexual maturity. The remarkably large sizes of juveniles are less susceptible to predation at time of recruitment.

1.1.2 The Application of Amphipods in Environmental Bioassays

In a polluted environment, the aquatic biota may suffer physiological stress that is revealed in change of growth rate, impaired reproductive capacity or behavioral modifications. Environmental bioassay is to evaluate the complex effects of environmental deterioration by the direct assessment on the physiological, biochemical and behavioral changes of the biota (APHA, AWWA, WPCF, 1985). According to Hellawell (1986), any aquatic biota employed in environmental bioassays should be abundant in the environment, sensitive to pollutants and ecologically relevant. These characteristics are the determining factors on whether the animal is suitable for use as a biomonitor, bioindicator or biological monitor in bioassays. Biomonitor is defined as a species which accumulates pollutants in its body tissues, and thus the bioavailability of the pollutants can be measured. Bioindicator is defined as a species to denote an ecological effect by the presence or absence of the species. Biological monitor is defined as a species to denote degrees of ecological change by behavioral, physiological or chemical responses of the species (Hellawell 1986; Rainbow 1995a).

Amphipods are one of the most commonly used organisms in environmental bioassays. In addition to lethal tests, sublethal responses of amphipods have also been

intensively studied in environmental assessments. Aspects on the abundance, sensitivity and ecological relevance of amphipods are discussed in the following section, with a view to show that amphipods are suitable organisms used in environmental bioassay.

1.1.2.1 Abundance and Availability

The abundance of the animal and its availability in sampling are both critical in the consideration of its application in bioassay. Amphipods are abundant in small productive lake and prairie pond bordering on agricultural land (Arts *et al.* 1995). The density of amphipods may achieve 2000 ind m^{-2}. In a study of *Orchestia gammarellus* associated with a sewage treatment works in the coastal town of Looe, Cornwall, the density of the amphipod living in biological filters was found to range from 280,000 ind m^{-2} in March to 4.34×10^6 ind m^{-2} in October (Jones & Wigham 1993).

In a local study, survival tests of the amphipod *Elasmopus rapax* and the postlarvae of the shrimp *Metapenaes ensis* to polycyclic aromatic hydrocarbons (PAHs) were performed by Wang (1996). She found that the postlarvae of *M. ensis* was more sensitive to PAHs than *E. rapax.* However, she suggested that the amphipod *E. rapax* was potentially better organism in ecological test because of its ease in culture and availability throughout the year.

1.1.2.2 Sensitivity

The sensitivity of the animal to the pollutants is another prime factor to be considered in the selection of suitable organism in different environmental bioassay. Zanders and Rojas (1992) pointed out that small crustacean species, such as amphipods which molt frequently, are likely to be more sensitive to heavy metals than larger species with lower molting frequency. The same study revealed that the amphipod *Elasmopus rapax* was the most sensitive marine organism yet studied concerning cadmium toxicity. The 48h and 96h LC_{50} values were 4.0 and 1.6 μmol L^{-1},

respectively. Phipps *et al.* (1995) compared the sensitivity of three freshwater benthic macroinvertebrates, the amphipod *Hyalella azteca,* the midge *Chironomus tentans* and the oligochaete *Lumbriculus variegatus,* to ten contaminants. The results showed that *H. azteca* was the most sensitive species to metal pollutants among the three animals.

Amphipod was also the first animal found missing from areas along the Venezuelan coast which was affected by pollution (Zanders & Rojas 1992). Because of their high sensitivity to pollutants, amphipods are suggested to be a good bioindicator to detect early environmental changes.

1.1.2.3 Ecological Relevance

The ecological relevance of the animal is one of the considerations in the selection of an organism in bioassays. Amphipods take a very important ecological role in ecosystem because they are common preys of higher consumers. The amphipod links up the energy flow between the detritus and macro-consumer biomass (Cooper 1965; Wen 1992). If they are contaminated, great and long-term effects may be exerted to the ecosystem through the bioaccumulation process. For example, certain pelagic seabirds were found to accumulate extremely high concentrations of cadmium and mercury in their kidneys (Muirhead & Furness 1988). Such high heavy metal concentration was most probably originated from their natural diet, the pelagic hyperiid amphipods *Thermisto* spp. The hyperiid amphipods were found to contain high cadmium level in their body (Rainbow 1989).

The concentration of pollutants accumulated in the body of the animal is correlated to the bioavailability of the pollutants. Due to the high bioavailability of heavy metals to amphipods, a measurement on accumulated metal contents may provide information on the degree of environmental contamination. Amphipods are suggested to be a good heavy metal biomonitor (Zanders & Rojas 1992). A suitable metal biomonitor should be a net accumulator of the relevant metal with a simple

correlation between the metal concentration accumulated in tissue and the average ambient bioavailable metal concentration over a recent time period (Rainbow 1995a).

In a study of the metal bioaccumulation in the amphipod *Allorchestes compressa*, the cadmium ion was found to accumulate inside the body without any regulation until the amphipods died (Ahsanullah & Williams 1991). Thus amphipod is commonly used to detect the cadmium contamination in the ambient environment (Hershelman *et al.* 1981; Ahsanullah & Williams 1991; Zanders & Rojas 1992; Bhat & Vamsee 1993; Caparis & Rainbow 1994). Another study showed that the cadmium accumulation in the burrowing amphipod *Corophium volutator* is highly correlated to the metal contents in the seabed around Californian sewage outfalls (Hershelman *et al.* 1981). Therefore, the measurement of the cadmium concentration of the deposit-feeder *C. volutator* may help to detect the level of sewage contamination around the outfalls.

Other than heavy metals, organic pollutants are commonly accumulated in the body tissues of amphipods. The amphipod *Gammarus lacustris* is a detritivore. The deposit-feeding habit attenuated its exposure to organic herbicides, such as triallate and diclofop-methyl. These hydrophobic herbicides were found to accumulate in lipid-rich tissues, such as gonads and digestive tract of the amphipod (Arts *et al.* 1995).

1.1.2.4 Living Habit

In the selection of a suitable animal in an environmental bioassay, its living habit is also required to take into consideration. Benthic animals with bottom-dwelling habit are particularly suitable for testing sediment toxicity. For example, *Ponyocrates arenarius* is a sand-dwelling inhabitant (Beare & Moore 1996) and *Leptocherius plumulosus* is a silty-clay sediment-dweller (Schlekat et al. 1991). They are potentially good biological monitors or heavy metal biomonitors in sediment toxicity assessments because of their extensive contact with the sediments.

In practice, the use of amphipod in whole-sediment toxicity tests has already become a standard method to assess sediment quality in marine and freshwater systems

(ASTM 1993). For example, the toxicity of sediment collected from McMurdo Station in Antarctica was assessed by the mortality of a phoxocephalid amphipod *Heterophoxus videns* (Lenihan *et al.* 1995). The sediment quality of Tampa Bay, Florida was also assessed by a 10-day amphipod survival test using *Ampelisca abdita* (Carr *et al.* 1996). *Hyalella azteca* is a widely used organism for bioassays of freshwater sediments because of its benthic habit (APHA, AWWA, WPCF, 1985; ASTM 1993; USEPA 1994). According to ASTM (1992), five species of marine and estuarine amphipods, *Rhepoxynius abronius, Eohaustorius estuarius, Ampelisca abdita, Grandidierella japonica,* and *Leptocheirus plumulosus* were well defined and recommended as testing organisms in 10-day static sediment toxicity tests.

1.1.2.5 Sublethal Response

Sublethal response of animals to pollutants is more significant than the lethal endpoint in the early detection of environmental deterioration (Pascoe *et al.* 1994). The following sublethal responses of amphipods are currently studied and employed in environmental bioassays.

Change in growth rate is a common biological response in environmental bioassays. Growth inhibition of *Hyalella azteca* was designed for the assessment of sediment toxicity (Kubitz *et al.* 1996). Not only growth inhibition, growth stimulation can also be the effect of the environmental deterioration. McGee *et al.* (1995) showed that *H. azteca* exposed to copper and tributyltin (TBT) contaminated sediments collected from marina basin of Chesapeake Bay in North America for 28 days, were larger in size than those exposed to sediments collected outside the marina. A stimulatory effect (i.e. hormesis) was exerted on the growth of the amphipod by the low level contamination of pollutants.

According to ASTM (1992), clean-sediment reburial ability of the amphipod *Rhepoxynius abronius* was also recommended as an alternative sublethal response. Pascoe *et al.* (1994) suggested that the prevention and disruption of precopulatory

guarding phase was an effective response for the detection of cadmium contamination. The time taken for male-female pairs to separate was noted to be directly correlated to the concentrations of cadmium exposed. Based on the time-dose correlation, a '*Gammarus pulex* precopula separation (GaPPS)' bioassay was suggested by Pascoe *et al.* (1994) for the detection of ambient cadmium.

1.2 Objectives of this Study

Rainbow & Smith (1992) found that the bioavailabilities of heavy metals to the barnacle *Balanus amphitrite* along Hong Kong coastal waters considerably increased between 1986 and 1989. The change was correlated to the increase in coastal development and land reclamation. As a result of the extensive construction works, water quality was deteriorated, such as in Hang Hau and Chai Wan Kok (Rainbow & Smith 1992). In the same study, Tolo Harbor was recognized to be polluted with heavy metals, such as Cu, Cd and Pb. As Tolo Channel in Hong Kong is a land-locked arm of the South China Sea with major centres of human activity on its shore, environmental assessment of the Channel had been widely studied (Emson *et al.* 1992). In 1982, a change in benthic fauna of the channel indicated an extensive pollution of the region (Wu 1982). The pollution source was organic in origin (Morton 1982; Hodgkiss & Chan 1983). No evidence for any heavy metal pollution in Tolo Harbor sites was observed in 1986 and before (Chan *et al.* 1990). However, a relative high degree of metal pollution had been found in inner Tolo Harbor since 1989 (Chan 1992). The dissolved concentrations of Cu, Pb and Ni in surface coastal water of inner Tolo Harbor were significantly higher than the levels recorded in the South China Sea (Chan 1992; Wang 1986). Due to industrial development, the progressive deterioration of water quality in local coastal waters becomes a serious environmental problem in recent years. The development of bioassays for early detection of pollution is an area of major concerns in the local environmental issues.

The amphipod is a universally acceptable animal used in environmental assessment. However, the study on local amphipod species and their application in

environmental assessment to Hong Kong's coastal waters are limited. Rainbow (1992) had suggested the talitroid amphipod *Platorchestia platensis* as a suitable biomonitor for copper and zinc around Hong Kong. *Hyale* sp. is an amphipod found in Tolo Harbor, Hong Kong. It inhabits underneath the leaflike thalli of the macroalga *Ulva lactuca* and rocks on seashore. Its availability makes it a potential candidate to be applied in local environmental bioassays. Thus the study on *Hyale* sp. provides basic information on the development of local environmental bioassay using a local amphipod.

The research is divided into two major parts. The first part focused on the general biology of *Hyale* sp., and the second part was to study the tolerance of the species to environmental stress. The objective of the first part of the research is to gain insights into the general biology of the amphipod *Hyale* sp. The basic biological knowledge of the species is a foundation for any further studies. The specific questions to be addressed are:

i. How to correctly identify the amphipod *Hyale* sp.?
ii. What are the characteristics of life history and growth patterns of the species?
iii. What are the optimal conditions for survival and growth of the species?

The objective of the second part of my research is to study the tolerance and sensitivity of the species to environmental stress and pollutant. In my project, temperature tolerance, salinity tolerance, and cadmium sensitivity of *Hyale* sp. were determined. Based on the results of the research, a conclusion is made on whether the local amphipod *Hyale* sp. is suitable to be used in local environmental assessments.

Chapter 2 Taxonomic Identification of *Hyale* sp.

2.1 Introduction

Hyale sp. is a member of the suborder Gammaridea Latreille 1803. The Gammaridea is the largest subordinal taxon with more than 4700 described species (Ruppert & Barnes 1994). Barnard (1975) stated that gammarideans are difficult to identify because they are numerous and highly diverse. Thus identifications may require systematic examination and dissection. The terminology and taxonomic features used in this chapter are mainly based on Barnard (1971, 1974, and 1975), Barnes (1994), Hayward (1995) and McLaughlin (1980).

The marine gammaridean fauna in Hawaii, Northwest Europe, Australia has been extensively described by Barnard (1971, 1974, 1975), Barnes (1994) and Hayward (1995), respectively. The gammaridean amphipods in Asian waters have also been studied, such as in China (Ren 1992) and Japan (Hiwatari & Kajihara 1981). In contrast, little taxonomic studies have been undertaken on amphipods in Hong Kong waters (Moore 1986). The following studies reveal some information on local species. *Hyale grandicornis* and *Allorchestes* sp. were found in the rocky intertidal of Tolo Harbor (Jiang and Zhou 1982). Fourteen gammaridean amphipod species were found in shallow waters in the vicinity of Hong Kong, in which four species of the genus *Hyale* were recorded (Moore 1986). The four *Hyale* spp. were identified to be *H. grandicornis, H. iole/barbicornis*, *H. schmidti* and *H. honoluluensis*. Twelve species of the genus *Corophium* were also reported in Hong Kong, from which four species and one subspecies were identified to be new to science (Hirayama 1986). Another four species of dexaminid amphipods were found in the same study, three of which belonging to the genus *Guernea* were also new to science (Hirayama 1986). In general, systematic information of local amphipods is still relatively limited and needed to be investigated.

The taxonomic features of *Hyale* sp. used in this research are described in this chapter. The species is identified based on the features and keys of gammarideans described in Barnard (1971, 1974, 1975).

2.2 Materials and Methods

2.2.1 Collection of Amphipods

The amphipod *Hyale* sp. was collected from outdoor concrete tanks in Marine Science Laboratory (MSL), The Chinese University of Hong Kong. The 3,000-liter concrete tanks (Figure 2.1) provided a semi-natural environment to the amphipods. Seawater in each tank was continuously exchanged and aerated. Natural seawater was pumped from Tolo Harbor and slowly introduced into the tanks in the rate of 30 mL s^{-1}. The turnover rate of seawater was about 2600 L day^{-1} in a tank. The tanks were covered with sheets of perforated nylon net to minimize direct sunlight illumination. Pieces of fiber-cotton in the tanks provided cool and dark substratum for the amphipods. The fiber-cotton also offered food sources to the animals by providing a large surface area for the attachment of detritus and microalgae. The amphipods were also found living underneath the leaf-like thalli of the macroalga *Ulva lactuca* in the tanks.

Amphipods attached to fiber-cotton were collected to a plastic container by flushing with natural seawater or by gentle mechanical agitation. The amphipods collected were carefully washed into a nylon net of 200 μm mesh. Any physical damage and desiccation to the animals was minimized. The amphipods attached on the mesh were rinsed into a plastic container with natural seawater. More than one amphipod species existed in the culture tanks. *Hyale* sp. was selected with the aid of a dissecting microscope (Nikon SMZ-2T). Amphipods of different sizes and sexes were grouped together for further treatments.

A

B

Figure 2.1 The outdoor concrete tanks in Marine Science Laboratory, The Chinese University of Hong Kong. A, the tanks are covered with sheets of perforated nylon net; B, pieces of fiber-cotton filter provide food and substratum for amphipods.

2.2.2 Preservation and Anesthesia

2.2.2.1 Preservation

Following Barnard (1971), the amphipods were sacrificed and fixed in 5% buffered formalin, and then preserved in 70% alcohol. The preserved specimens were stored in small vials with tight covers to prevent alcohol leakage. The vials were kept in a cool and dark place for further inspection and dissection.

2.2.2.2 Anesthesia

For photography of live specimens, amphipods were collected from outdoor tanks and inspected under a dissecting microscope for any physical damage before the application of anesthesia. Physically intact individuals were anesthetized by immersing them in a mixture of lignocaine HCl 1% and seawater (1:1 v/v). Lignocaine HCl 1% is a common anesthetic agent used for local anesthesia in human. The mixture could completely paralyze the amphipods within five minutes.

2.2.3 Dissection

2.2.3.1 Dissecting Instruments

Amphipods for dissection were placed into a drop of glycerine on a glass slide and dissected under a dissecting microscope by using a couple of 25-G syringe needles. The sharp edge of the needle tip was used to cut any musculature while the blunt bevel of the tip was to hold any part in the position and pull any tissues out from the body.

2.2.3.2 Dissecting Medium

Following Barnard (1975), the specimens prepared for slide mounting were dissected under glycerine. Glycerine was slowly added to gradually replace the

alcohol. The replacement process was prolonged for more than two hours in order to eliminate all air bubbles. The mounted slides in glycerine medium could be stored for a period of two weeks.

The dissecting medium used for photography of live amphipod was different from that used for slide mounting. Fresh specimen was dissected under 20 μm-filtered natural seawater (29-32‰) instead of glycerine because gas bubbles formation could be effectively minimized under the seawater medium.

2.2.3.3 Photographic Recording and Illustration

The specimen was observed under a compound microscope (Nikon model SE) or an inverted microscope (Nikon model SMZ-10). Images of the specimen were captured with a color video camera (Teli® CCD CS 5110) connected to a television set (Sharp VT-1428). Photographs were taken with a color camera (Ricoh Data back 7). The line drawings of the body parts were traced from the specimens and the color photographs with waterproof fine line pens.

2.3 Results

2.3.1 Figure Index

The morphological structures of *Hyale* sp. required for identification are presented in Figures 2.2–2.27. The structures were grouped into five categories according to their anatomical relationships. The categories are:

1. The body and head: This category is composed of the carcass of an amphipod with its left side up (Figure 2.2), the cephalon (Figure 2.3), the left antenna 1 (Figure 2.4) and the left antenna 2 (Figure 2.5).

A

B

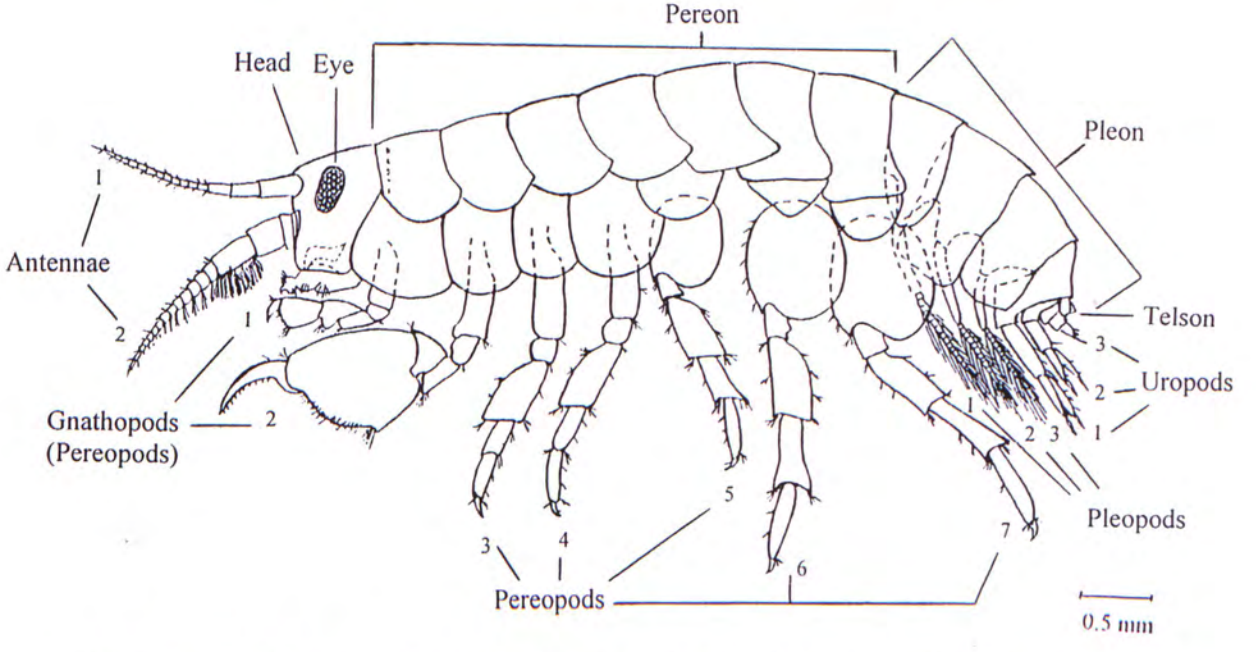


Figure 2.2 The amphipod *Hyale* sp., male, whole animal, lateral view. A, photograph; B, line drawing.

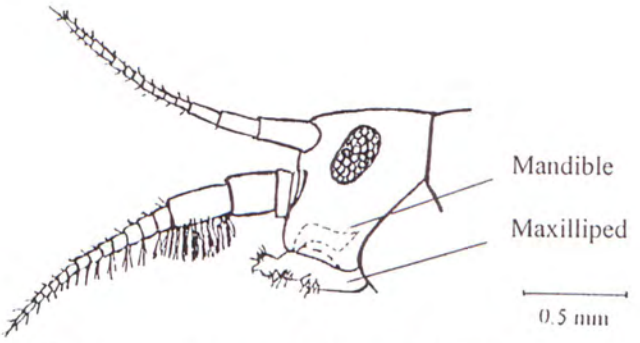
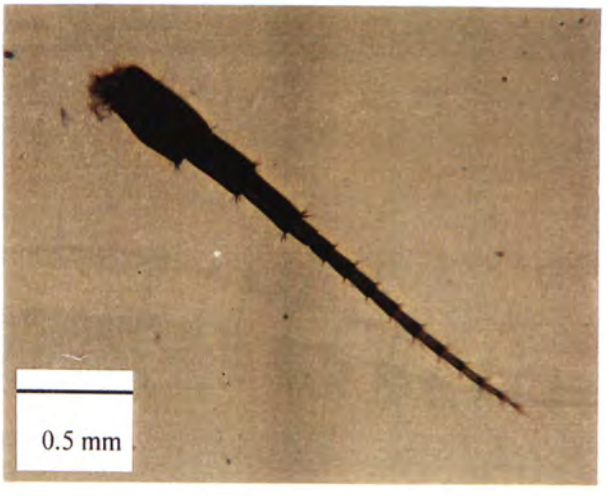


Figure 2.3 The line drawing of the cephalon of *Hyale* sp., male, lateral view.

A



B

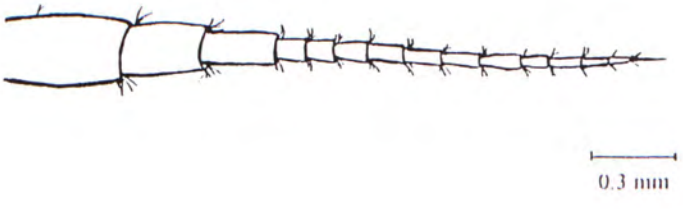


Figure 2.4 The left antenna 1 of *Hyale* sp., male, medial aspect, lateral view. A, photograph; B, line drawing.

A

B

Figure 2.5 The left antenna 2 of *Hyale* sp., male, medial aspect, lateral view. A, photograph; B, line drawing.

2. The mouthparts: This category is composed of the several components of the compact mouthparts. They are the lower (Figure 2.6) and upper lips (Figure 2.7), the mandible (Figure 2.8), the left maxilliped (Figure 2.9), the left maxilla 1 (Figure 2.10) and the left maxilla 2 (Figure 2.11).

3. The gnathopods: This category is composed of the left gnathopod 1 (Figure 2.12) and 2 (Figure 2.13) of a male, and the left gnathopod 2 of a female (Figure 2.14).

4. The pereopods: This category is composed of 5 left pereopods. They are the pereopod 3 (Figure 2.15), the pereopod 4 (Figure 2.16), the pereopod 5 (Figure 2.17), the pereopod 6 (Figure 2.18) and the pereopod 7 (Figure 2.19).

5. The abdominal appendages: This category is composed of 3 left pleopods, 3 left uropods and the telson (in a dorsal view). They are the pleopod 1 (Figure 2.20), the pleopod 2 (Figure 2.21), the pleopod 3 (Figure 2.22), the uropod 1 (Figure 2.23), the uropod 2 (Figure 2.24), the uropod 3 (Figure 2.25) and the telson (Figure 2.26).

2.3.2 Description of Key Characters

The amphipod was identified to be a member of the superfamily Talitroidea. This is the only taxon classified as a superfamily in the suborder Gammaridea (Barnard 1971). The superfamily Talitroidea is composed of two families. One is Hyalidae and the other is Hyalellidae. The amphipod species was determined to be a member of the family Hyalidae based on the following characteristics (Barnard 1971; 1974):

1. Body compressed laterally (Figure 2.2).

2. Coxa 1 visible and not less than half-surface area of coxa 2. Coxa is article 1 of pereonal appendage, expanding into a lateral lamella (Figure 2.2).

A B

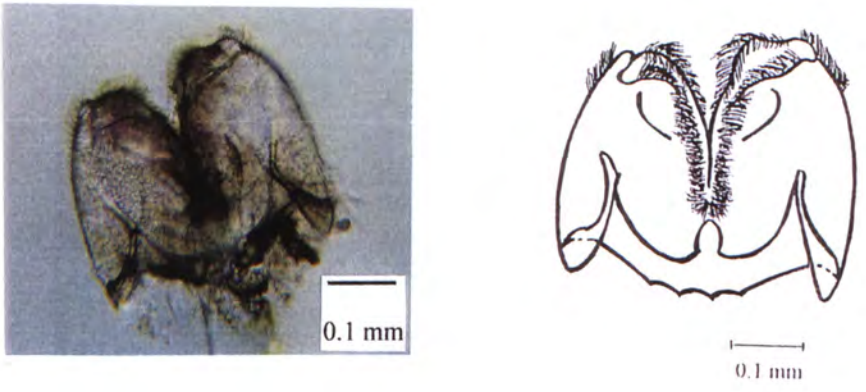


Figure 2.6 The lower lip (labium) of *Hyale* sp., male. A, photograph; B, line drawing.

A B

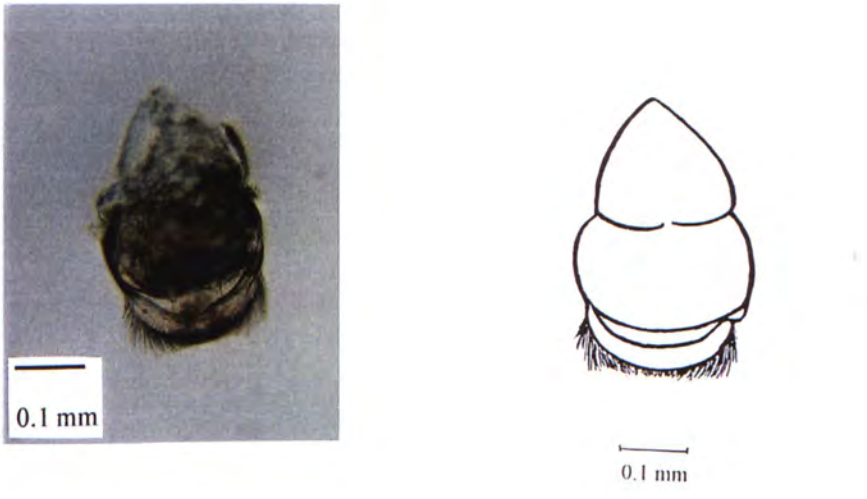


Figure 2.7 The upper lip (labrum) of *Hyale* sp., male. A, photograph; B, line drawing.

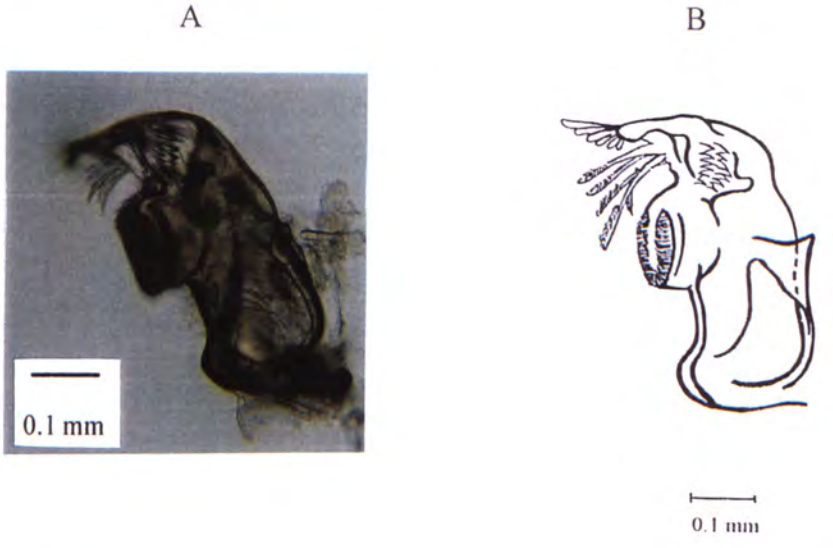


Figure 2.8 The mandible of *Hyale* sp., male. A, photograph; B, line drawing.

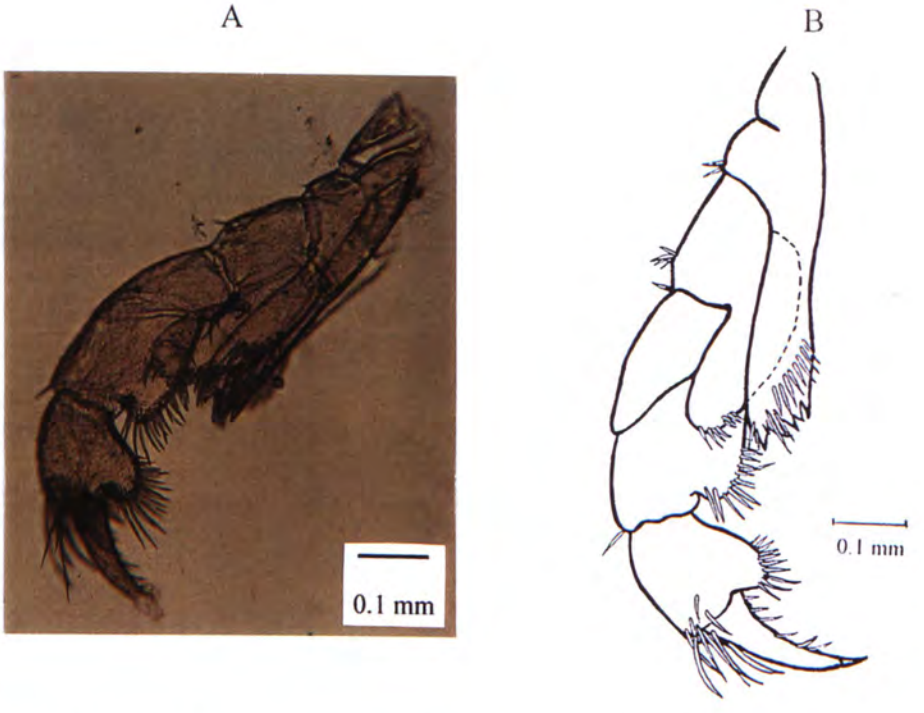


Figure 2.9 The left maxilliped of *Hyale* sp., male. A, photograph; B, line drawing.

A B

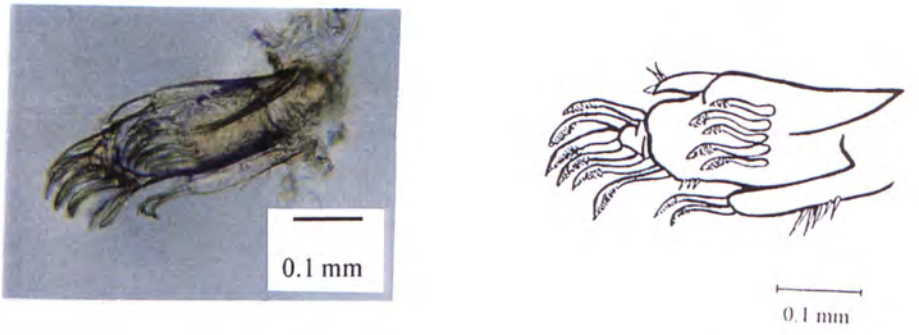


Figure 2.10 The left maxilla 1 of *Hyale* sp., male. A, photograph; B, line drawing.

A B

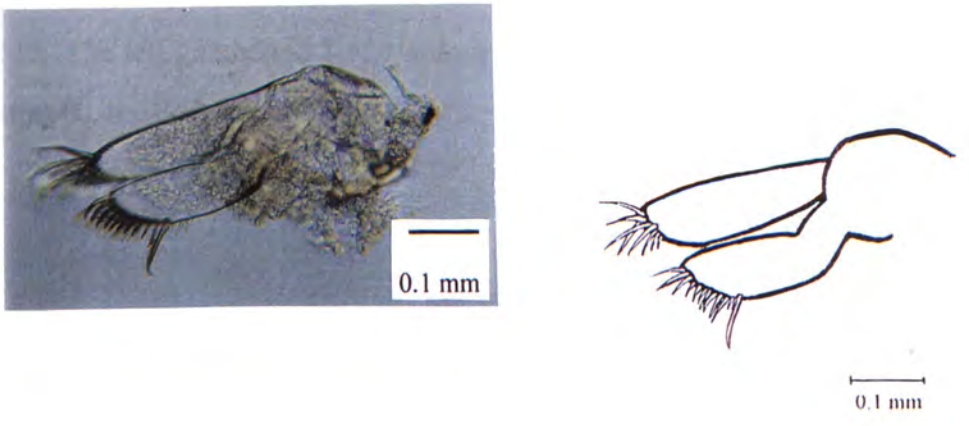


Figure 2.11 The left maxilla 2 of *Hyale* sp., male. A, photograph; B, line drawing.

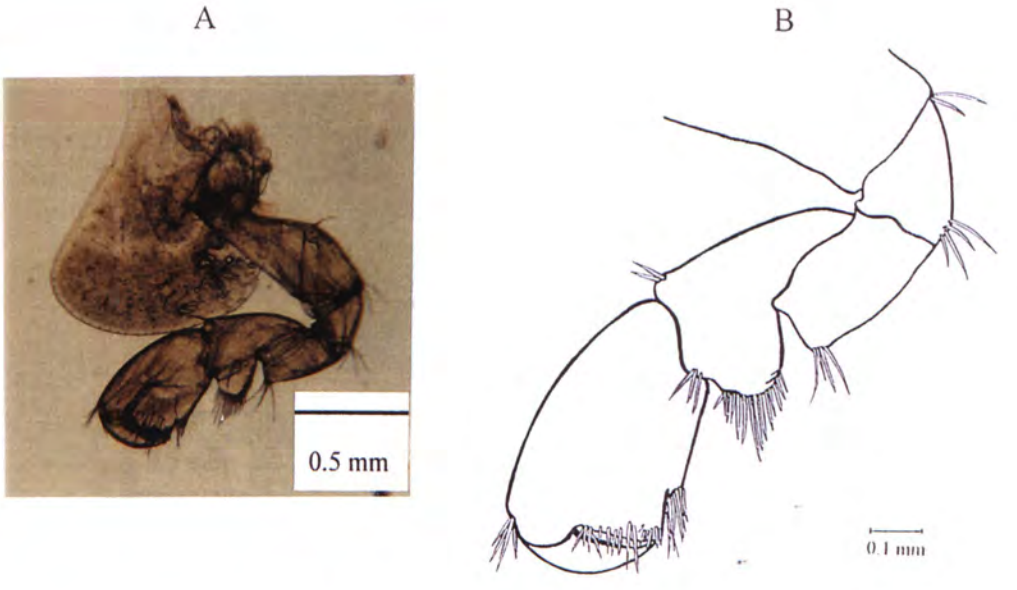


Figure 2.12 The left gnathopod 1 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

A

B

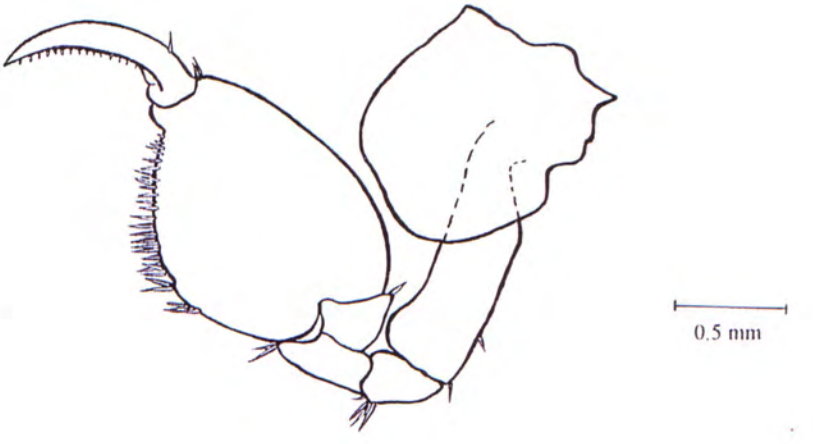


Figure 2.13 The left gnathopod 2 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

A

B

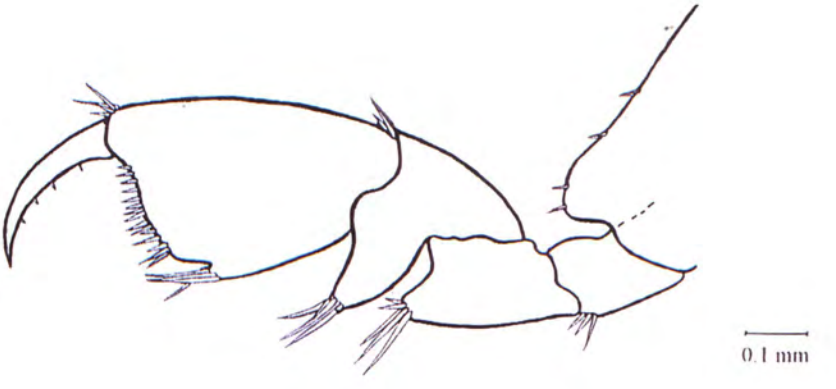


Figure 2.14 The left gnathopod 2 of *Hyale* sp., female, outer aspect, lateral view. A, photograph; B, line drawing.

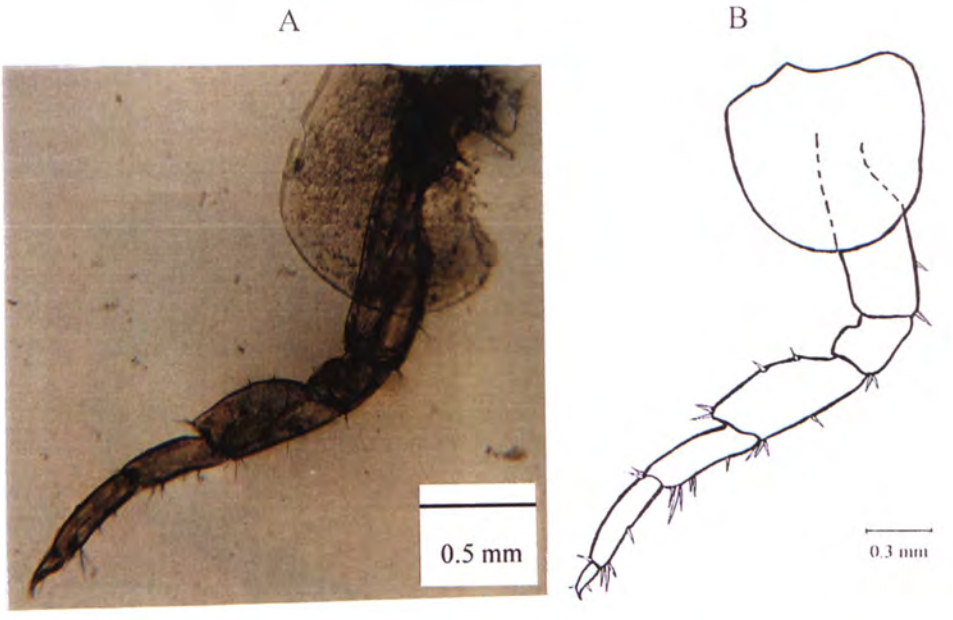


Figure 2.15 The left pereopod 3 of *Hyale* sp., male, outer aspect, lateral view.

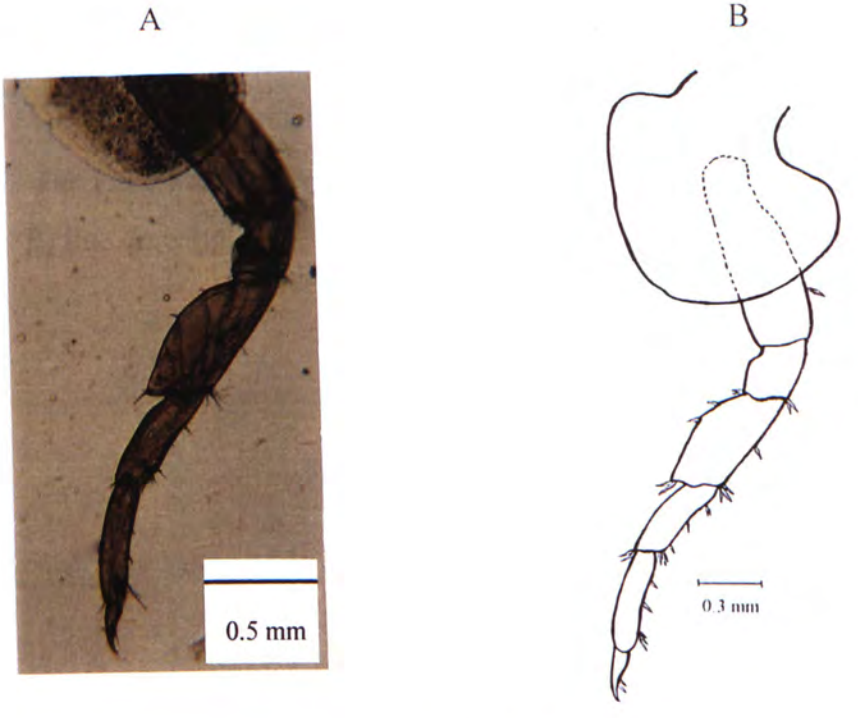


Figure 2.16 The left pereopod 4 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

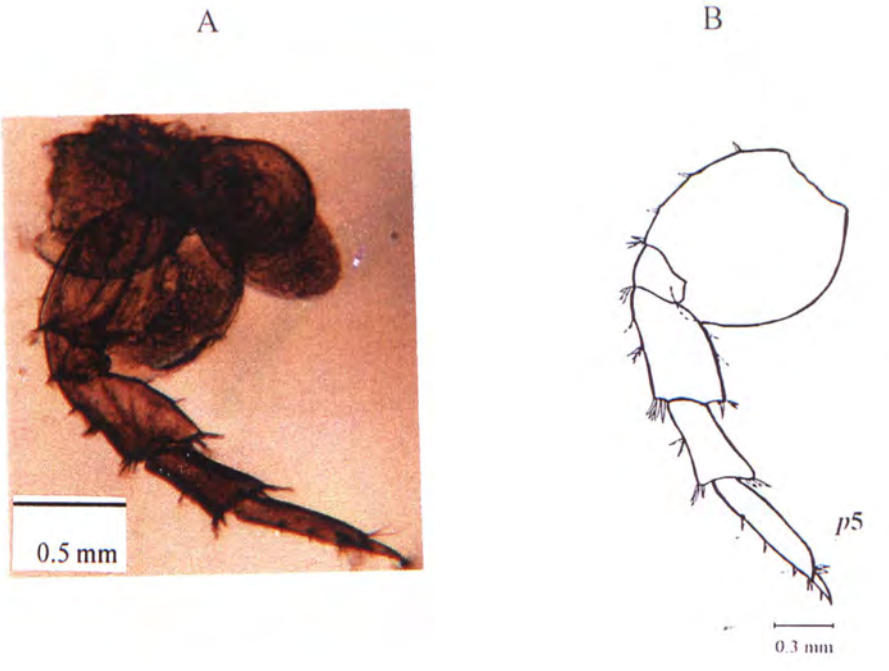


Figure 2.17 The left pereopod 5 of Hyale sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

A

B

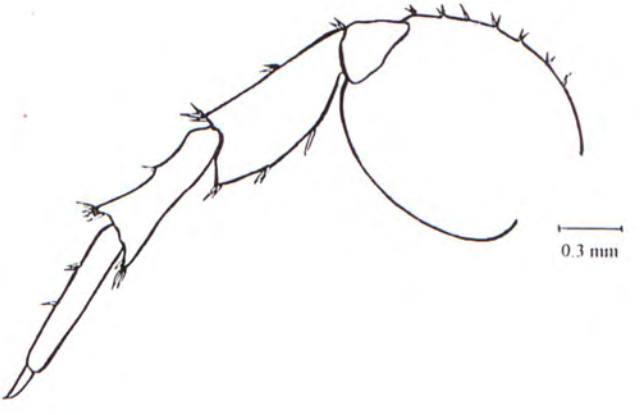
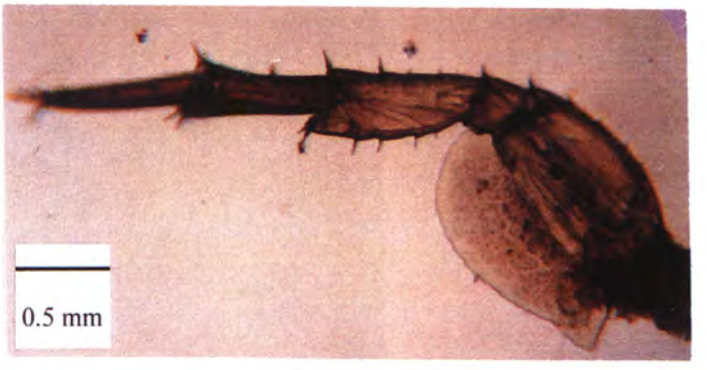


Figure 2.18 The left pereopod 6 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

A



B

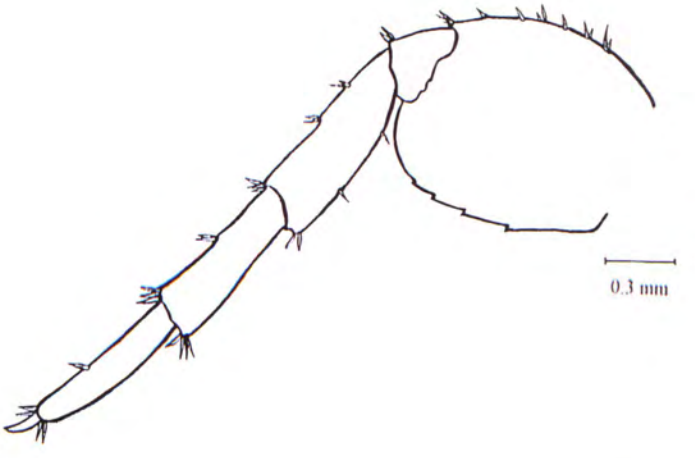


Figure 2.19 The left pereopod 7 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

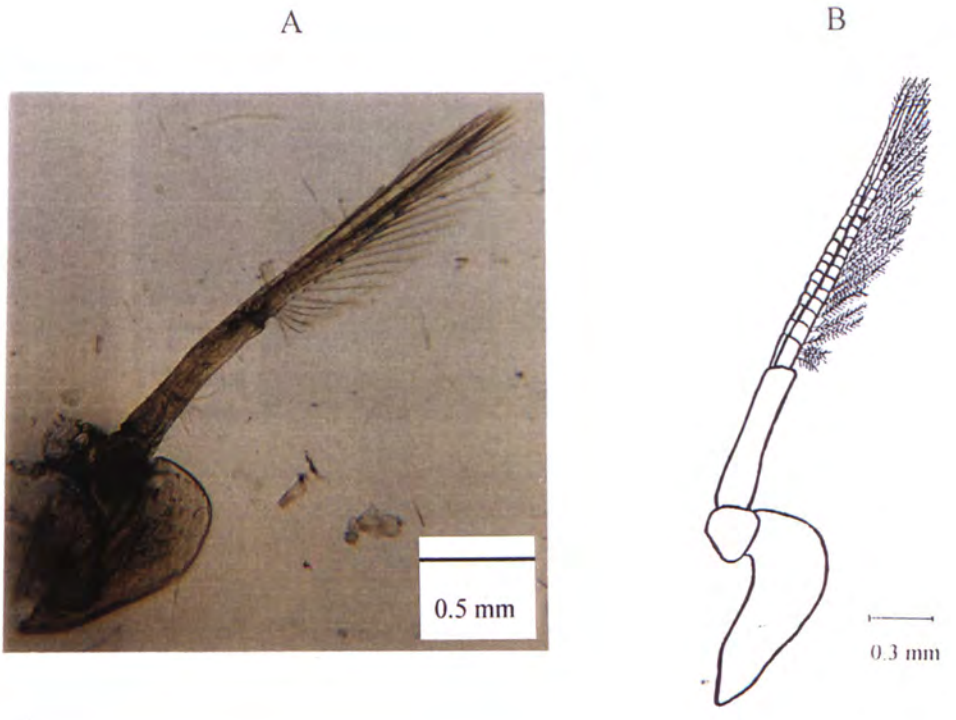
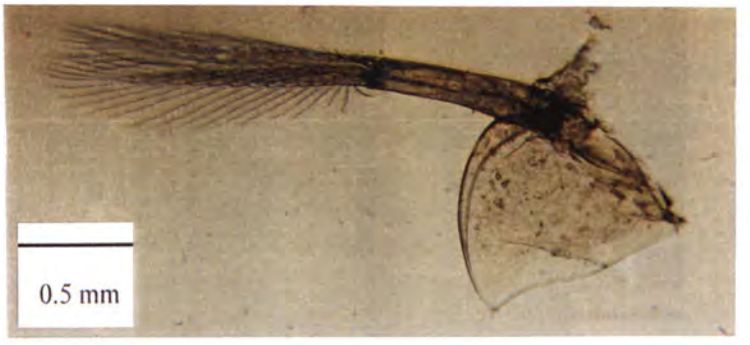


Figure 2.20 The left pleopod 1 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

A



B

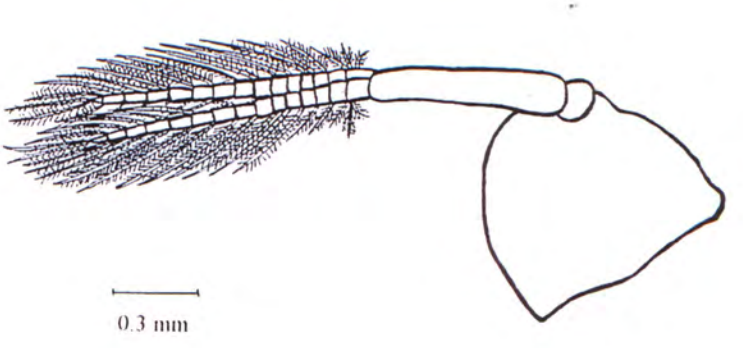
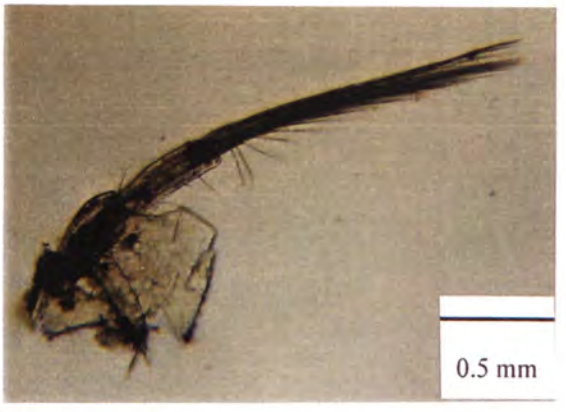


Figure 2.21 The left pleopod 2 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

A



B

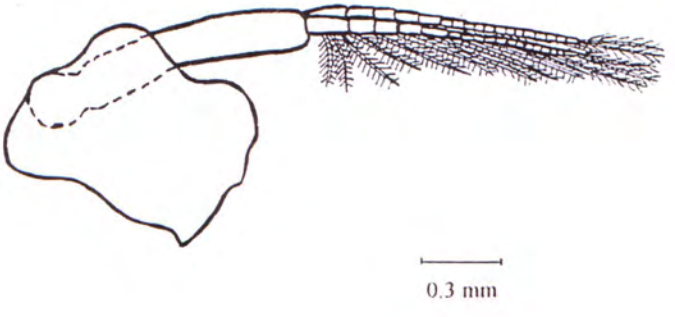
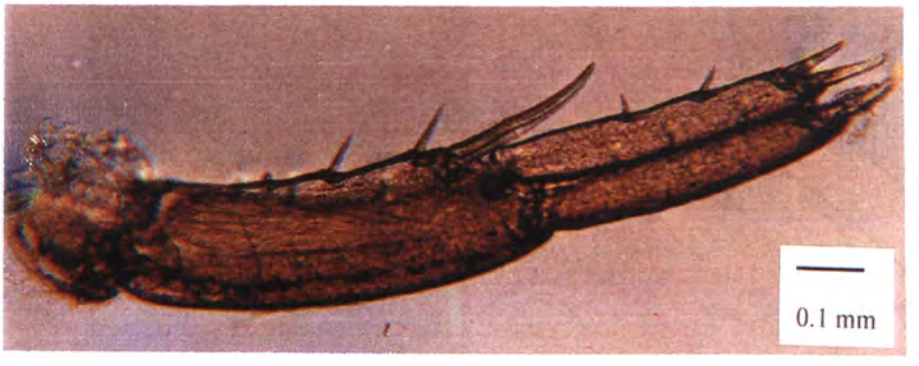


Figure 2.22 The left pleopod 3 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

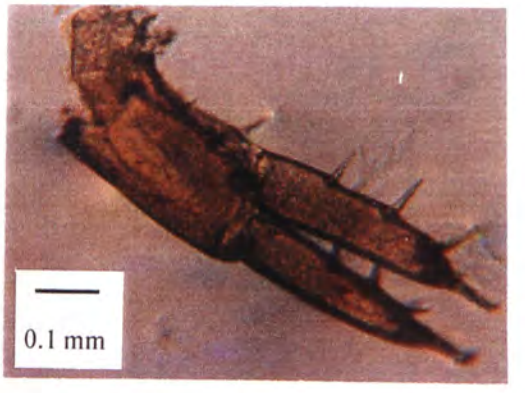
A



B

Figure 2.23 The left uropod 1 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

A B



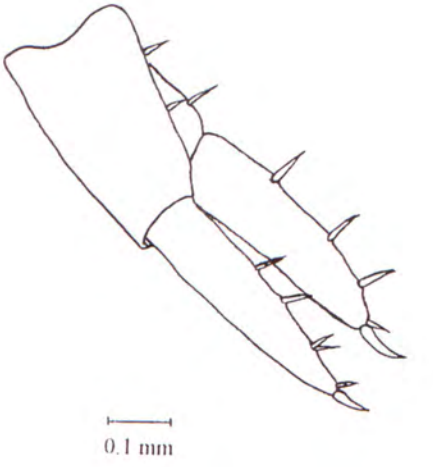


Figure 2.24 The left uropod 2 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

A B

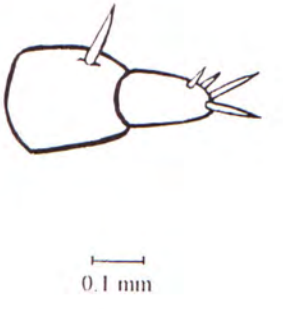


Figure 2.25 The left uropod 3 of *Hyale* sp., male, outer aspect, lateral view. A, photograph; B, line drawing.

A B

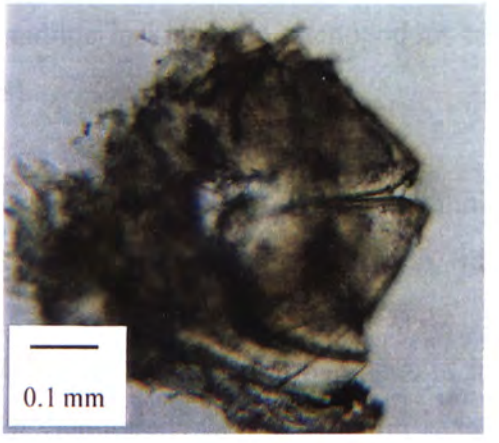


Figure 2.26 The telson of *Hyale* sp., male, dorsal view. A, photograph; B,

3. Accessory flagellum absent on antennae 1. No secondary ramus attached medially to peduncular article 3 on antennae 1 (Figure 2.4).

4. Mandible lacking palp (stenopodous terminal article of buccal appendage) (Figure 2.8).

5. Mandible with strongly triturative molar. The molar process subcylindrical, with rasp-like surface composed of teeth, ridges and cusps (Figure 2.8).

6. Gnathopods 1 & 2 (first two pairs of free thoracic appendages) subchelate. Article 6 of gnathopods with distal palm against which article 7 closes (Figures 2.12, 2.13 & 2.14).

7. Brood setae curl-tipped, with tangled apices (Figure 2.27).

8. Uropod 3 lacking inner ramus (Figure 2.25).

9. Telson cleft with coalesced base (Figure 2.26)

Following Barnard (1971), the amphipod was further identified to be a member of the genus *Hyale* Rathke based on the characteristics shown below.

1. Uropod 3 uniramous (Figure 2.25).

2. Article 5 (carpus) of the male gnathopod 2 masked between the article 4 (merus) and article 6 (propodus), unlobed (Figure 2.13).

The genus of the amphipod has been clarified but the species name is not yet confirmed at this stage. According to Barnard (1971, 1974), the species in the genus *Hyale* are differentiated by the specific locking spine, setae, patterns of setules on antenna 2 and the other specific microscopic structures. The identification of the

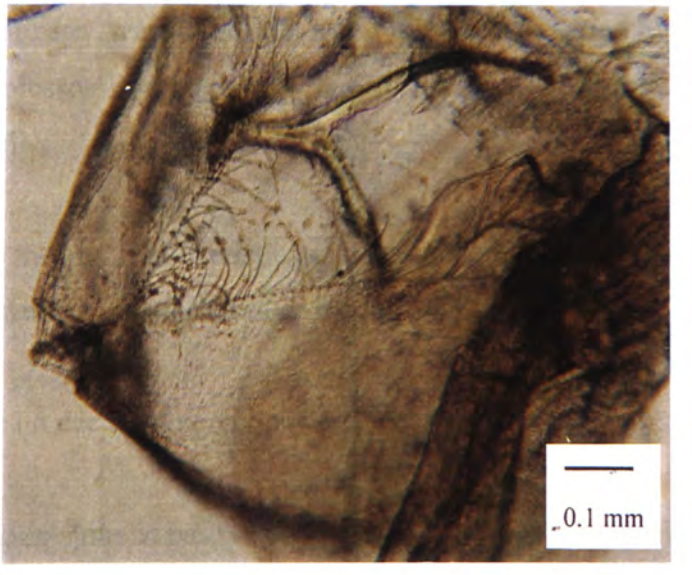


Figure 2.27 Brood pouch with curl-tipped brood setae in an ovigerous female *Hyale* sp., female, anterior view.

amphipod at the species level requires expert knowledge and literature on amphipod taxonomy. Thus specimens were sent to Professor Ren Xianqiu of the Institute of Oceanology, The Chinese Academy of Sciences, in Qingdao, China for identification in October 1997. Professor Ren suggested that the amphipod is most probably *Hyale crassicornis* Haswell.

According to the key characteristics of the amphipod shown below, the amphipod was preliminarily identified to be *Hyale crassicornis*.

1. Marginal setule on dactyl of pereopods 1-5 very stout.

2. Distalmost locking spine at base of dactyl larger than next proximal spine.

The amphipod specimens were also sent to Professor Eric A. Lazo-Wasem of the Peabody Museum of Natural History, Yale University, U.S.A. and Dr. Penny Berents of the Australian Museum, Sydney, Australia in March 1999 for identification. The two scientists have not yet completed the identification. Thus, the amphipod species being studied is still referred as *Hyale* sp. in this thesis.

Chapter 3 Growth of *Hyale* sp.

3.1 Introduction

Amphipods are widely used as biological indicators in environmental bioassays (Zander & Rojas 1992). Their wide applications are based on the well-documented biology of the amphipod species (ASTM 1992). Growth and reproduction of a particular species are the fundamental knowledge required to develop the species as a good biological indicator. In toxicological studies, the growth patterns and reproductive behaviors of amphipods are commonly employed for monitoring the level of environmental deterioration (McGee *et al.* 1995; Nipper & Roper 1995; Rice *et al.* 1995; Kubitz *et al.* 1996). Pascoe *et al.* (1994) suggested that the prevention and disruption of precopulatory guarding phase in *Gammarus pulex* is an effective response for the detection of cadmium contamination.

Conventionally, growth of amphipods is expressed as growth curves. A growth curve is simply represented by the variations of magnitude in one dimension of space (such as body length) to successive intervals of time (Thompson 1942). For example, the body lengths of both sexes of *Caprella okadai* and female *Caprella danilevskii* increased in sigmoidal patterns with successive instars, but the male *C. danilevskii* grows exponentially (Takeuchi & Hirano 1991, 1992). Growth can also be expressed by the relationship between the percentage molt increment on body length to the pre-molt body length. For example, the percentage molt increment of *Gammarus duebeni* decreases in a linear proportion with size (Hartnoll 1982).

Alternatively, growth of amphipods can be expressed in relative pattern. In relative growth studies, relationship between two growth dimensions at a time is analyzed simultaneously (Hartnoll 1982). Different patterns of relative growth occurs at the time of sexual maturation. In a study of allometric relationship between the number of flagellar articles on antenna 2 and body length of the male *Orchomene nanus*, the number of articles increased at greater rates than the body length did in

stages of post-maturation. Thus the flagellum was more elongated at each molt after sexual differentiation (Moore & Wong 1996). In *Orchestia gammarella*, both sexes have two immature phases and one mature phase in growth pattern as evidenced by the change in the level of allometry in the relative growth between the propodus of gnathopod 2 and the basis of pereopod 3 (Charniaux-Cotton 1957).

According to Hartnoll (1982), salinity is one of the most important extrinsic factors directly affecting growth of marine crustaceans. The salinity can exert highly variable effects on both molt increment and intermolt period. For example, the molt duration of postlarvae of the crab *Rhithropanopeus harrisii* prolonged at the salinities of 2.5 and 40‰ (Costlow *et al.* 1966). According to Chen *et al.* (1992), the growth rate of the juvenile fleshy prawn *Penaeus chinensis* was highest at isosmotic point (16.5‰). However, limited information is available to the effect of salinity on growth and molting patterns of amphipods.

In this project, the growth study of *Hyale* sp. was divided into two main parts. The first part focused on the growth patterns and sexual dimorphism in standardized laboratory conditions. Developmental phase change was studied based on the relative growth of different parameters. The relative growth of the two parameters Y and X, is expressed by the allometric equation, $Y = aX^b$ (Hartnoll 1982). The equation can be transformed into the logarithmic scale to obtain an equation $\log Y = b \log X + \log a$. In the equation, X, Y, and b represent the generalized body size (e.g. body length), sexually dimorphic characters (e.g. the propodus length of the left gnathopod 2) and the allometric coefficient (the slope of the regression line). Any change in allometric coefficient indicates the change in relative growth rate. The growth phase transition is revealed by the change in level of allometry.and dimension. The increase and decrease in allometric coefficient indicate a positively and negatively allometric growth, respectively. Yet an isometric growth is revealed by a constant value of allometric coefficient. Sexual dimorphism and maturation is thus identified by the change in relative growth rate of sexually dimorphic characters to body length. The length of body and head, the left eye length, the left antennae 1 and 2 and the propodus of the left

gnathopod 1 and 2, and the merus length of the left pereopod 7 were the growth parameters studied in this research. In the second part of the study, the growth patterns of juvenile *Hyale* sp. reared in salinities ranged from 10 to 40‰ were investigated. Since lengthening of the intermolt duration is observed in crustaceans under extremely high or low salinities (Hartnoll 1982), intermolt duration was also investigated in this study.

3.2 Materials and Methods

3.2.1 Growth in Standardized Environmental Conditions

3.2.1.1 Experimental Animals

Ovigerous females of *Hyale* sp. were randomly collected from outdoor tanks in MSL and placed individually in a 100-mL beaker in June 1998. They were reared in standardized laboratory conditions (see Section 3.2.1.2) to allow spawning. Two juveniles just released from the marsupium were randomly selected from each female. Juveniles were reared in 100-mL glass beakers individually. Each beaker was filled with 80 mL artificial seawater and covered with a sheet of perforated plastic film to avoid excessive evaporation. A total of 72 juveniles were used in the study but nearly two third of them died at the initial stages. Only 25 animals (including 9 males and 16 females) were followed throughout their life.

3.2.1.2 Culture Conditions

Commercially artificial seawater was used instead of natural seawater to rear the amphipods, because the source of natural seawater in Tolo Harbor was suspected to be contaminated (Morton 1982; Wu 1982; Hodgkiss & Chan 1983; Chan *et al.* 1990; Chan 1992; Emson *et al.* 1992; Rainbow & Smith 1992). The use of artificial seawater could standardize the water quality and eliminate any contamination. Artificial seawater was made by mixing 33 g of commercial sea salt (Instant Ocean®) into one liter distilled

water. The salinity of artificial seawater was adjusted to 30‰ by the addition of either the commercial salt or distilled water. The pH value of the artificial seawater was adjusted to 7.8 by the addition of either 1M hydrochloric acid or 1M sodium hydroxide. Artificial seawater was actively aerated for at least one hour before use. Each individual was cultivated in 80 mL of the artificial seawater at 25°C and the photoperiod of 14:10 h light-dark cycle by incubating the experimental vessels inside an environmental chamber (Shel-lab model 2015). Each individual was fed with a small piece (1–2 mm^3) of chopped freeze-dried krill. A piece of the green alga thallus *Ulva lactuca* (10 cm^2) was also provided as substratum and supplementary food source. Its presence could improve the water quality by assimilation of metabolic waste and oxygen liberation during light period. Regular replacement of the thallus piece was not necessary unless it was severely grazed.

Water quality was maintained by means of static-renewal technique (APHA, AWWA, WPCF 1985). Half volume of water (about 40 mL) was removed from a culture beaker every two days and then replaced by the same volume of freshly prepared and well-aerated artificial seawater. All freshly prepared artificial seawater was actively aerated for at least one hour and checked for the dissolved oxygen content (with more than 80% O_2 saturation, > 4.85 mg L^{-1}). Any fecal matter, uneaten food, exuvium and any undesirable algal colony were removed during the procedure. A small piece of freshly prepared krill flesh was added to the culture beaker after water exchange.

The physical parameters of the environmental conditions and water quality in experimental vessels were monitored every two days. The water temperature was measured with a digital long stem thermometer (model NEW 61220-416). The salinity of artificial seawater was measured with a refractometer (Sper Scientific, model W/ATc 300011). The pH value of the artificial seawater was measured by a pH meter (pH Testr. 3 model 59000-30). The dissolved oxygen content was monitored by a D.O. meter (YSI model 5000). The light intensity was recorded by means of a photometer (LI-COR model LI-189).

The experimental temperature was recorded to vary within the range of 25 ± 2°C (mean ± standard deviation). The salinity and pH value were measured in 30 ± 3‰ and 7.8 ± 1.0, respectively. The dissolved oxygen content and light intensity varied within the ranges of 4.9 – 7.0 mg L^{-1} and 857 – 1212 lux, respectively.

3.2.1.3 Examination and Recording

Amphipods were examined for any evidence of molting every day. Any exuvium left in the culture beakers showed the molting of the amphipod. The day of an exuvium discovery was regarded as the day of molting. Any exuvium found was removed from the beaker after examination and recording. The developmental stage of the juvenile just emerged from the marsupium of ovigerous females was designated as instar 1 (Takeuchi & Hirano 1991).

The growth study was based on the measurement of the following parameters, the body length, the head length, the eye length, the length of the left antennae 1 and 2, the articular number of the left antennae 1 and 2, the merus length of the left pereopod 7, and the propodus length of the left gnathopod 1 and 2. Figure 3.1 illustrates the measurement of the different growth parameters in this study.

i. The body length (BL) was measured from the dorsal tip (rostrum) of the head to the posterodistal end of urosomite 3 in a straight posture as suggested by Hirayama (1986). In the present study, the measurement basically followed Hirayama (1986). However, it was difficult to measure the body length of a live *Hyale* sp. in a straight posture because any manipulation applied on amphipods could cause physical damage to such small animals. Thus the measurement was only made on its naturally curved body to minimize any disturbance to the animals. In a preliminary study, the body length measured in the straight posture was significantly greater than that in naturally curved posture ($P < 0.001$; Student's *t*-test). In 20 individuals, the mean percentage difference in body length measured between two methods was 6.9 ± 0.8% (mean ± standard error).

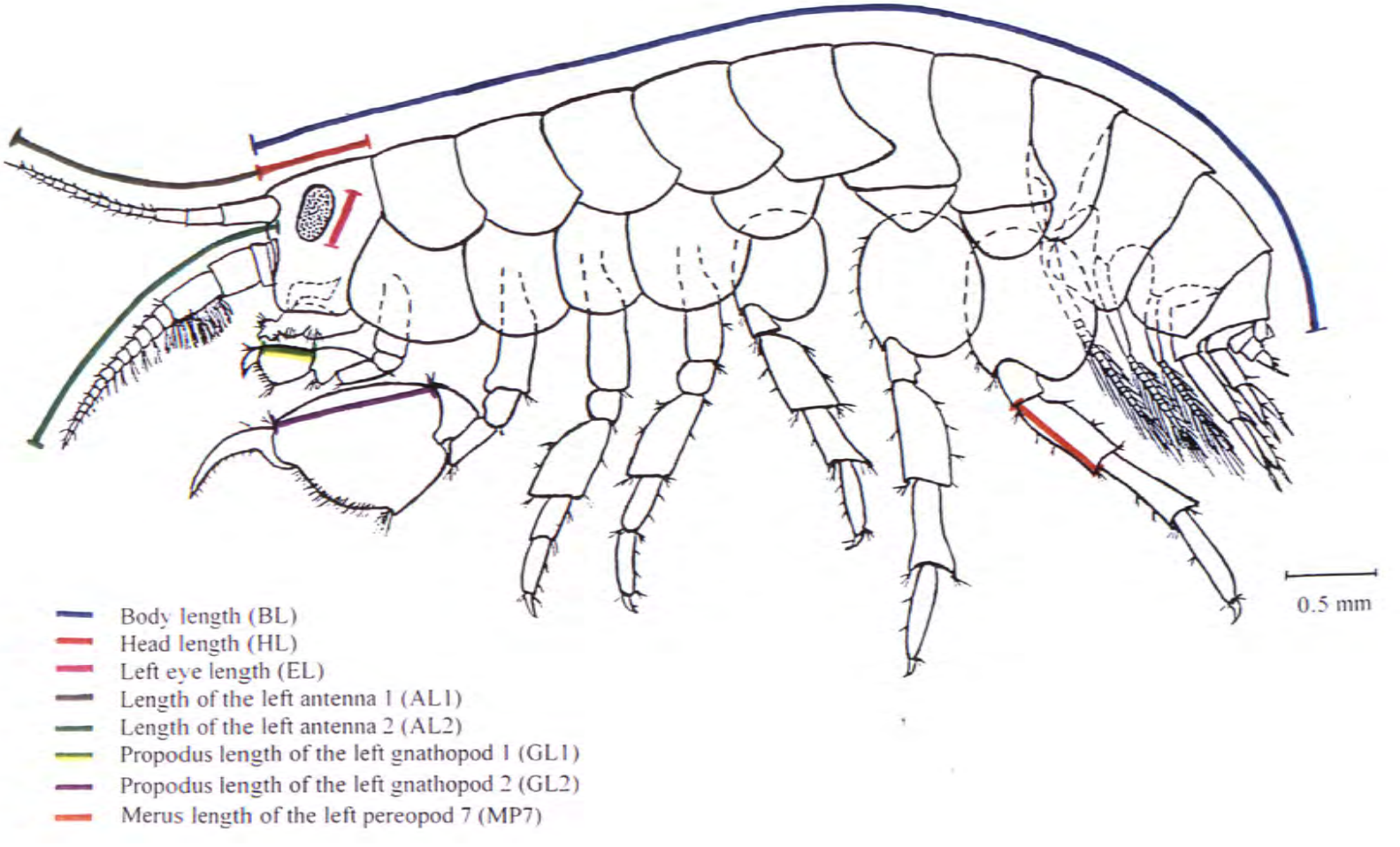


Figure 3.1 An illustration of the measurements on the growth parameters of *Hyale* sp. in the growth study.

ii. The head length (HL) was measured from the dorsal tip to the posterior margin of the head.

iii. The eye length (EL) was defined as the longest diameter across the slightly elliptical left eye.

iv. The length of the antennae 1 (AL1) and 2 (AL2) were measured from the posterior end of the peduncle to the anterior tip of the flagellum on the left antenna 1 and 2, respectively.

v. The number of articles on the antennae 1 (AA1) and 2 (AA2) were the total number of articles, including peduncular and flagellar articles, on the left antennae 1 and 2, respectively.

vi. The merus length of the pereopod 7 (MP7) was measured on the anterior shaft of the article merus in between 2 adjacent joints of ischium and carpus on the left pereopod 7.

vii. The propodus length of the left gnathopods 1 (GL1) and 2 (GL2) were measured on the straight length of the dorsal side on the propodus in between 2 adjacent joints of the dactylus and carpus on the left gnathopods 1 and 2, respectively.

The parameters were measured one day after each molting (the beginning of each instar). Each amphipod was followed throughout its life span in this study, any physical damage to the animal was minimized during the measurement. The amphipod was sedated and secured by covering it with a sheet of plastic film (area, 3 x 2 cm^2). The amphipod was then held and trapped by a layer of water film in between the glass slide and the plastic film. The measurement was limited within two minutes to minimize any influence on subsequent growth of the amphipod due to hypoxia and environmental stress. All the parameters stated were examined under a compound microscope (Nikon model SE) and recorded in a videotape with a color camera (Teli ®

CCD CS 5110) connected to a television set (Sharp VT-1428). The body parts were captured in video images. The length of the growth parameters in study were measured by using an image analyzing programme (Quantimet 500C).

3.2.2 Growth of Juveniles in Different Salinities

3.2.2.1 Experimental Animals

Ovigerous females of *Hyale* sp. were randomly collected from the outdoor tanks. They were acclimated individually in 100-mL glass beakers. The laboratory conditions were standardized as described in Section 3.2.1.2 in the acclimation. All females were acclimated under the standardized conditions for at least three days. Two newly hatched juveniles were randomly selected from each female. A total of 112 juveniles were used in this study. They were reared in a 100-mL beaker individually. Each beaker was covered with a sheet of perforated plastic film so as to prevent any change of the salinity due to excessive evaporation. Juveniles were followed until 50 days of age, so that all individuals had completed their juvenile phase during the study.

3.2.2.2 Culture Conditions

The growth of the juvenile *Hyale* sp. was studied in four different salinities, 10, 20, 30 and 40‰. Artificial seawater with different salinity was made by mixing different amounts of commercial sea salt (Instant Ocean®) in distilled water. The other environmental conditions were standardized as the same as described in Section 3.2.1.2. The pH value of the artificial seawater was adjusted to 7.8. Each juvenile was cultivated in 80 mL artificial seawater with selected salinity at 25°C and the photoperiod of 14:10 h light-dark cycle by incubating the experimental vessels inside an environmental chamber (Shel-lab model 2015). Newly introduced juveniles were acclimated to the testing salinities by adjusting the salinity at a rate of 5‰ per day.

The procedures of feeding and water exchanging were basically the same as those described in Section 3.2.1.2. But the food materials were specially treated before added. Pieces of krill flesh and thallus were immersed into the artificial seawater of respective salinity for two hours before being introduced into the culture beakers. The physical parameters of the experimental environment and water quality were monitored every two days by the equipment as described in Section 3.2.1.2.

The salinity was recorded within the range of 10 ± 2‰, 20 ± 2‰, 30 ± 3‰ and 40 ± 3‰ independently. The experimental temperature and the pH value varied in 25 ± 2.0°C (mean ± standard deviation) and 7.8 ± 1.0, respectively. The dissolved oxygen content and light intensity varied within the ranges of 4.9 – 8.0 mg L^{-1} and 984 – 1566 lux, respectively.

3.2.2.3 Examination and Recording

Amphipods were examined for any evidence of molting every day. The procedures and the equipment employed for the parameter measurement were the same as those described in Section 3.2.1.2. All the parameters were measured one day after each molting. The amphipods were followed for 50 days of age. Based on the results obtained from the growth study in standardized conditions (Section 3.3.1), no significant difference between the two sexes was observed in the following parameters at the first 6 instars: BL ($P = 0.238$; Student's *t*-test), HL ($P = 0.180$), EL ($P = 0.485$), AL1 ($P = 0.094$), AA1 ($P = 0.169$), AA2 ($P = 0.117$), and MP7 ($P = 0.053$). The parameters generally started the significant deviation between the two sexes at instar 7. Therefore, the growth parameters of juveniles measured in this study were only limited within the first 6 instars.

3.2.3 Morphometric Analysis on Length - Weight Relationship

Two hundred and five amphipods, including 60 males, 60 non-gravid females, 62 gravid females and 23 sexually undifferentiated juveniles, were randomly collected from the outdoor tanks in MSL.

They were anesthetized with lignocaine HCl 1%. The body length of the inactivated amphipods was measured under the compound microscope and the television set as described in Section 3.2.1.3. The amphipods were air-dried and placed into pre-weighed aluminium weighing capsules (N241-1255) individually. They were weighed with an analytical balance (CAHN C-31 model 10931-01F) to the nearest 0.001mg. The air-dried amphipod with capsule was incubated at 60°C for 3 days and the dry weight was determined.

3.2.4 Statistical Analysis

All statistical analysis applied in this study, including linear regression, Student's *t*-test, and 2-factor analysis of variance (ANOVA) were based on Zar (1996). Differences in growth, intermolt duration and juvenile growth in different salinities were determined by using 2-factor ANOVA and Tukey test. The computer software Sigmastat version 2.01 was employed.

3.3 Results

3.3.1 Growth in Standardized Environmental Conditions

3.3.1.1 Life Span

The relationship between life span and number of instars is shown in Table 3.1. The mean life span of males and females were 178 ± 53 days (mean ± standard deviation) and 175 ± 45 days, respectively. There was no significant difference in the

Table 3.1. Life span of *Hyale* sp. The table shows the life span and the total no. of instar of each individual. A. male; B. female.

A. Male

Individual number	1	2	3	4	5	6	7	8	9	Mean ± SD (sample size)
Life span (day)	262	164	248	191	199	167	111	136	121	177.7 ± 52.9 (9)
Total no. of instar (instar)	19	13	15	15	16	15	12	12	12	14.3 ± 2.3 (9)

B. Female

Individual number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Mean ± SD (sample size)
Life span (day)	170	201	211	159	254	150	99	143	143	258	213	138	122	185	197	150	174.6 ± 45.2 (16)
Total no. of instar (instar)	17	17	18	18	21	15	13	15	16	18	21	13	18	20	17	11	16.8 ± 2.8 (16)

mean life span between the two sexes (P = 0.878; Student's *t*-test). The maximum life span of males and females were 262 and 258 days, respectively.

The mean number of instars over the life span of males and females were found to be 14 ± 2 and 17 ± 3, respectively. There was a significant difference between the two sexes (P = 0.041; Student's *t*-test). The result indicated that the female has more instar numbers than the male in their life span. The maximum number of instar in males and females reached was 19 and 21, respectively.

3.3.1.2 Molt cycle

3.3.1.2.1 Age

The relationship between the number of instars and the corresponding age of *Hyale* sp. is shown in Table 3.2 and Figure 3.2. Statistical analysis showed that the mean age of males was significantly greater than that of females since instar 13 (P = 0.039; Student's *t*-test). At instar 13, the mean age of males was 131 ± 10 (N = 6) and that of females was 110 ± 25 days (N = 15). Data analysis only limited to instar 15 in males (N = 5) and instar 18 in females (N = 7) in this growth study. Data collected from any developmental stages greater than instar 16 (N = 2) in males and instar 19 (N = 3) in females were rejected because of small sample size (N < 5). The same situation was also applied to the data analysis of the other growth parameters.

3.3.1.2.2 Intermolt Duration

The mean duration of each instar was shown in Table 3.3 and Figure 3.3. The mean intermolt duration of males increased gradually from 2 ± 1 days at instar 1 to 10 ± 4 days at instar 3, after which the intermolt duration became relatively stable in the range between 10 ± 6 and 11 ± 4 days from instar 4 to 9. From instar 10 to 14, the intermolt duration ranged between 16 ± 6 and 17 ± 3 days. In females, the mean intermolt duration increased gradually from 3 ± 2 days at the instar 1 to 9 ± 5 days at

Table 3.2 The mean age of *Hyale* sp. at different no. of instar. A, male; B, female.

A. Male

No. of instar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Age (day)	0.0	2.4	9.2	19.4	28.8	39.2	49.9	57.8	69.2	80.7	97.0	112.1	131.3	148.6	165.8	177.0	215.0	226.0	251.0
± SD	0.0	0.9	4.1	6.4	5.1	7.9	7.5	6.9	10.1	12.4	12.0	12.3	10.1	10.1	10.4	11.3	/	/	/
Sample size	9	9	9	9	9	9	9	9	9	9	9	9	6	5	5	2	1	1	1

B. Female

No. of instar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Age (day)	0.0	3.4	8.2	14.4	23.5	32.6	42.8	55.0	64.5	75.4	87.3	99.8	110.3	121.7	133.0	144.9	159.4	166.6	172.0	191.0	220.5
± SD	0.0	1.7	3.7	5.6	45.8	9.2	11.7	15.0	16.3	17.8	21.8	23.4	25.0	28.9	29.1	31.4	35.6	45.5	29.2	30.8	44.5
Sample size	16	16	16	16	16	16	16	16	16	16	16	16	15	13	13	11	10	7	3	3	2

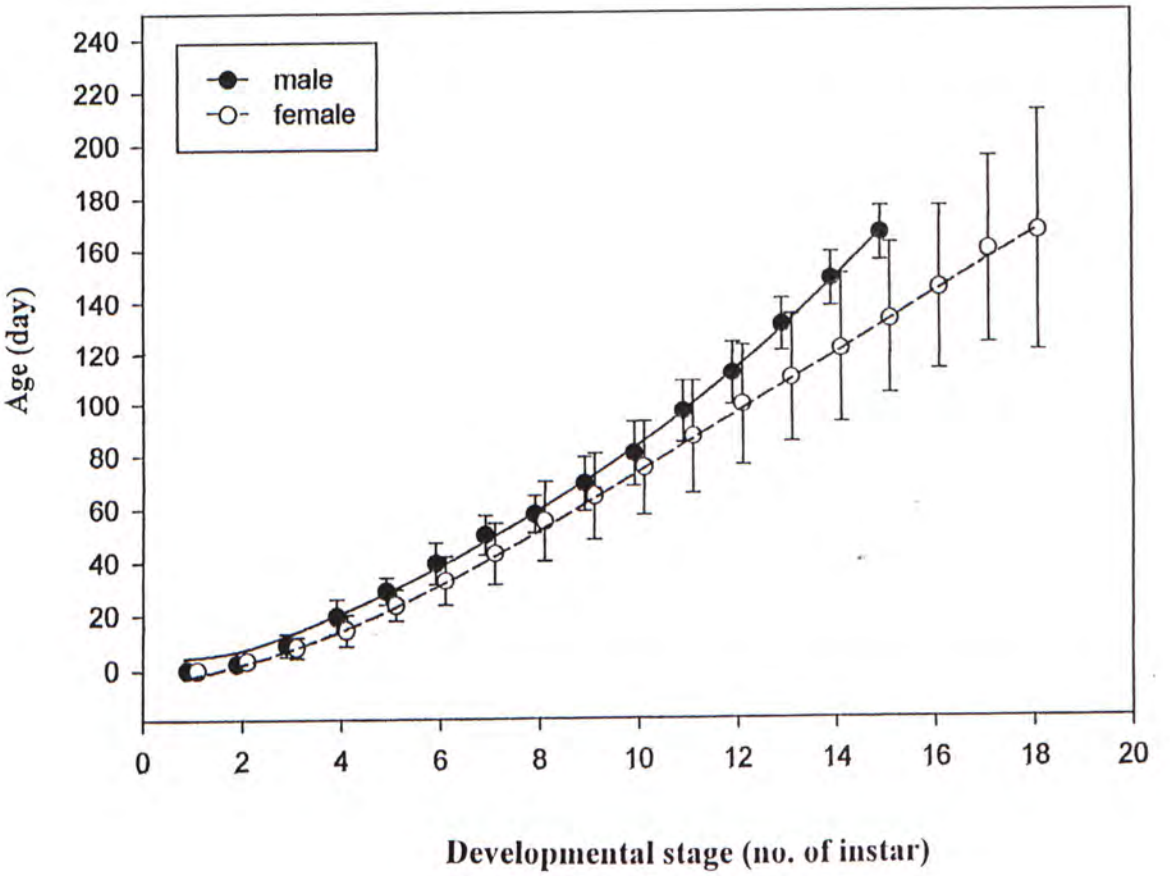


Figure 3.2 Age of *Hyale* sp. in different instars. The regression equations of the growth curves of males and females are $Y = 0.49X^2 + 4.01X - 5.32$ ($r^2 = 0.999$) and $Y = 0.19X^2 + 6.88X - 12.43$ ($r^2 = 0.998$), respectively. X represents number of instar and Y represents age (age of the first day of each instar). The error bars represent the standard deviation.

Table 3.3 The mean duration of each instar of *Hyale* sp. A, male; B, female.

A. Male

No. of instar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Duration (day)	2.4	6.8	10.2	10.3	10.4	10.7	7.1	11.6	11.4	16.3	15.1	17	16.2	17.4	*16.5	46.0	11.0	25.0	/
± SD	0.9	3.7	3.7	5.8	7.1	4.7	1.5	4.2	4.3	6.2	3.2	2.9	1.8	3.3	0.7		/	/	/
Sample size	9	9	9	9	9	9	9	9	9	9	9	9	6	5	2	1	1	1	/

B. Female

No. of instar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Dduration (day)	3.4	4.8	6.1	8.4	9.4	8.9	12.2	9.5	10.9	11.3	12.1	13.5	11.6	11.3	12.0	13.9	14.7	19.7	19.0	18.0	/
± SD	1.7	2.9	3.0	5.3	5.8	4.9	6.5	3.5	4.0	5.5	5.3	5.7	4.5	5.8	3.5	5.8	7.2	8.5	6.9	11.3	/
Sample size	16	16	16	16	16	16	16	16	16	16	16	15	13	13	11	10	7	3	3	2	

* The readings in italic are rejected data.

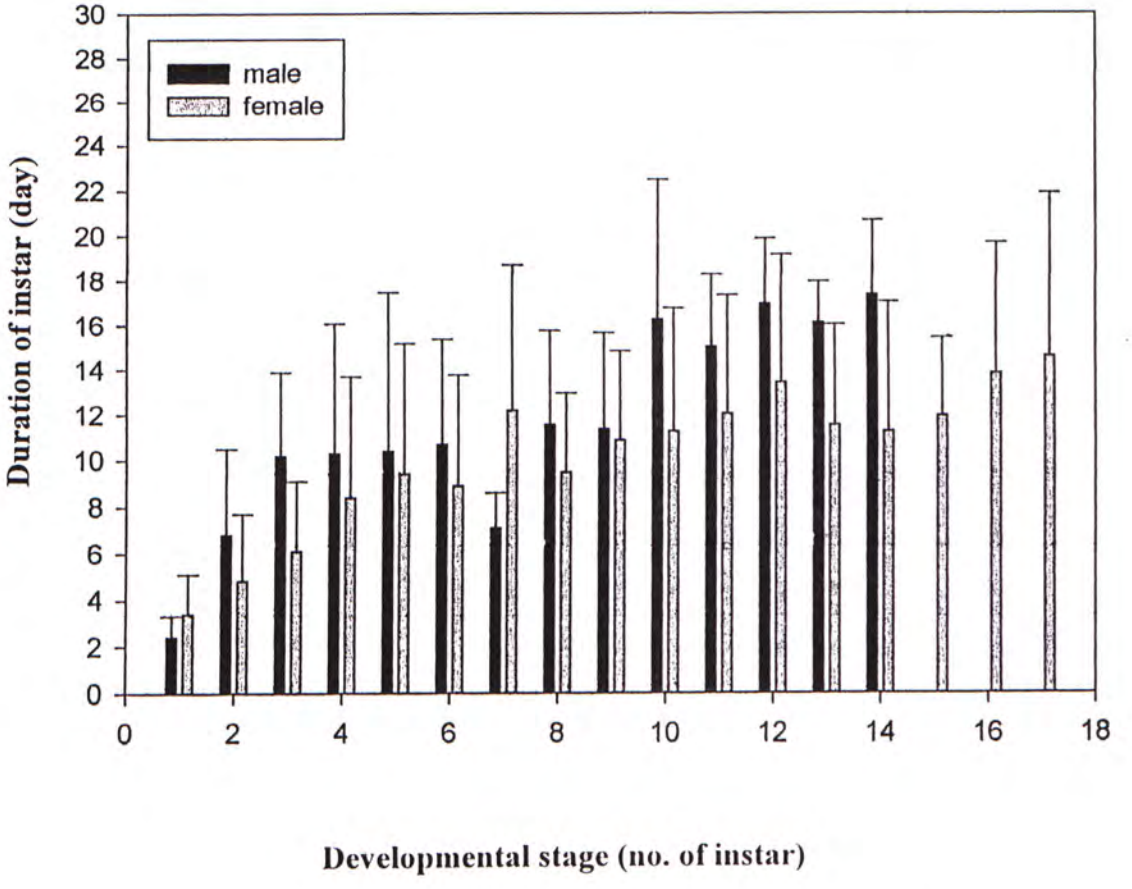


Figure 3.3 Mean intermolt duration of each instar. The error bars represent the standard deviation.

instar 5, after which the intermolt duration became slightly increased from 9 ± 5 days at instar 6 to 15 ± 7 days at instar 17.

The effects of sex and number of instar on the intermolt duration were analyzed in 2-factor ANOVA. The analysis showed that there was no significant difference between the two sexes in intermolt duration ($P = 0.075$), but there was a significant difference in intermolt duration among instars ($P < 0.001$).

3.3.1.3 Body Length (BL)

Growth curve constructed by integrating data on mean values of growth increment in BL and age is shown in Figure 3.4. The growth curves could also be expressed in the mean BL versus the number of instar (Figure 3.5). Body length of the two sexes increased gradually in sigmoid patterns. The increase in BL stabilized after instar 14 in males and instar 17 in females. The maximum BL measured in males and females were 11.02 mm at instar 16 and 11.38 mm at instar 17, respectively. The effects of sex and number of instar on body length increment were analyzed in 2-factor ANOVA. The analysis showed that BLs were significantly different among instars ($P < 0.001$), and the mean BL of males was significantly greater than that of females ($P < 0.001$). The significant difference in mean BL between the two sexes started at instar 7 ($P = 0.016$; Tukey test after 2-factor ANOVA).

The relationships of the percentage molt increment in BL to the pre-molt BL of males and females are shown in Figures 3.6A and B, respectively. The maximum percentage increment of males and females were 70.8% with 4.21 mm of pre-molt BL at instar 5 and 91.3% with 2.42 mm pre-molt BL at instar 4, respectively. Such high percentage increment did not frequently occur. Only 14.2% of males and 4.0% of females grew in the increment above 40% on the pre-molt BL at instar 1 to 7.

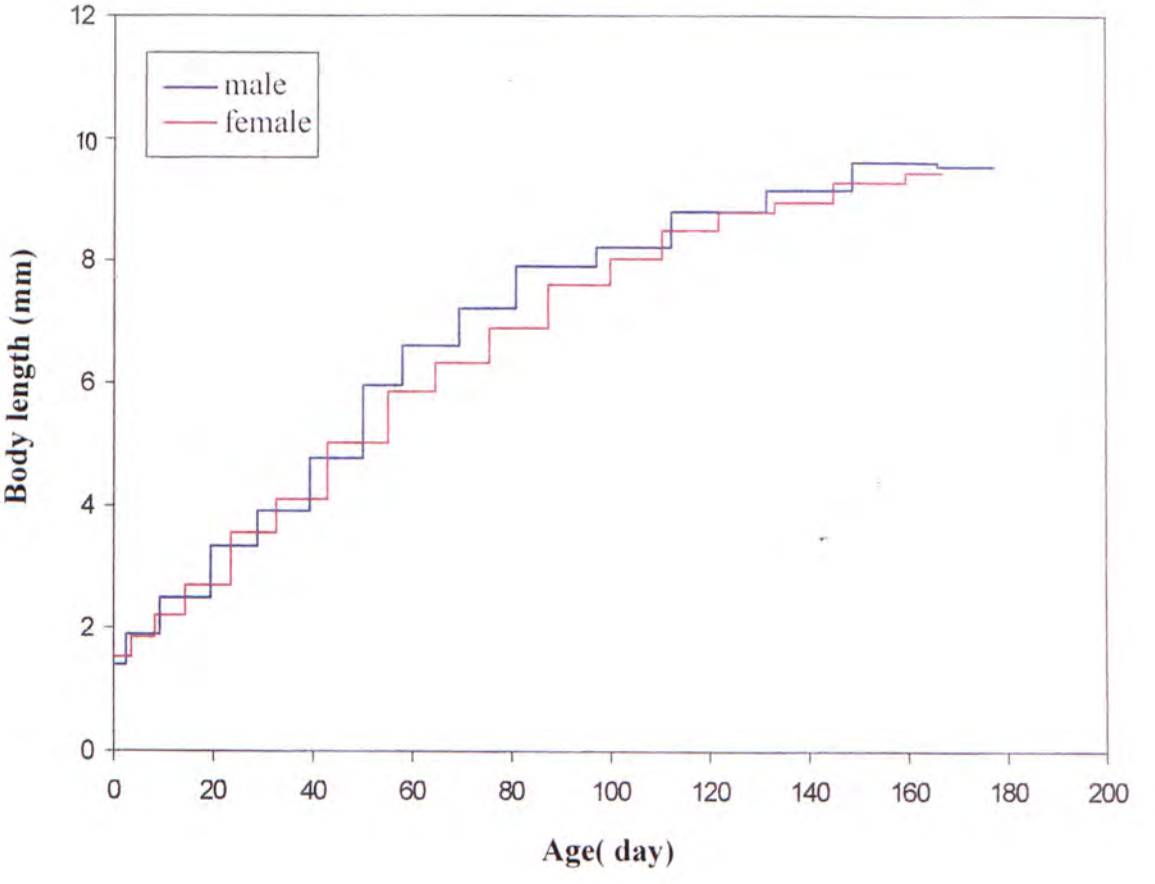


Figure 3.4 Growth curves of *Hyale* sp. in mean body length at different mean ages. Each increment represents the increase in mean body length between two subsequent instars. The intermolt growth is excluded from the study.

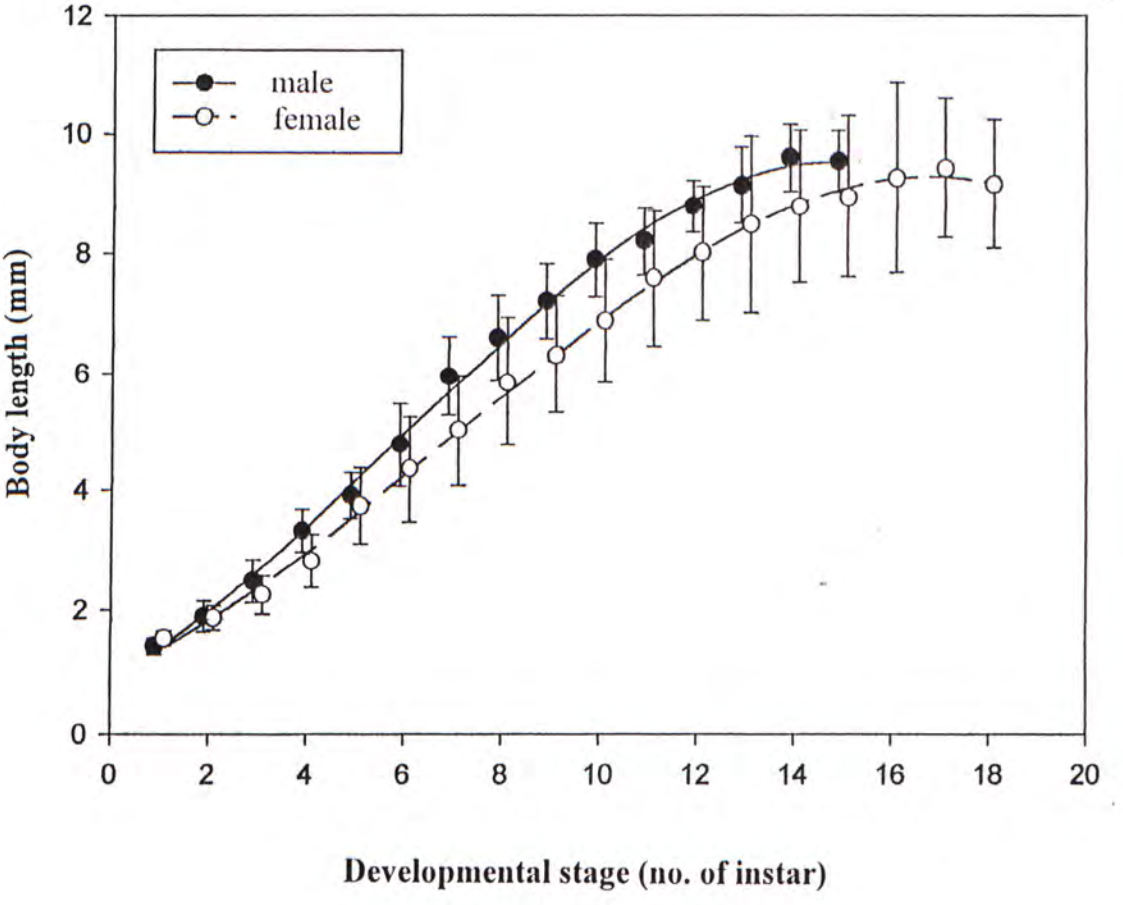


Figure 3.5 Growth curves of *Hyale* sp. in mean body length (BL) at different instars. The regression equations of the growth curves in males and females are $Y = 0.003X^3 + 0.058X^2 + 0.456X + 0.862$ ($r^2 = 0.998$) and $Y = 0.002X^3 + 0.047X^2 + 0.356X + 0.948$ ($r^2 = 0.999$), respectively. In the equations, X represents number of instar and Y represents BL. The error bars represent the standard deviation.

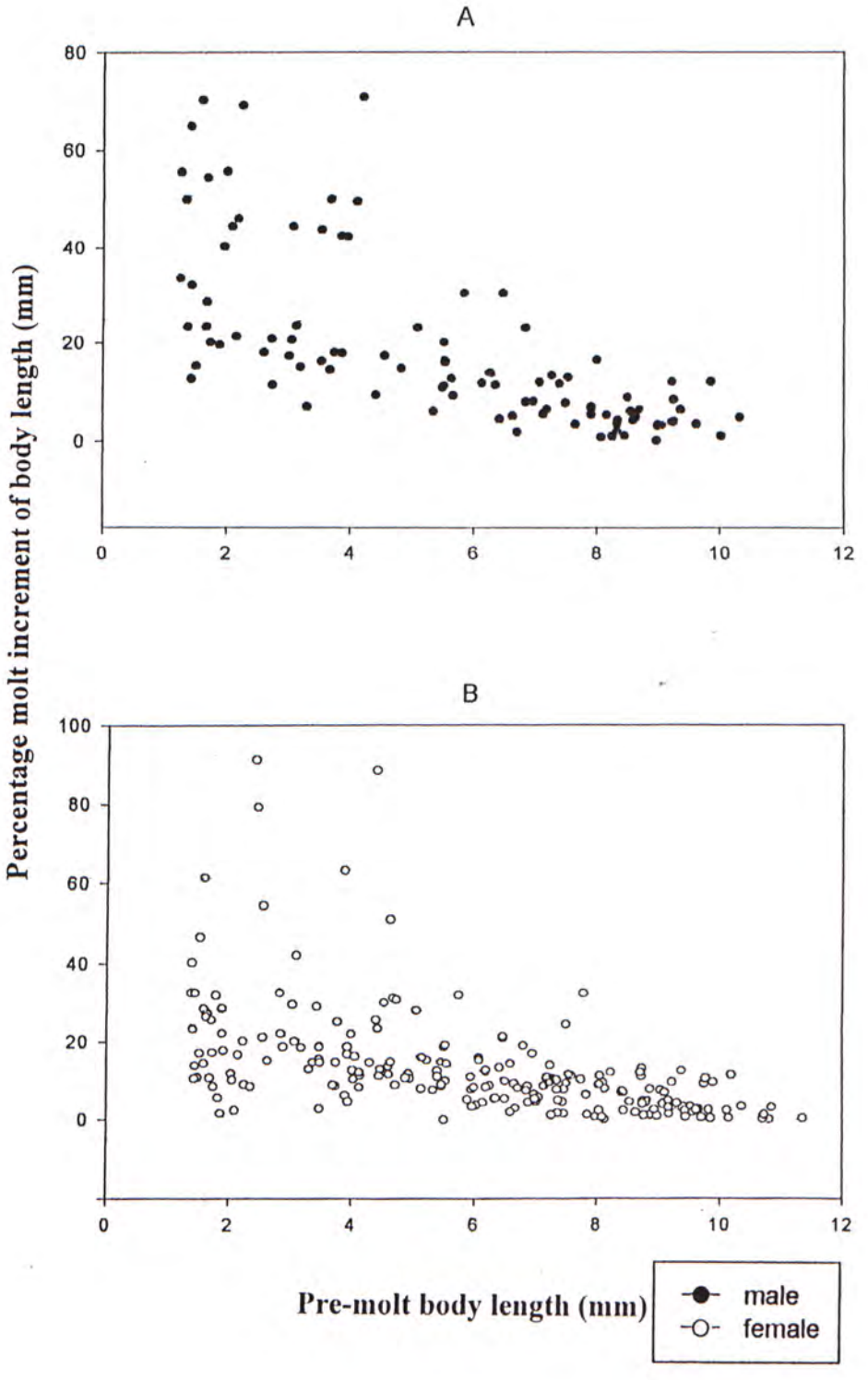


Figure 3.6 Percentage molt increment of body length versus pre-molt body length. A, males, N = 120, correlation coefficient r = 0.699; B, females, N = 253, r = 0.578.

A linear correlation could be obtained as the percentage molt increment in BL was logarithmically transformed and plotted against the pre-molt BL. The regression equations of the linear curve for males and females were $\log_{10}Y = -0.13X + 1.79$ ($r^2 = 0.567$) and $\log_{10}Y = -0.11X + 1.60$ ($r^2 = 0.400$), respectively (Figure 3.7). In the equations, X represents pre-molt BL and Y represents the percentage molt increment. The correlation coefficients in the population of males and females were 0.754 and 0.632, respectively. The analysis on the standard error of the correlation coefficients showed that the percentage molt increment of BL was significantly correlated to the pre-molt BL in the two sexes ($P < 0.0001$). The slopes of the two lines were significantly different from zero ($P < 0.0001$; Student's *t*-test). There was a significant correlation between the logarithmic percentage molt increment in BL and pre-molt BL in population of the two sexes. The analysis also indicated that there was no significant difference between the slopes of the two regression lines ($P = 0.127$).

3.3.1.4 Head Length (HL)

The mean HL of two sexes grew along with the number of instars in sigmoid patterns (Figure 3.8). The increase in HL stabilized at instar 13 in males and instar 16 in females. The effects of sex and number of instar on growth of HL increment were analyzed in 2-factor ANOVA. The analysis showed that HLs were significantly different among instars ($P < 0.001$), and the mean head length of males was significantly greater than females in general ($P < 0.001$). The mean HL of the two sexes were significantly different since instar 7 ($P = 0.021$; Tukey test after 2-factor ANOVA). The maximum HL recorded in males was 0.82 mm. Two male amphipods reached the maximum head length at instar 15 as their body lengths reached 9.69 mm and 10.12 mm individually. The ratio of head to body length was 0.085 and 0.081, respectively. The maximum HL recorded in females was 0.86 mm. A female reached the maximum HL as the body length reached 10.07 mm at instar 21. The ratio of head to body length was 0.085.

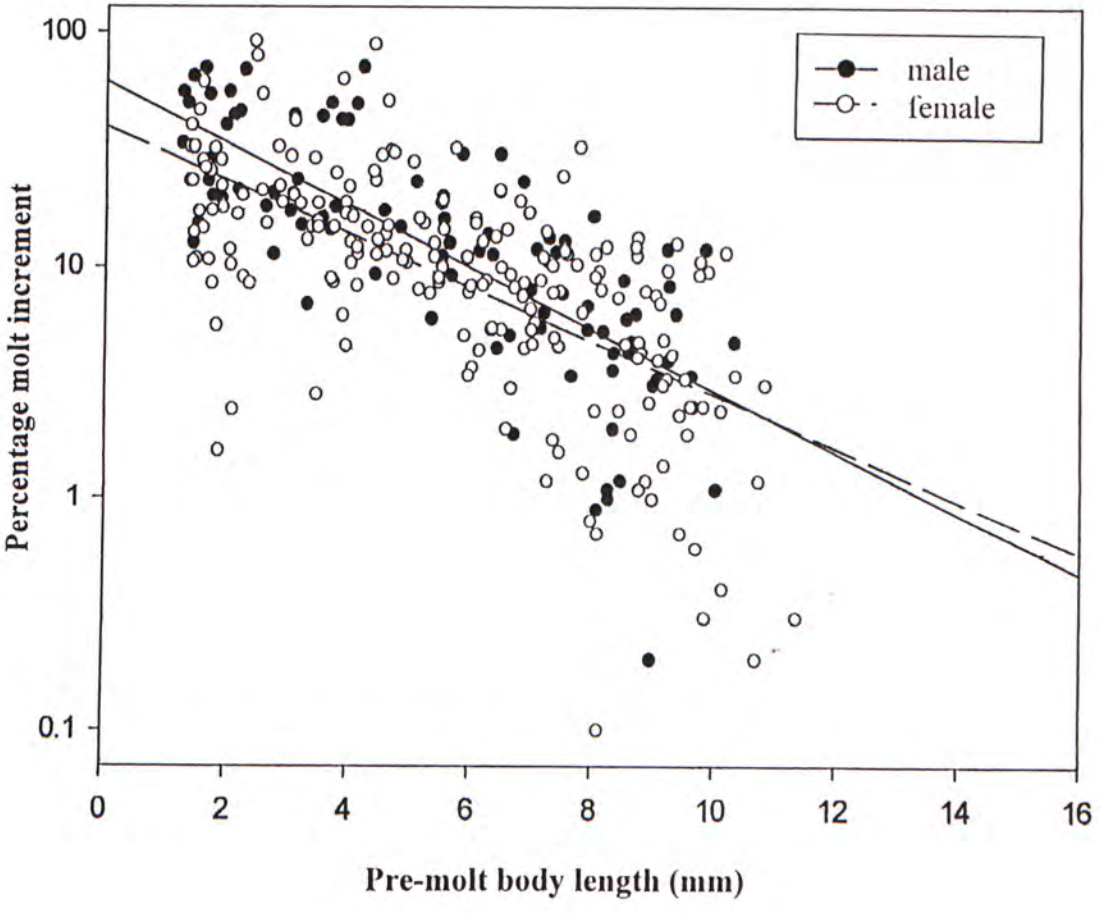


Figure 3.7 Percentage molt increment of body length in $\log_{10}$ scale versus pre-molt body length (BL). The regression equations of the growth curves in males and females are $\log_{10}Y = -0.13X + 1.79$ ($r^2 = 0.567$) and $\log_{10}Y = -0.11X + 1.60$ ($r^2 = 0.400$), respectively. In the equations, X represents the pre-molt BL and Y represents percentage molt increment in logarithmic scale. N (male) = 120, N (female) = 253

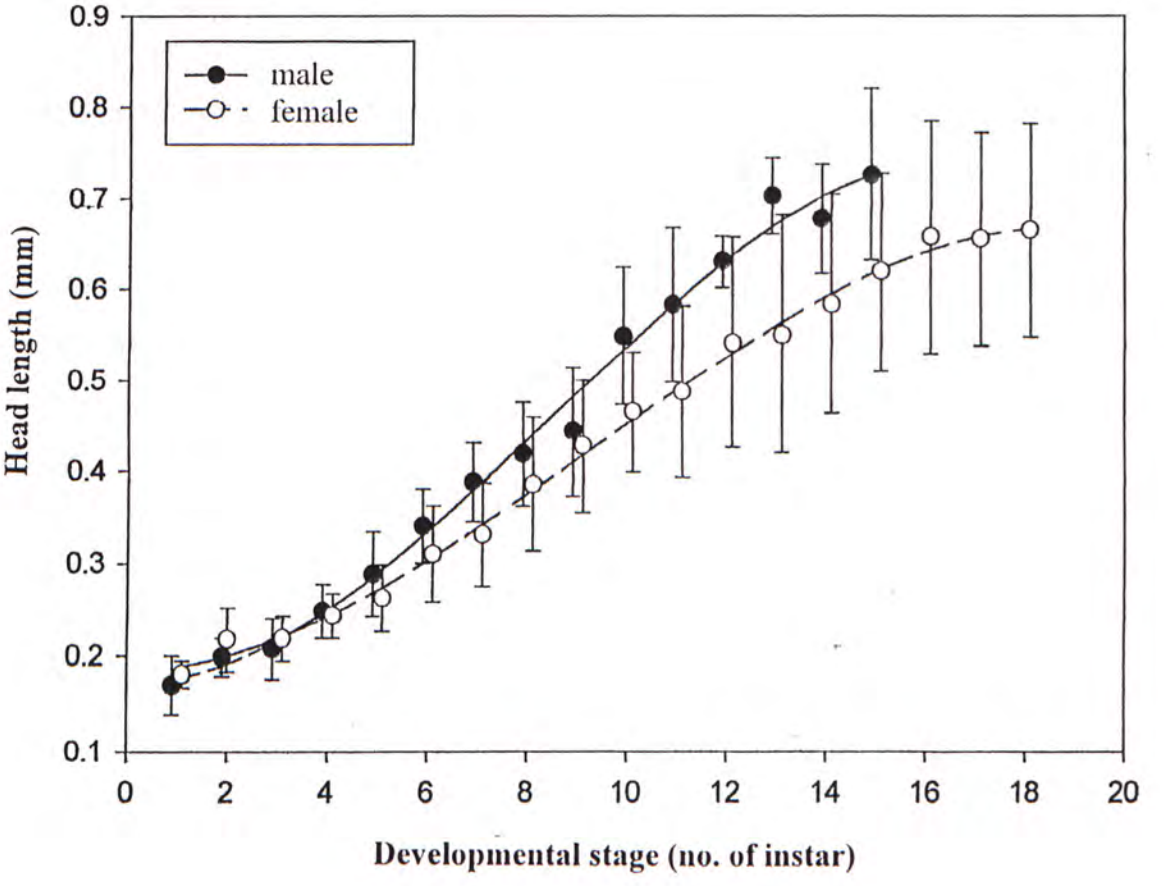


Figure 3.8 Growth curves of *Hyale* sp. in mean head length (HL) at different instars. The regression equations of the growth curves in males and females are $Y = -3 \times 10^{-4}X^3 + 0.007X^2 - 0.005X + 0.174$ ($r^2 = 0.993$) and $Y = -1.5 \times 10^{-4}X^3 + 4 \times 10^{-4}X^2 + 0.001X + 0.183$ ($r^2 = 0.997$), respectively. In the equations, X represents number of instar and Y represents HL. The error bars represent the standard deviation.

3.3.1.5 Eye Length (EL)

The eye length of the two sexes grew in very similar patterns (Figure 3.9). The effects of sex and number of instar on growth of EL increment were analyzed by 2-factor ANOVA. The analysis showed that ELs were significantly different among different instars ($P < 0.001$), but there was no significant difference in mean eye length between the two sexes ($P = 0.536$). The growth of EL in the two sexes stabilized at instar 15, the mean EL reached the range from 0.39 ± 0.05 mm at instar 15 ($N = 13$) to 0.40 ± 0.06 mm at instar 18 ($N = 7$).

The relative growth pattern of logarithmic values of both EL to BL is shown in Figure 3.10. Two different growth phases were differentiated by a change in the level of allometry from the phase AB to the phase BC as the body length reached the range of 4-5 mm (equivalent to instar 5-6 in males and instar 5-8 in females). The regression equations of the growth phase AB in males and females were $\log_{10}Y = 1.19 \log_{10}X - 1.44$ ($r^2 = 0.844$) and $\log_{10}Y = 1.28 \log_{10}X - 1.40$ ($r^2 = 0.857$), respectively. In the equations, X represents BL and Y represents EL. There was no significant difference in the allometric coefficients (b value) of the regression lines between the two sexes ($P = 0.192$; Student's *t*-test). The regression equations of the growth phase BC in males and females are $\log_{10}Y = 0.83 \log_{10}X - 1.20$ ($r^2 = 0.875$) and $\log_{10}Y = 0.77 \log_{10}X - 1.14$ ($r^2 = 0.821$), respectively. There was no significant difference in the allometric coefficients of the regression lines between the two sexes ($P = 0.097$). However, the allometric coefficients of the regression lines was significantly smaller in BC than in AB in males ($P < 0.0001$) and females ($P < 0.0001$). This indicated that EL of the two sexes grew in a smaller proportion to BL when BL reached 4 to 5 mm (growth phase BC), which was equivalent to instar 5 to 7 in males and instar 5 to 8 in females. It was a negatively allometric growth pattern.

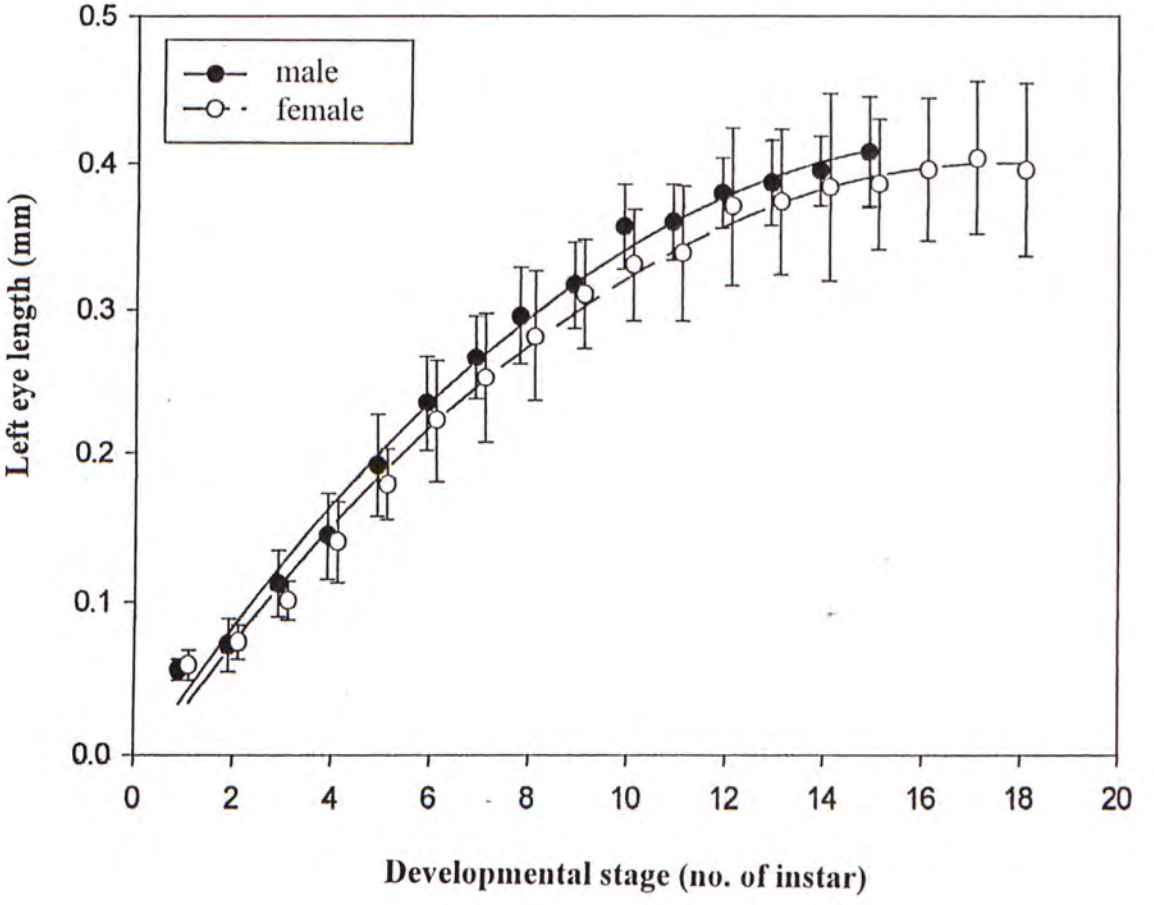


Figure 3.9 Growth curves of *Hyale* sp. in mean eye length (EL) at different instars. The regression equations of growth curves in males and females are $Y = -0.001X^2 + 0.050X - 0.015$ ($r^2 = 0.993$) and $Y = -0.001X^2 + 0.047X - 0.012$ ($r^2 = 0.996$), respectively. In the equations, X represents number of instar and Y represents EL The error bars represent the standard deviation.

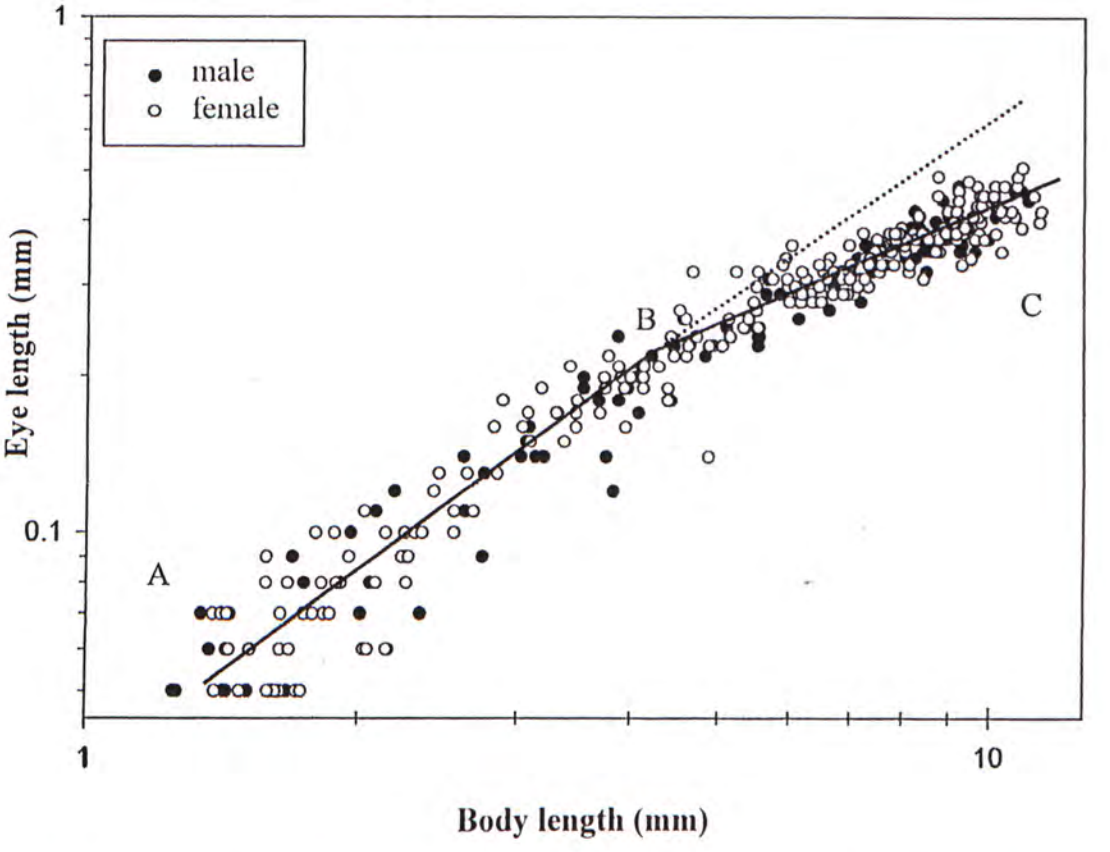


Figure 3.10 The logarithmic relationships between the left eye length (EL) and body length (BL). Two growth phases AB and BC are marked on the figure. The dotted line indicates the deviation of the growth rate from the isometric growth pattern. The regression equations of the growth phase AB in males and females are $\log_{10}Y = 1.19 \log_{10}X - 1.44$ ($r^2 = 0.844$) and $\log_{10}Y = 1.28 \log_{10}X - 1.40$ ($r^2 = 0.857$), respectively. The regression equations of the growth phase CD in males and females are $\log_{10}Y = 0.83 \log_{10}X - 1.20$ ($r^2 = 0.875$) and $\log_{10}Y = 0.77 \log_{10}X - 1.14$ ($r^2 = 0.821$), respectively. In the equations, X represents BL and Y represents EL. N (male) = 120, and N (female) = 253.

3.3.1.6 Length and Articles of Antenna 1 (AL1 & AA1)

The growth of the AL1 in the two sexes is shown in Figure 3.11. The effects of sex and number of instars on growth of AL1 were analyzed by 2-factor ANOVA. The analysis showed that AL1s were significantly different among instars ($P < 0.001$), and the mean AL1 of males was significantly greater than that of females ($P < 0.001$). The significant difference in mean AL1 between the two sexes started at instar 7 ($P = 0.001$; Tukey test after 2-factor ANOVA). The deviation became greater in the later stages. For example, the mean AL1 of males and females were 0.76 ± 0.10 mm ($N = 9$) and 0.74 ± 0.07 mm ($N = 15$), respectively at instar 5. At instar 15, the mean AL1 of males was 2.31 ± 0.09 mm ($N = 5$) while that of the females was 1.71 ± 0.27 mm ($N = 12$). The mean AL1 of males was 35.8% longer than that of females at instar 15.

The relative growth pattern of AL1 to BL in the two sexes is shown in Figure 3.12A. Two growth phases were differentiated in males (Figure 3.12B). Two significantly different regression lines indicated the two different growth phases. The regression equations of the lines AB and CD are $\log_{10}Y = 0.76 \log_{10}X - 0.16$ ($r^2 = 0.848$) and $\log_{10}Y = 1.23 \log_{10}X - 0.85$ ($r^2 = 0.939$), respectively. In the equations, X represents BL and Y represents AL1. The allometric coefficients of the two lines were significantly different from each other ($P < 0.0001$; Student's *t*-test). When BL was below 3.5 mm, AL1 of males grew in the phase AB. When BL was greater than 4.5 mm (equivalent to instar 4-6), the level of allometry changed. AL1 subsequently grew in the phase CD. This indicated that AL1 grew in a greater proportion to BL when BL reached 4.5 mm (growth phase CD). It was a positively allometric growth. The phases transition (represented by CB region) between two successive growth phases AB and CD involved the overlapping of the body size ranged from 3.5 to 4.5 mm. In females, only one growth phase EF was observed in the relative growth of AL1 to BL (Figure 3.12C). Neither change in the level of allometry nor dimension in AL1 was observed during development of females. This indicated that AL1 of females grew in a constant proportion to BL throughout their life span. It was an isometric growth pattern. The regression lines of the growth phases AB in males (Figure 3.12B) and EF (Figure

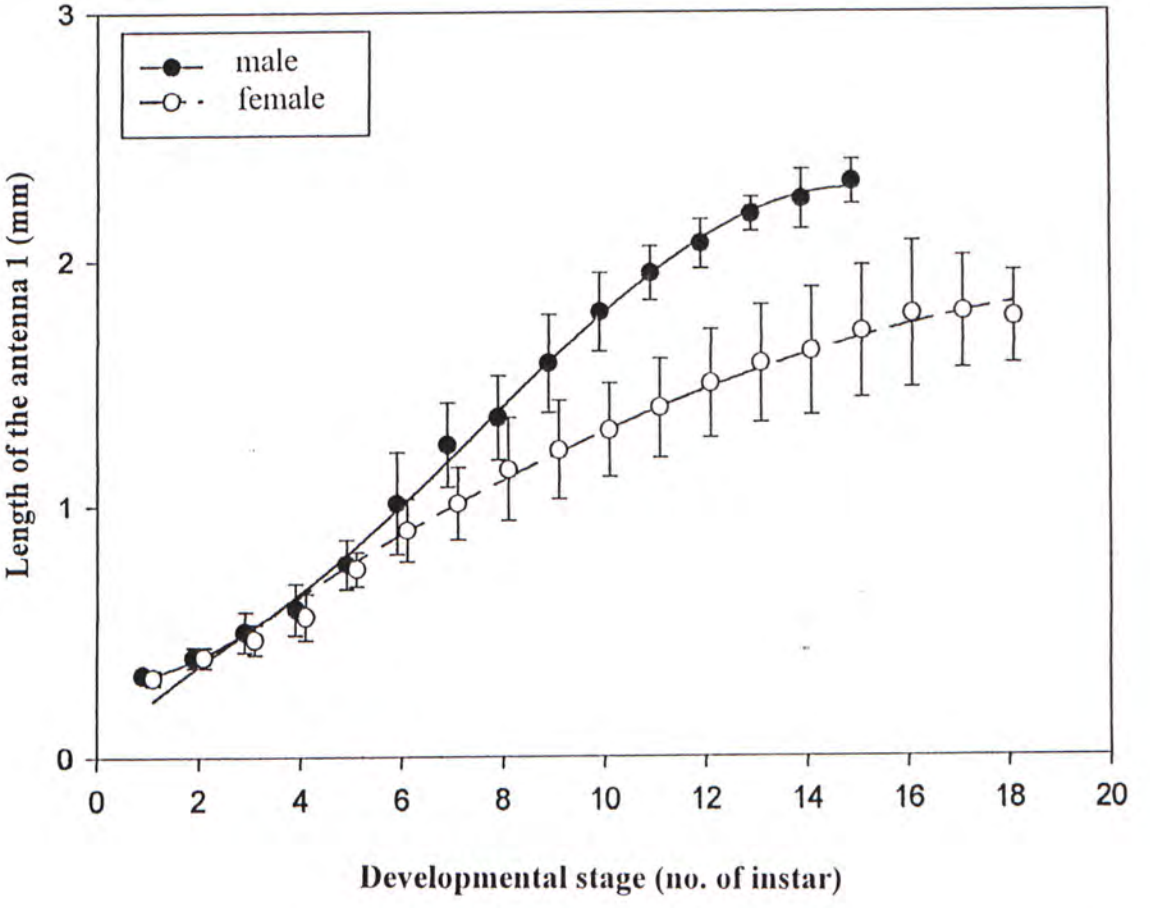
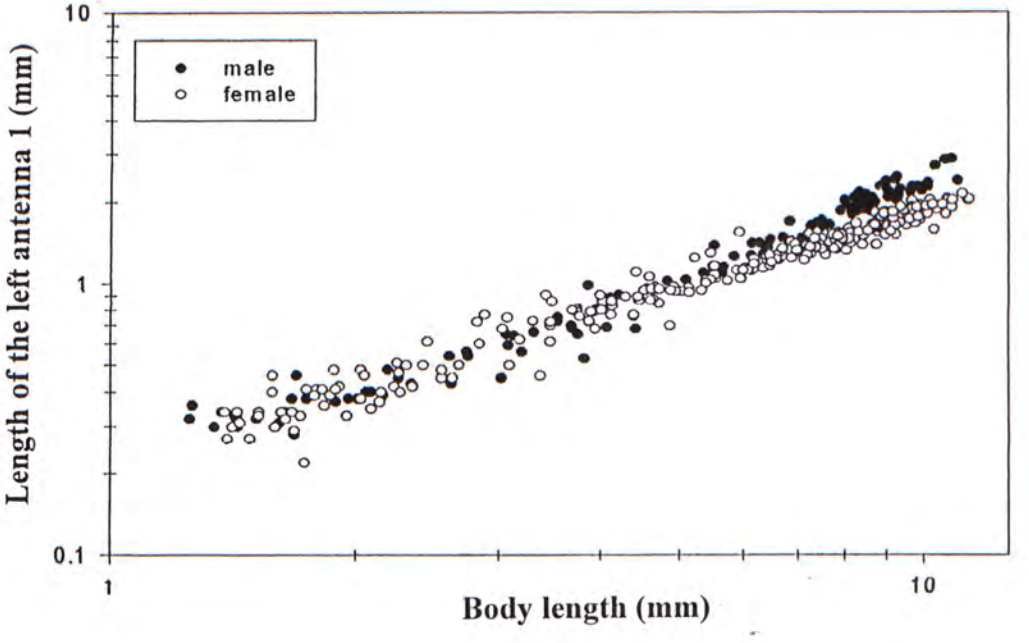
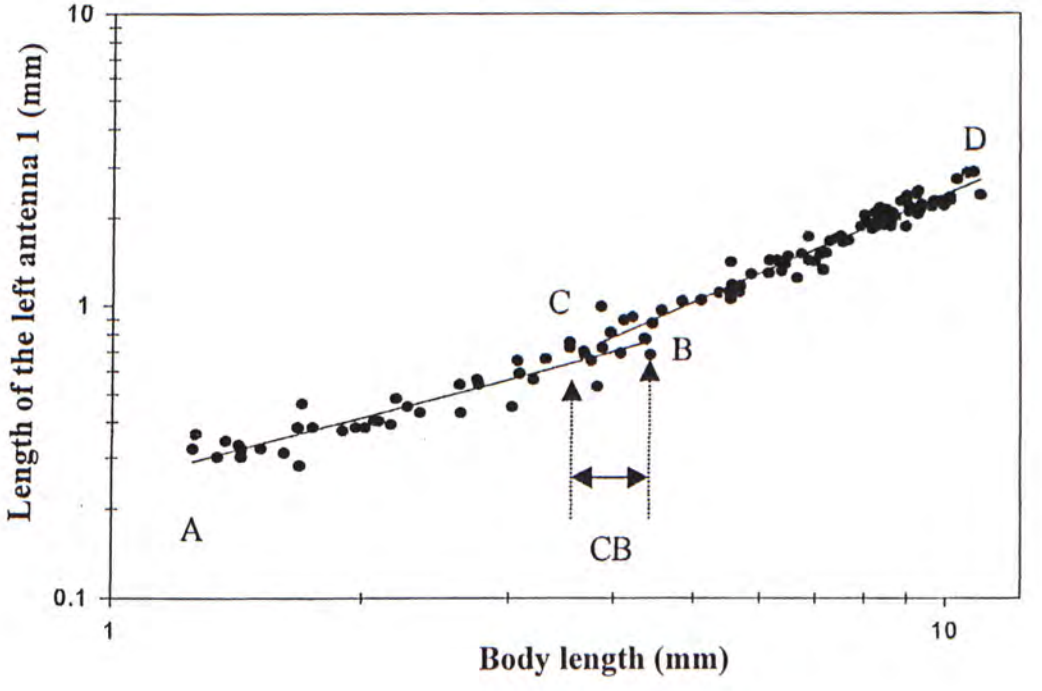


Figure 3.11 Growth curves of *Hyale* sp. in mean length of the left antenna 1 (AL1) at different instars. The regression equations of the growth curves in males and females are $Y = -0.001X^3 + 0.028X^2 - 0.004X + 0.294$ ($r^2 = 0.998$) and $Y = -0.003X^2 + 0.158X + 0.068$ ($r^2 = 0.994$), respectively. In the equations, X represents number of instar and Y represents AL1. The error bars represent the standard deviation.

3.12A



3.12B



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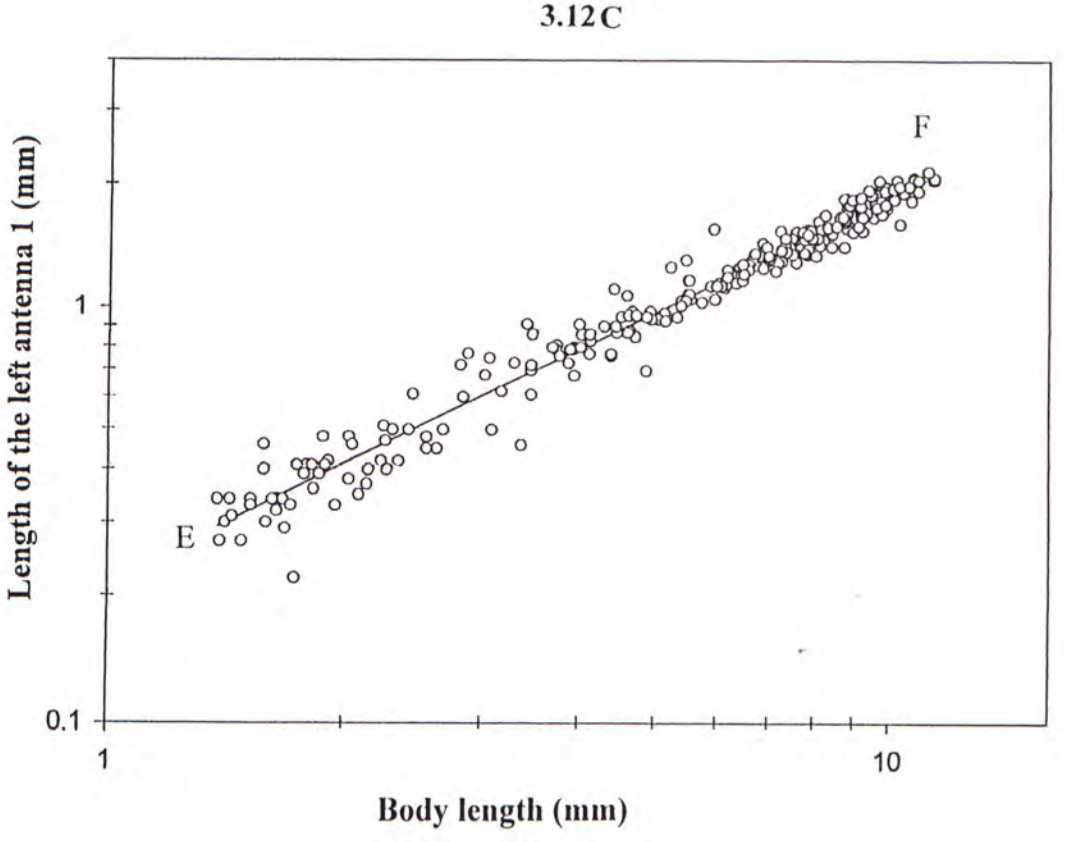


Figure 3.12 The relationships between the logarithmic values of the length of the left antenna 1 (AL1) and the body length (BL). A, the two sexes; B, male, two growth phases AB and CD are marked on the figure, the CB region indicates the body length with overlapping of the two growth phases, the regression equations of the lines AB and CD are $\log_{10}Y = 0.76 \log_{10}X - 0.16$ ($r^2 = 0.848$) and $\log_{10}Y = 1.23 \log_{10}X - 0.85$ ($r^2 = 0.939$), respectively; C, female, one growth phase EF is marked on the figure, the regression equation of the linear curve was $\log_{10}Y = 0.93 \log_{10}X - 0.66$ ($r^2 = 0.970$). In the equations, X represents BL and Y represents AL1. N (male) = 120, and N (female) = 253.

3.12C) in females were compared by the Student *t*-test. It was found that there was no significant difference in the allometric coefficients between the two growth phases (P = 0.059). This indicated that AL1 of males (growth phase AB) and females (growth phase EF, the whole life span) grew in an equal proportion to BL.

The number of articles on the left antenna 1 (AA1) was the total numbers of articles, including three peduncular and variable flagellar articles, counted on the left antenna 1. The number of flagellar articles increased at each molt could vary from 0 to 3. But in general, one flagellar article was built on the antenna 1 at each molt (Figure 3.13). The effects of sex and number of instar on growth of AA1 were analyzed by 2-factor ANOVA. The analysis showed that AA1 was significantly different among instars ($P < 0.001$), and the mean AA1 of males was significantly greater than that of females ($P < 0.001$). The mean AA1s between the two sexes started to be significantly different from each other at instar 7 ($P = 0.030$). The maximum AA1 of males and females were 20 at instar 18 and 17 at instar 17, respectively.

The relationship between the mean AL1 and the mean AA1 in the two sexes is shown in Figure 3.14. The elongation of the mean AL1s in males and females were directly proportional to the increment of AA1s throughout development. The regression lines of males and females are expressed by the equations $Y = 0.184X - 0.855$ ($r^2 = 0.987$) and $Y = 0.169X - 0.751$ ($r^2 = 0.917$), respectively. In the equations, X represents AA1 and Y represents AL1. The slopes of the regression lines of the two sexes were significantly different from each other ($P = 0.0001$; Student's *t*-test). The length of an article added on the flagellum was a constant and equivalent to the slope of the regression line in the two sexes. The mean length of an article was 0.18 mm in males and 0.17 mm in females..

3.3.1.7 Length and Articles of Antenna 2 (AL2 & AA2)

The growth curve of AL2 is shown in Figure 3.15. The effects of sex and number of instar on growth of AL2 were analyzed by 2-factor ANOVA. Statistical

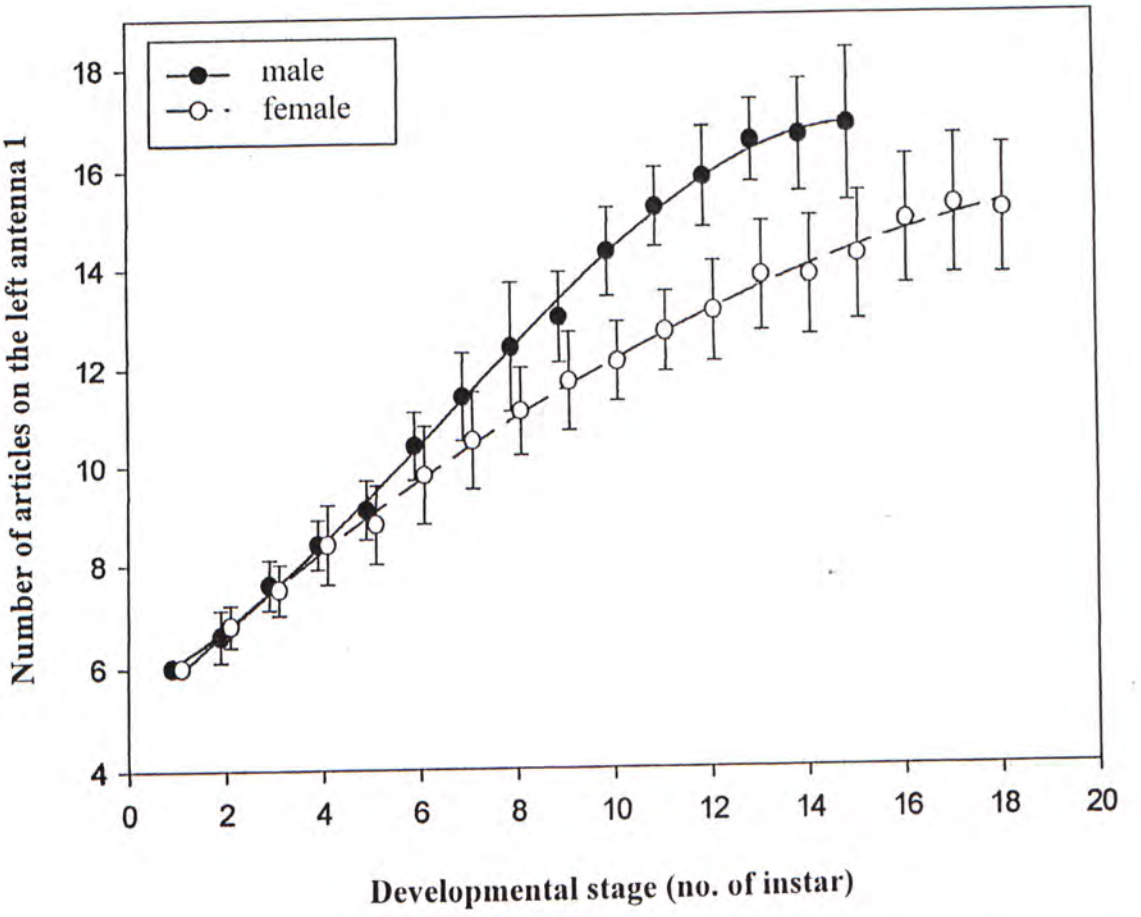


Figure 3.13 Growth curves of *Hyale* sp. in mean number of articles on the left antenna 1 (AA1) at different instars. The regression equations of the growth curves in males and females are $Y = -0.005X^3 + 0.098X^2 + 0.391X + 5.608$ ($r^2 = 0.999$) and $Y = -0.018X^2 + 0.892X + 5.052$ ($r^2 = 0.997$), respectively. In the equations, X represents number of instar and Y represents AA1. The error bars represent the standard deviation.

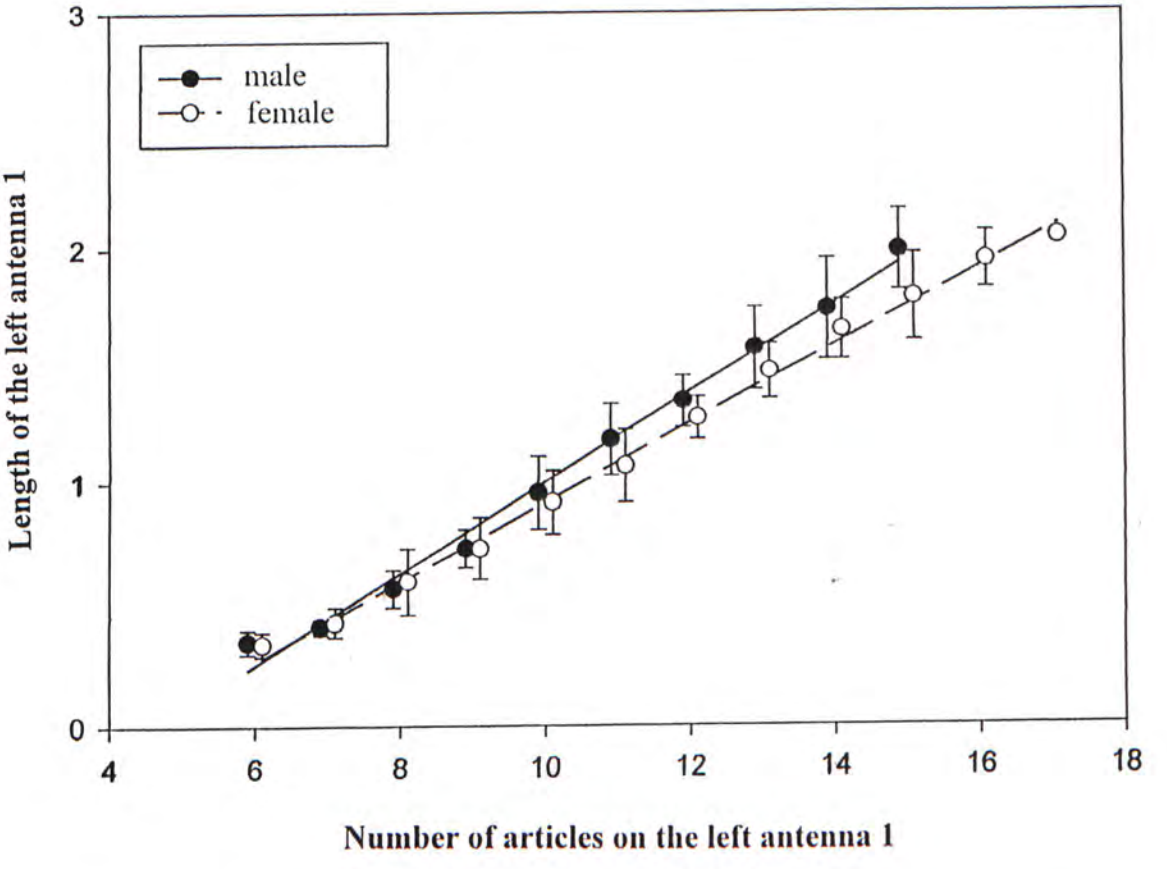


Figure 3.14 The relationships between the length (AL1) and the number of articles (AA1) on the left antenna 1. The regression equations of the curves in males and females are $Y = 0.19X - 0.90$ ($r^2 = 0.992$) and $Y = 0.17X - 0.74$ ($r^2 = 0.997$), respectively. In the equations, X represents AA1 and Y represents AL1. The error bars represent the standard deviation.

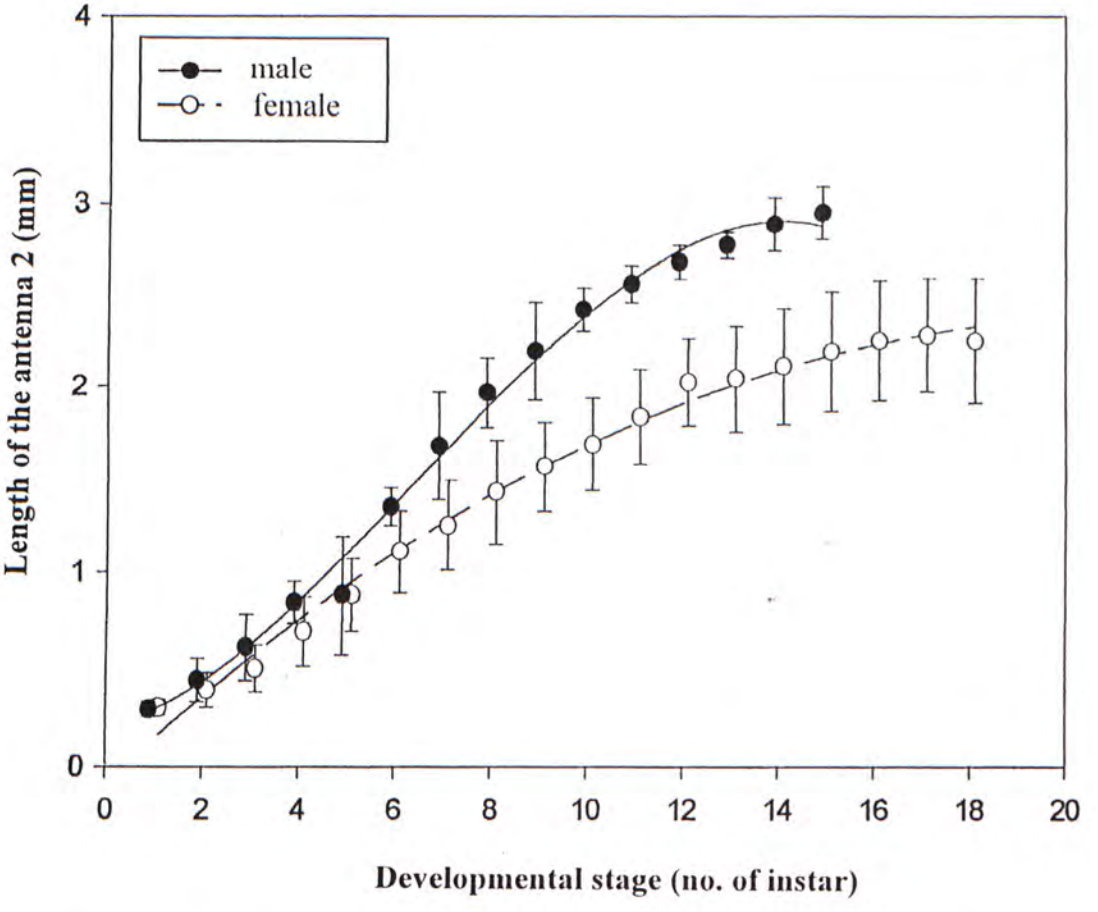


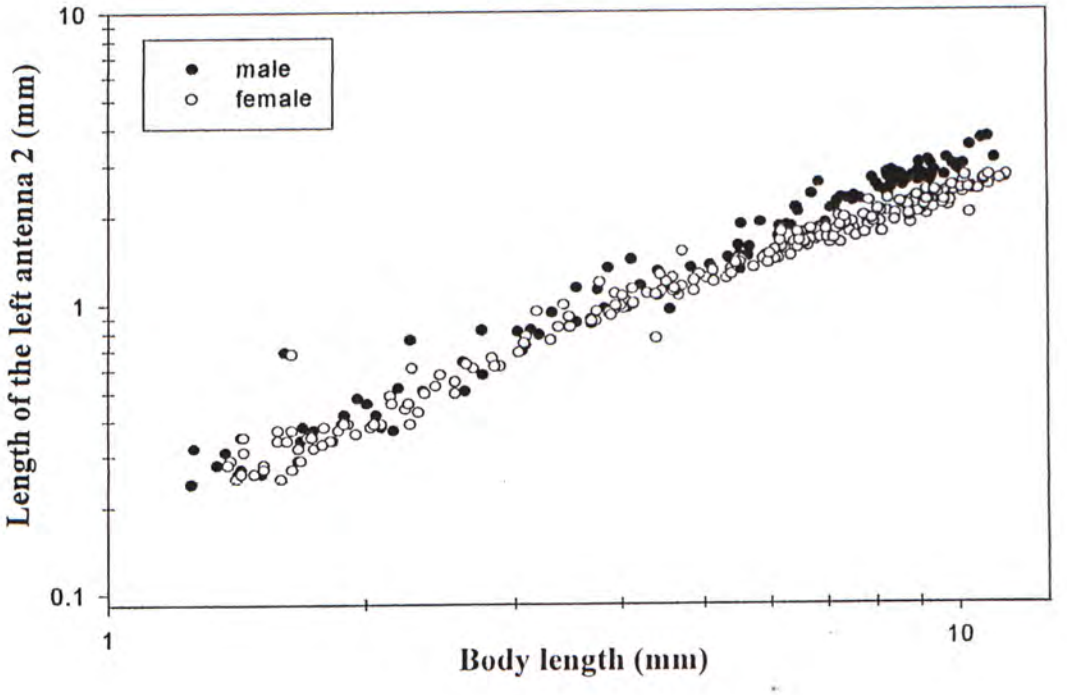
Figure 3.15 Growth curves of *Hyale* sp. in mean length of the left antenna 2 (AL2) at different instars. The regression equations of the growth curves in males and females are $Y = -0.002X^3 + 0.033X^2 + 0.056X + 0.203$ ($r^2 = 0.995$) and $Y = -0.005X^2 + 0.230X - 0.065$ ($r^2 = 0.993$), respectively. In the equations, X represents the number of instar and Y represents AL2. The error bars represent the standard deviation.

analysis showed that AL2s were significantly different among instars ($P < 0.001$), and the mean AL2 of males was significantly greater than that of females ($P < 0.001$). The significant deviation in the mean AL2 between the two sexes started at instar 6 ($P = 0.005$; Tukey test after 2-factor ANOVA). At instar 15, the mean AL2 of males was 2.95 ± 0.14 mm while that of females was 2.20 ± 0.33 mm. The mean AL2 of males was 34.2% larger than that of females at instar 15.

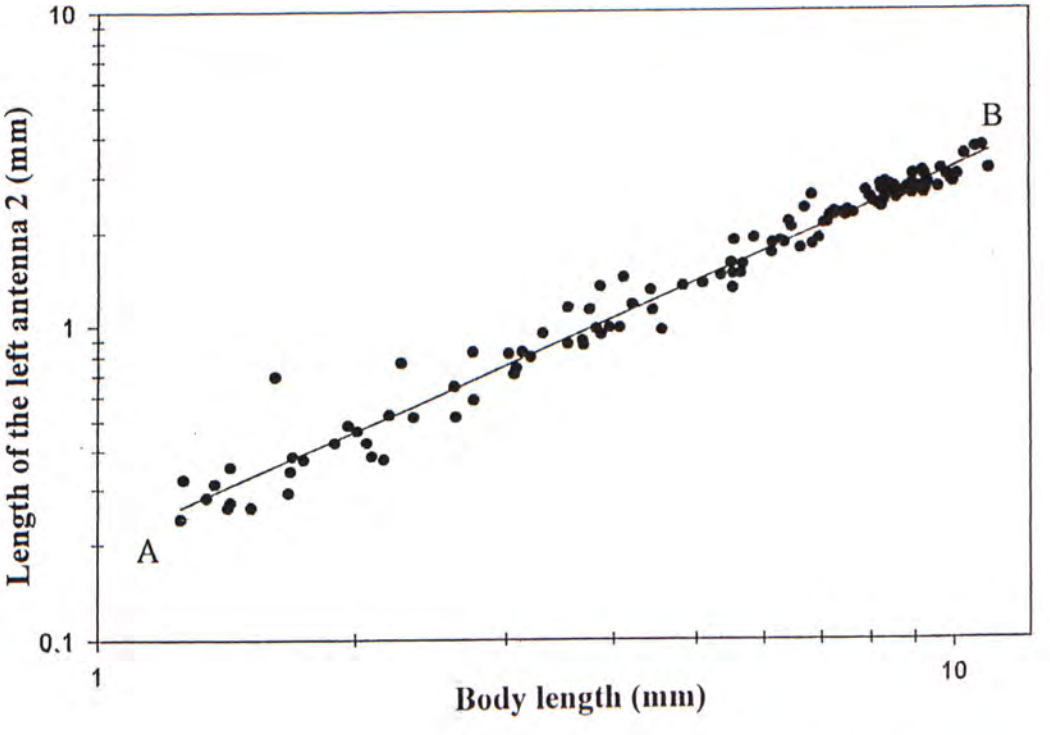
The relative correlation between the logarithmic values of both AL2 and BL is shown in Figure 3.16A. Only one growth phase was observed in males (Figure 3.16B). No change in the level of allometry or dimension in AL2 was observed during the development of males. This indicated that AL2 grew in a constant proportion to BL throughout their life span. It was an isometric growth pattern. However two growth phases were differentiated in females (Figure 3.16C). The two different growth phases were indicated by two significantly different regression lines. The regression equations of the lines CD and EF are $\log_{10}Y = 1.29 \log_{10}X - 0.76$ ($r^2 = 0.912$) and $\log_{10}Y = 0.95 \log_{10}X - 0.57$ ($r^2 = 0.922$), respectively. In the equations, X represents BL and Y represents AL2. The allometric coefficients of the two lines were significantly different from each other ($P < 0.0001$; Student's *t*-test). When BL was below the range of 3.5-4.5 mm, AL2 grew in the phase CD. As the body length became greater than 4.5 mm, AL2 subsequently grew in the phase EF. The phases transition (represented by ED region) between two successive phases CD and EF involved the overlapping of the body size (from 3.5 to 4.5 mm). This indicated that AL2 of females grew in a smaller proportion to BL when BL was greater than 4.5 mm (growth phase EF). There was no significant difference in the allometric coefficients between the growth phases AB in males (Figure 3.16B) and CD in females (Figure 3.16C) ($P = 0.054$). This indicated that AL2 of males (growth phase AB, whole life span) and females (growth phase CD) grew in an equal proportion to BL.

The effects of sex and number of instar on growth of AA2 were analyzed by 2-factor ANOVA. The analysis showed that AA2s were significantly different among instars ($P < 0.001$), and the mean AA2 of males was significantly greater than that of

3.16A



3.16B



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3.16C

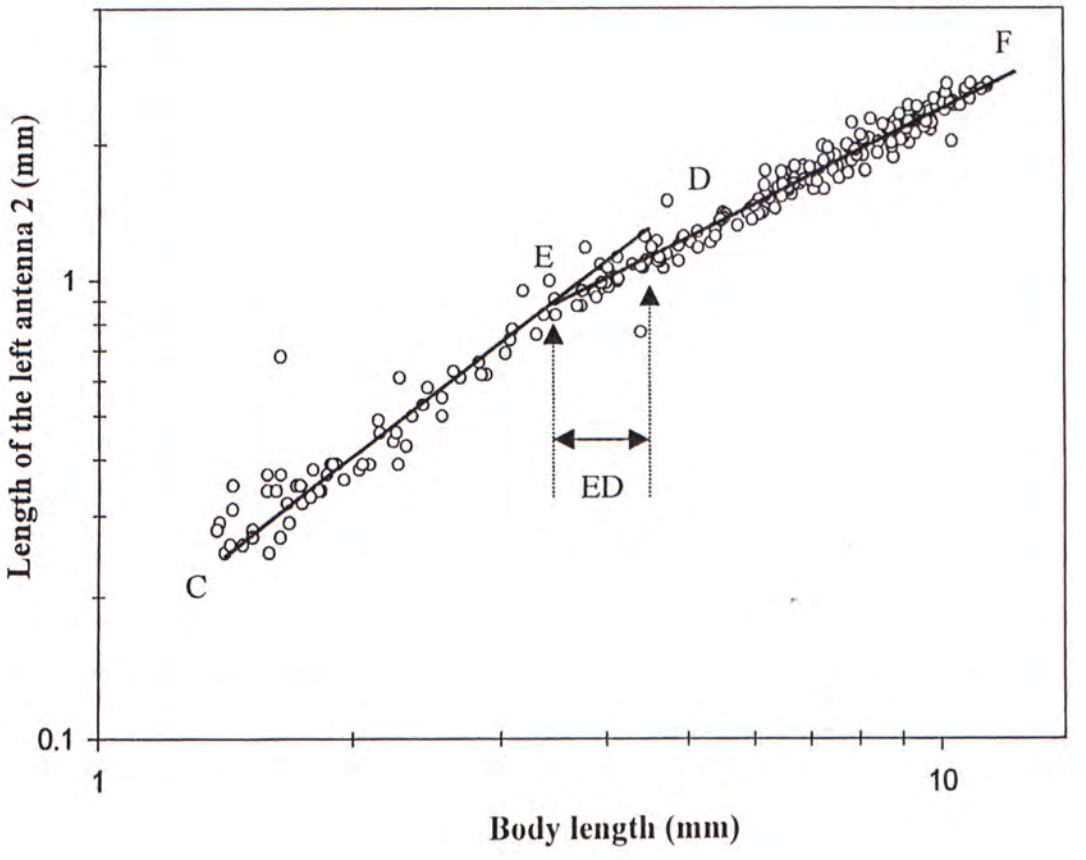


Figure 3.16 The relationships between the logarithmic values of the length of the left antenna 2 (AL2) and the body length (BL). A, the two sexes; B, male, one growth phase AB is marked on the figure, the regression equation of the linear curve was $\log_{10}Y = 1.20 \log_{10}X - 0.70$ ($r^2 = 0.973$).; C, female, two growth phases CD and EF are marked on the figure, the ED region indicates the body length with overlapping of the two growth phases, the regression equations of the lines CD and EF are $\log_{10}Y = 1.29 \log_{10}X - 0.76$ ($r^2 = 0.912$) and $\log_{10}Y = 0.95 \log_{10}X - 0.57$ ($r^2 = 0.922$), respectively. In the equations, X represents BL and Y represents AL2. N (male) = 120, and N (female) = 253.

females ($P < 0.001$). The mean AA2s of the two sexes were started to deviate at instar 7 ($P = 0.011$; Tukey test after 2-factor ANOVA). The maximum AA2 of males and females were 22 at instar 18 and 20 at instar 15, respectively (Figure 3.17).

Figure 3.18 shows the correlation between the elongation of antenna 2 and the increment of article. The elongation of the mean AL2 of females was directly proportional to the increment of the article numbers. The articles built on the antenna were all similar in length. The length of each flagellar article was a constant (0.18 mm) and equivalent to the slope of the regression line. For males, AL2 grew in the same rate as females when AA2 was less than 16 articles. The mean AL2s of the two sexes started to deviate as AA2 became greater than 16 articles ($P = 0.003$; Student's *t*-test). This indicated that when the mean AA2 was greater than 16 articles, the mean AL2 grew in a faster rate than the increment of AA2. Thus the distal articles (> 16 articles) were longer in length than the basal articles.

3.3.1.8 Propodus Length of Gnathopod 1 (GL1)

The growth of the mean GL1 in both sexes is shown in Figure 3.19. The effects of sex and number of instar on growth of GL1 were analyzed by 2-factor ANOVA. The analysis showed that GL1s were significantly different among instars ($P < 0.001$), and the mean GL1 of males was significantly longer than that of females ($P < 0.001$). The significant deviation in the mean GL1 between the two sexes started at instar 6 ($P = 0.008$; Tukey test after 2-factor ANOVA). For example, the mean GL1 of males was 0.49 ± 0.02 mm at instar 15 while that of females was 0.36 ± 0.04 mm. The mean GL1 was 36.5 % longer in males than in females. The maximum GL1 recorded was 0.56 mm of a male at instar 19 and 0.48 mm of a female at instar 17.

The relative growth of logarithmic values of GL1 to BL in the two sexes is shown in Figure 3.20A. GL1 of males grew in only one phase AB during the development (Figure 3.16B). No change in the level of allometry or dimension in GL1 was observed throughout the development of males. This indicated that GL1 of males

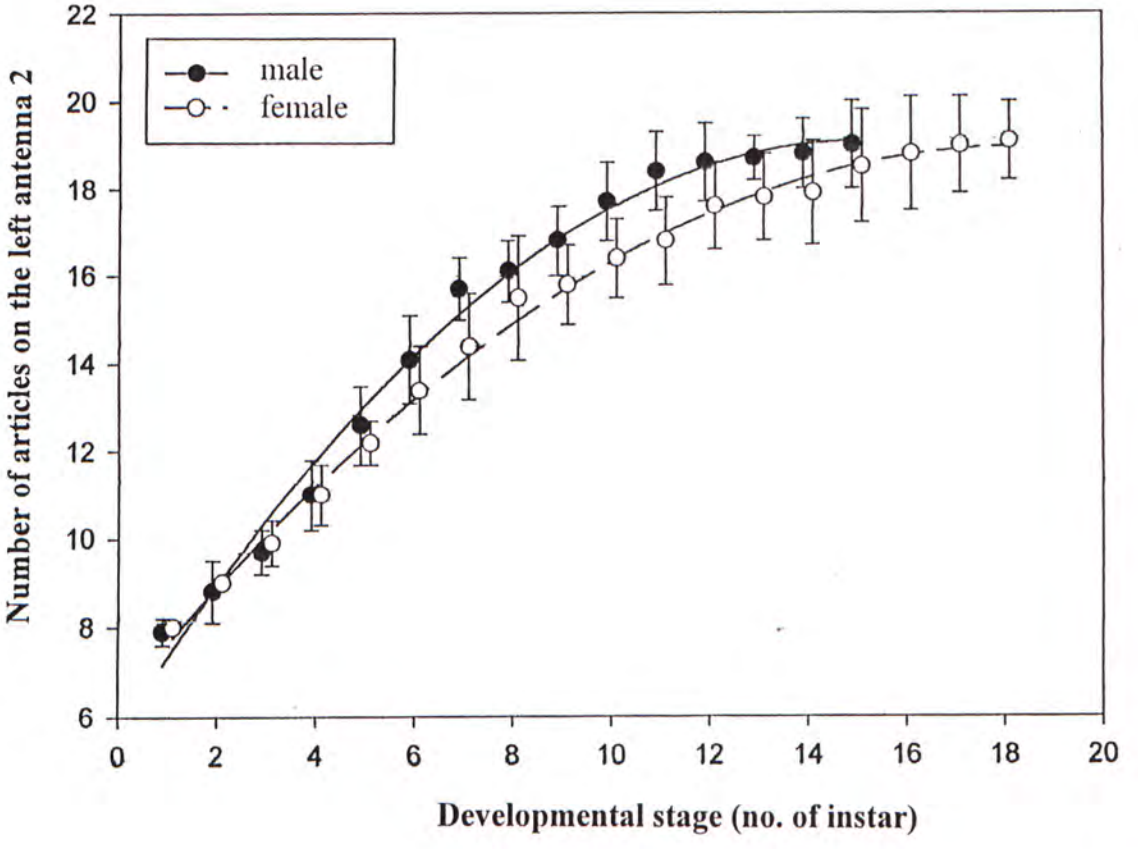


Figure 3.17 Growth curves of *Hyale* sp. in mean number of articles on the left antenna 2 (AA2) at different instars. The regression equations of the growth curves in males and females are $Y = -0.06X^2 + 1.81X + 5.37$ ($r^2 = 0.991$) and $Y = -0.04X^2 + 1.37X + 6.44$ ($r^2 = 0.996$), respectively. In the equations, X represents number of instar and Y represents AA2. The error bars represent the standard deviation.

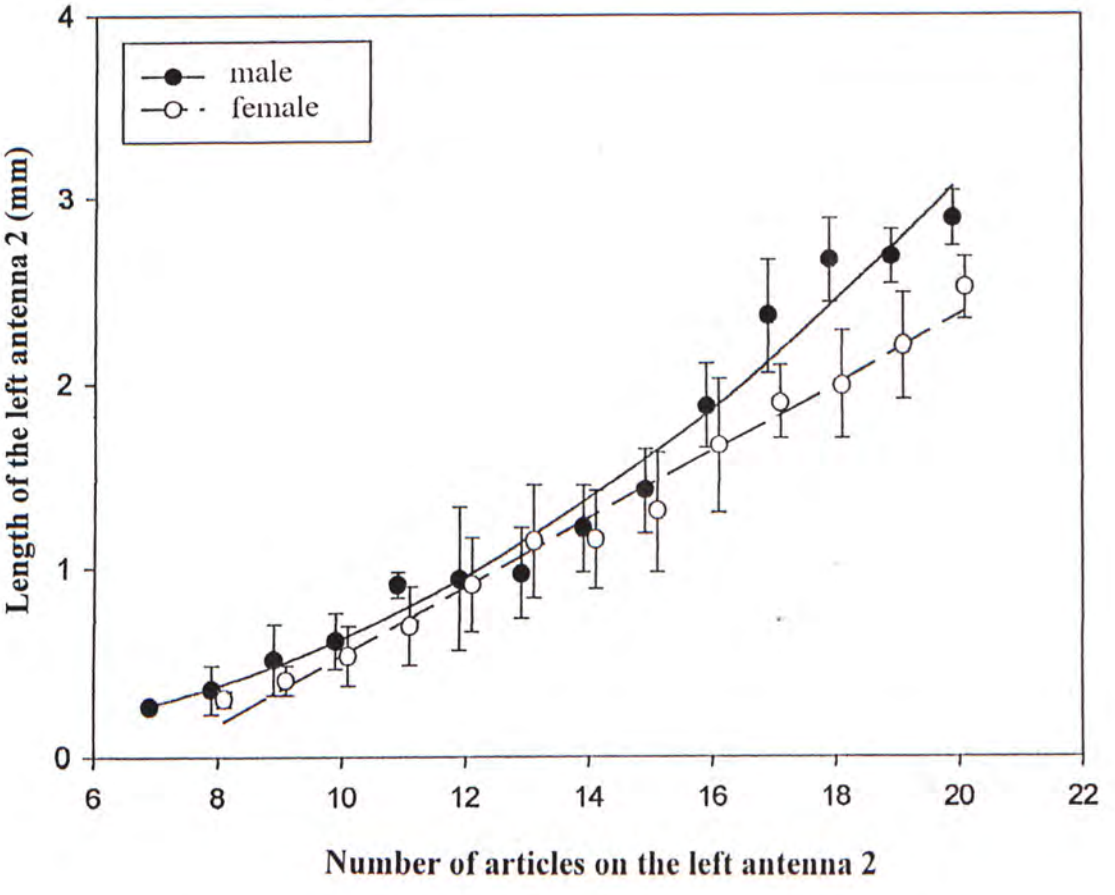


Figure 3.18 The relationships between the length (AL2) and the number of articles on the left antenna 2 (AA2). The regression equations of the curves in males and females are $Y = 0.01X^2 - 0.056X + 0.170$ ($r^2 = 0.977$) and $Y = 0.18X - 1.31$ ($r^2 = 0.986$), respectively. In the equations, X represents AA2 and Y represents AL2. The error bars represent the standard deviation.

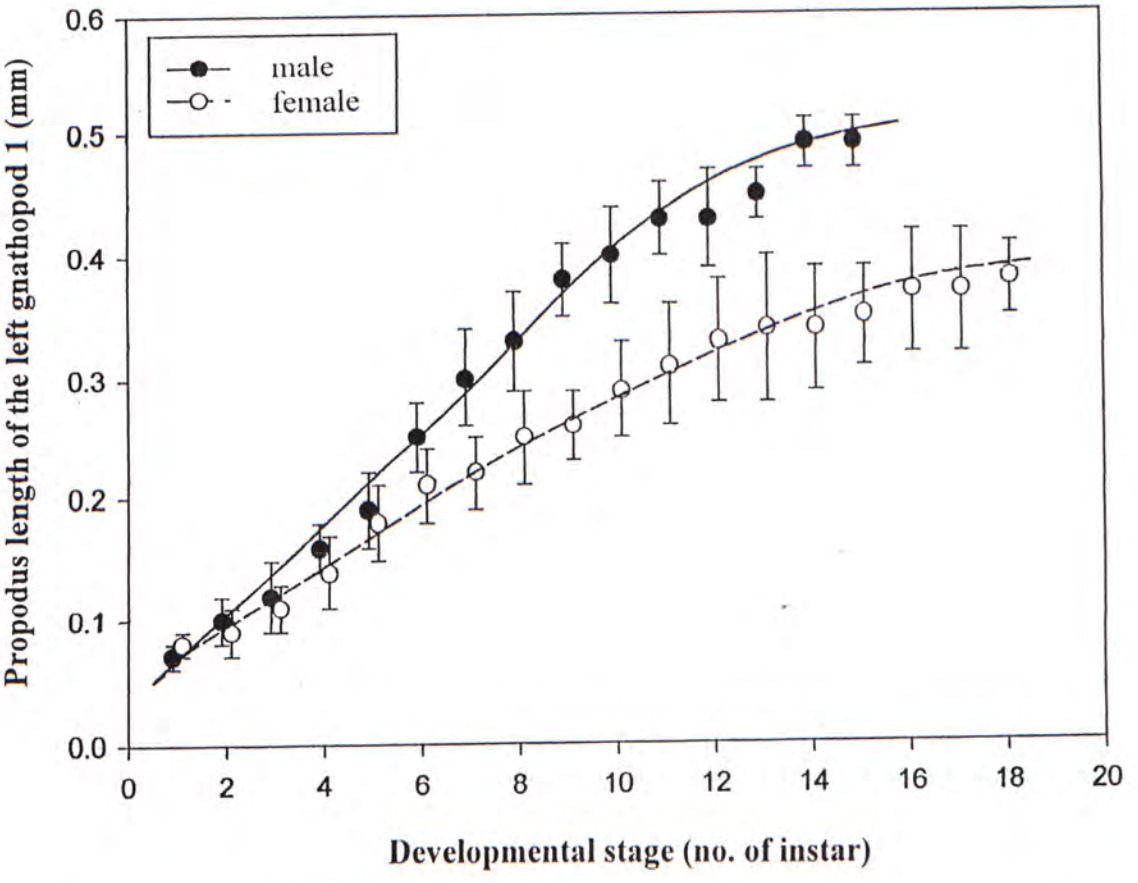
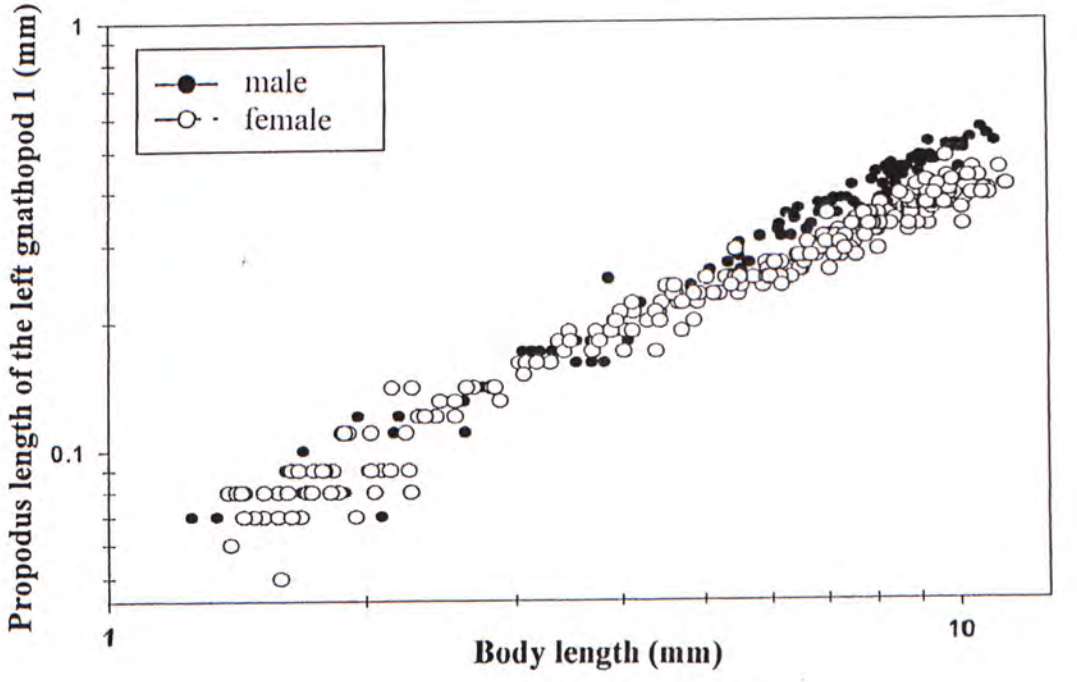
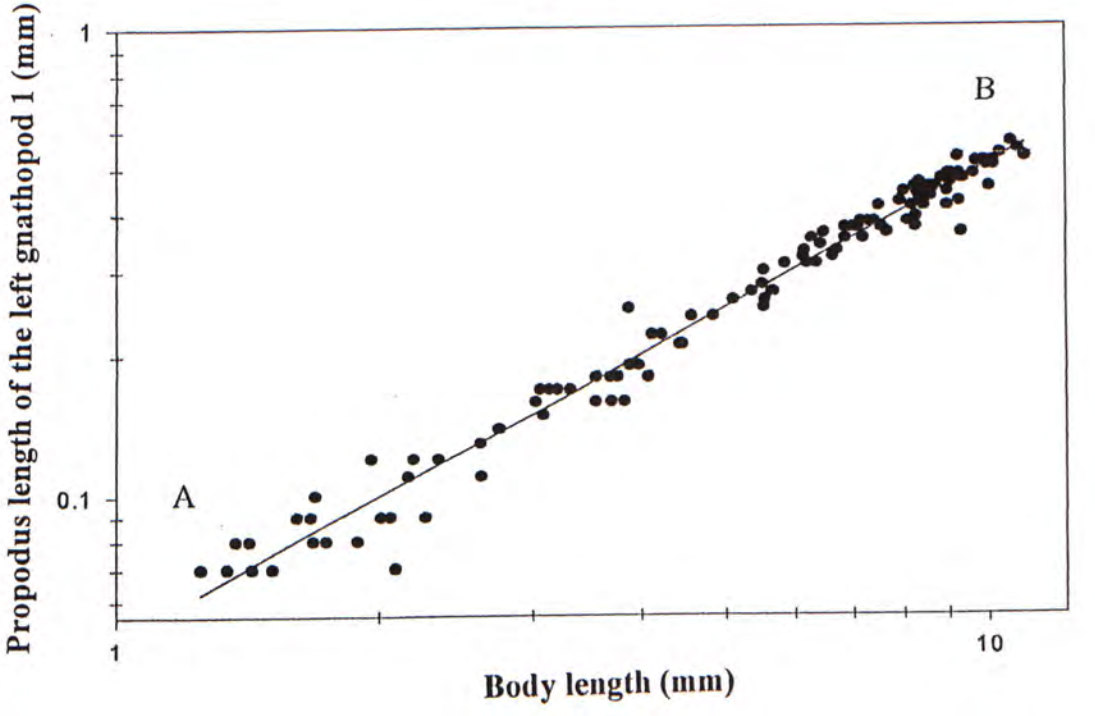


Figure 3.19 Growth curves of *Hyale* sp. in mean propodus length of the left gnathopod 1 (GL1) at different instars. The regression equations of the growth curves in males and females are $Y = -0.001X^2 + 0.049X - 0.001$ ($r^2 = 0.988$) and $Y = -0.001X^2 + 0.035X + 0.026$ ($r^2 = 0.995$), respectively. In the equations, X represents number of instar and Y represents GL1. The error bars represent the standard deviation.

3.20A



3.20B



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3.20C

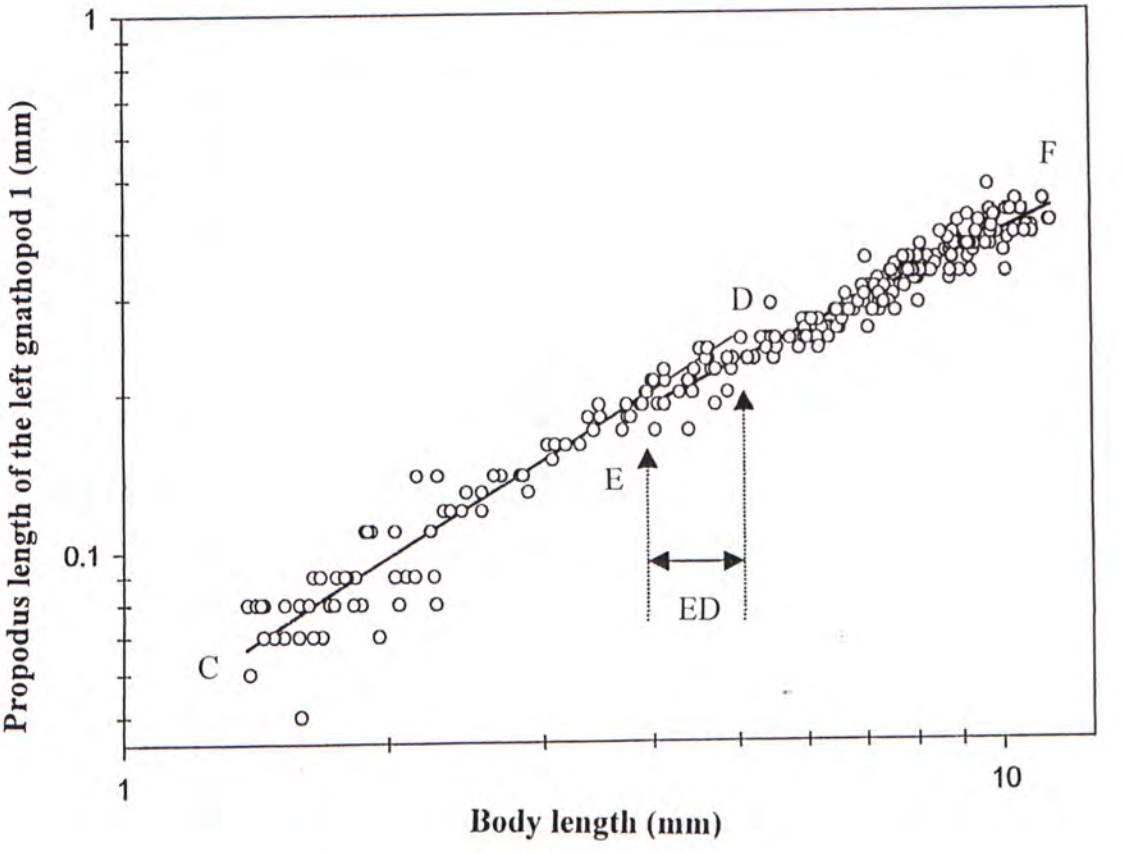


Figure 3.20 The relationships between the logarithmic values of both the propodus length of the left gnathopod 1 (GL1) and the body length (BL). A, the two sexes; B, male, one growth phase AB is marked on the figure, the regression equation of the linear curve AB was $\log_{10}Y = 1.01 \log_{10}X - 1.30$ ($r^2 = 0.980$); C, female, two growth phases CD and EF are marked on the figure, the ED region indicates the body length with overlapping of the two growth phases, the regression equations of the lines CD and EF are $\log_{10}Y = 1.04 \log_{10}X - 1.32$ ($r^2 = 0.889$) and $\log_{10}Y = 0.79 \log_{10}X - 1.20$ ($r^2 = 0.914$), respectively. In the equations, X represents BL and Y represents GL1. N (male) = 120, and N (female) = 253.

grew in a constant proportion to BL. It was an isometric growth pattern. However two growth phases were differentiated in females (Figure 3.20C). Two significantly different regression lines indicated the two different growth phases. The regression equations of the lines CD and EF are $\log_{10}Y = 1.04 \log_{10}X - 1.32$ ($r^2 = 0.889$) and $\log_{10}Y = 0.79 \log_{10}X - 1.20$ ($r^2 = 0.914$), respectively. The allometric coefficients of the two lines were significantly different from each other ($P < 0.0001$; Student's *t*-test). When BL was below 4 mm, GL1 grew in the phase CD. When BL became greater than 5 mm, GL1 subsequently grew in the phase EF. The phases transition (represented by ED region) between two successive phases CD and EF involved the overlapping of the body size in the range of 4 to 5 mm (equivalent to instar 5 –8). This indicated that GL1 of females grew in a smaller proportion to BL when BL became greater than 4-5 mm (growth phase EF). It was a negatively allometric growth pattern. There was no significant difference in the allometric coefficients between the growth phases AB in males and CD in females ($P = 0.268$). This indicated that GL1 of males (growth phase AB, throughout the whole life span) and females (growth phase CD) grew in an equal proportion to BL.

The change in mean ratio of GL1 to BL along development of the two sexes is shown in Figure 3.21. Regression lines of the two sexes were plotted. The mean ratio of males was kept in a constant level at 0.05 along the development. This indicated that GL1 grew in a constant ratio to BL. The slope of the regression line was not significantly different from zero ($P = 0.1003$; Student's *t*-test) and thus there was no significant correlation between GL1 / BL and the number of instar in males. The ratio in females decreased gradually until instar 10 and stabilized at a nearly constant 0.041. The change of ratio was observed in the development of females.

3.3.1.9 Propodus Length of Gnathopod 2 (GL2)

The growth of GL2 in the two sexes is shown on Figure 3.22. The effects of sex and number of instar on growth of GL2 were analyzed by 2-factor ANOVA. The analysis showed that GL2s were significantly different among instars ($P < 0.001$), and

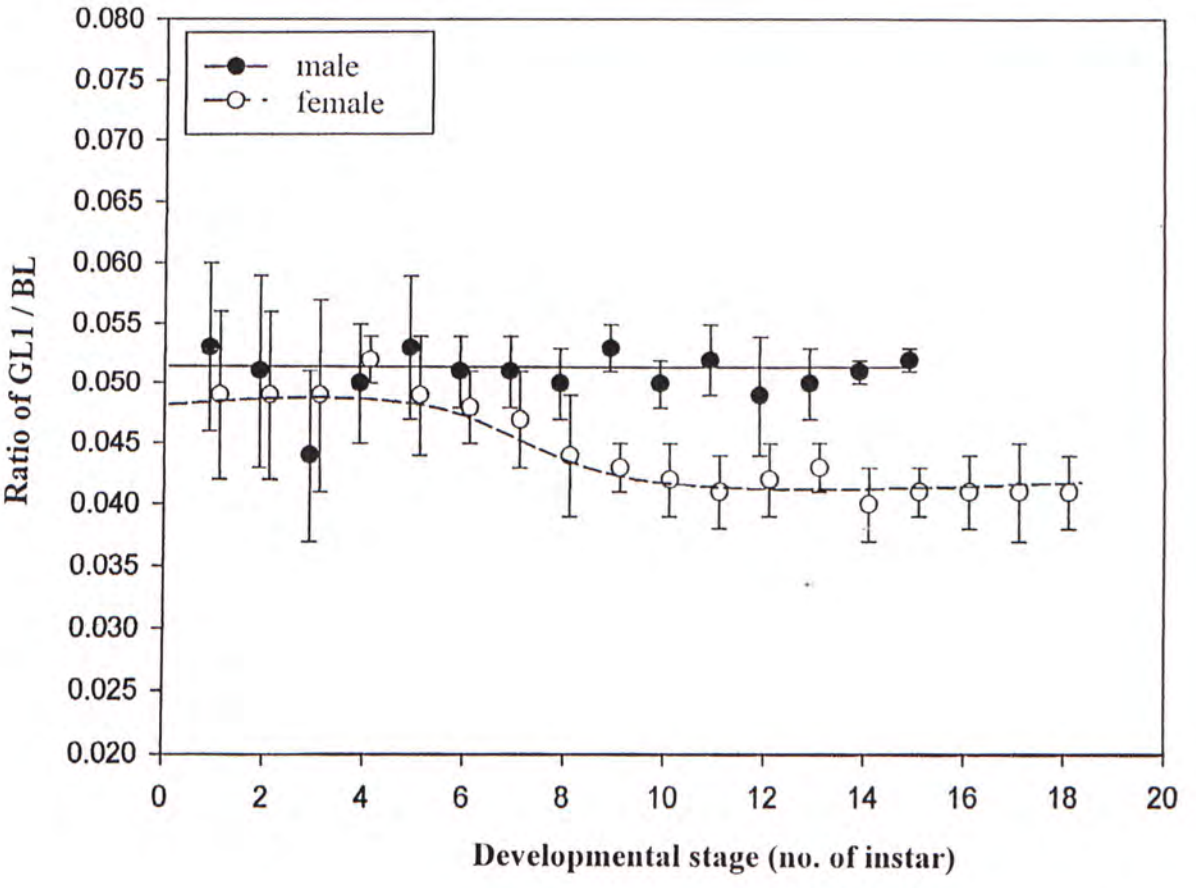


Figure 3.21 The variation on the ratio of the propodus length of the left gnathopod 1 to the body length (GL1 / BL) in different instar. The regression equations of the curves in males and females are $Y = 6 \times 10^{-4}X + 0.050$ ($r^2 = 0.013$) and $Y = 3 \times 10^{-5}X^2 - 0.001X + 0.053$ ($r^2 = 0.840$), respectively. In the equations, X represents number of instar and Y represents GL1 / BL. The error bars represent the standard deviation.

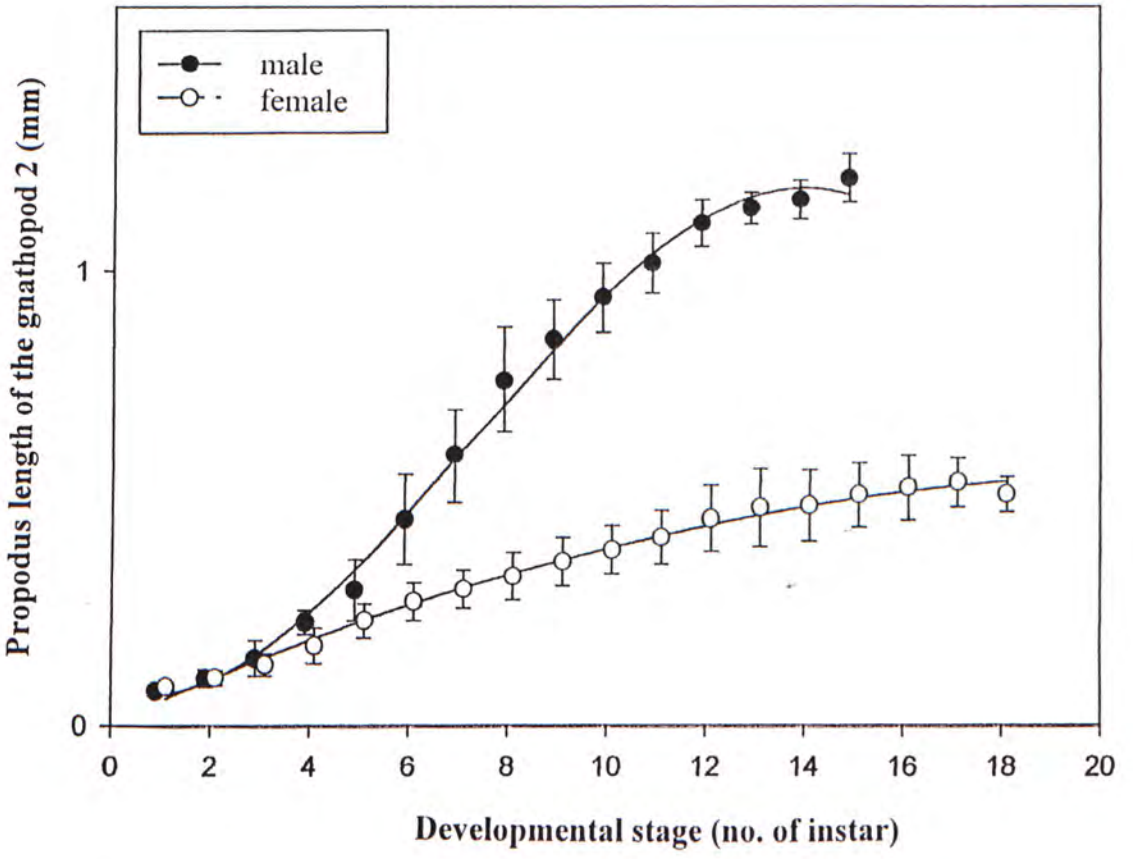


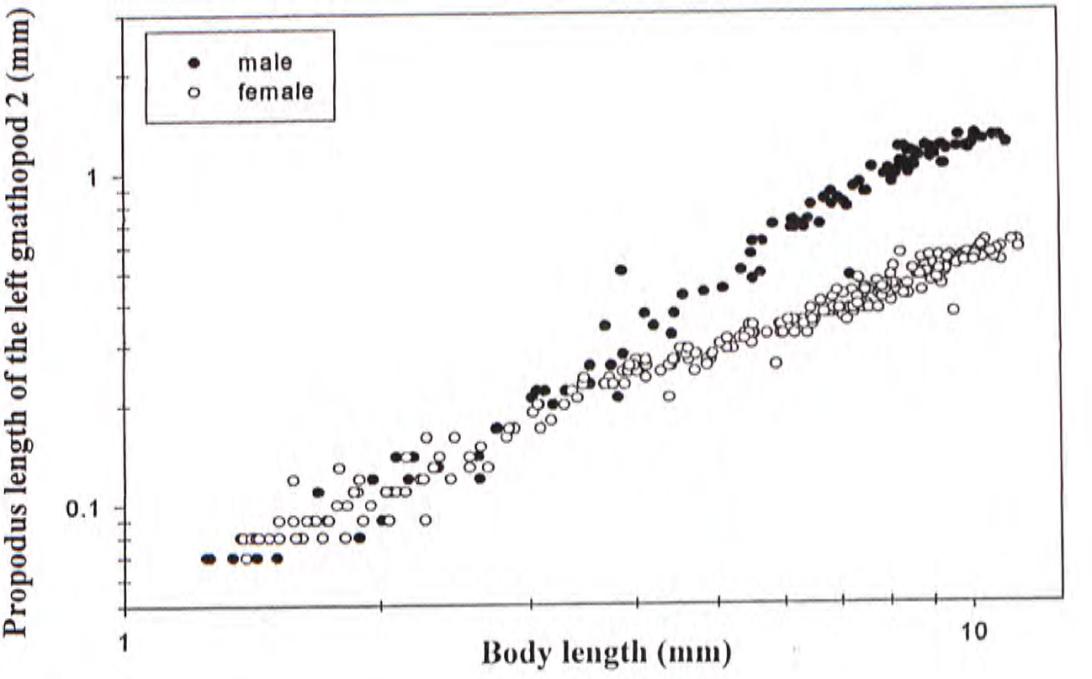
Figure 3.22 Growth curves of *Hyale* sp. in mean propodus length of the left gnathopod 2 (GL2) at different instars. The regression equations of the growth curves in males and females are $Y = 9 \times 10^{-4}X^3 + 0.021X^2 - 0.0250X - 0.070$ ($r^2 = 0.996$) and $Y = -0.001X^2 + 0.049X + 0.010$ ($r^2 = 0.993$), respectively. In the equations, X represents number of instar and Y represents GL2. The error bars represent the standard deviation.

the mean GL2 of males was significantly greater than that of females ($P < 0.001$). The mean GL2 of the two sexes started to significantly deviated at instar 4 ($P = 0.006$; Tukey test after 2-factor ANOVA). At instar 15, the mean GL2 of males and females were 1.22 ± 0.05 mm and 0.50 ± 0.07 mm, respectively. The mean GL2 of males at instar 15 was 2.44 times larger than that of females. The maximum GL2 of males and females recorded were 1.28 mm at instar 15 and 0.61 mm at instar 16, respectively.

The relative growth of logarithmic values of both GL2 to BL in the two sexes is shown in Figure 3.23A. Only one growth phase was observed in males (Figure 3.23B). Neither change in allometric level nor dimension was observed in the relative growth of GL2 to BL in males (Figure 3.23B). This indicated that GL2 grew in a constant proportion to BL in their life span. It was an isometric growth pattern. However, two growth phases were differentiated in females (Figure 3.23C). Two regression lines indicated the two different growth phases. The regression equations of the lines CD and EF are $\log_{10}Y = 1.10 \log_{10}X - 1.28$ ($r^2 = 0.951$) and $\log_{10}Y = 0.90 \log_{10}X - 1.17$ ($r^2 = 0.931$), respectively. The allometric coefficients of the two regression lines were significantly different from each other ($P < 0.0001$; Student's *t*-test). When BL was below 4 mm, GL2 grew in the phase CD. When BL became greater than 4 to 5 mm, GL2 subsequently grew in the phase EF. The phases transition (represented by ED region) between two successive phases CD and EF involved the overlapping of the body size in the range of 4 to 5 mm (equivalent to instar 5-8). This indicated that GL2 grew in a smaller proportion to BL in the growth phase CD than in EF. It was a negatively allometric growth pattern. The analysis also revealed that the allometric coefficients of the regression line of the growth phase AB (in males) was significantly greater than that of the growth phase CD (in females) ($P < 0.0001$). This indicated that GL2 of males (growth phase AB) grew in a greater proportion to BL than those of females (growth phases CD and EF).

The mean ratio of GL2 to BL increased continuously in development of males (Figure 3.24). The ratio stabilized at 0.13 when instar 12 was reached. The highest GL2 / BL ratio reached 1.43 at instar 13 in males. However, the mean ratio of females

3.23A



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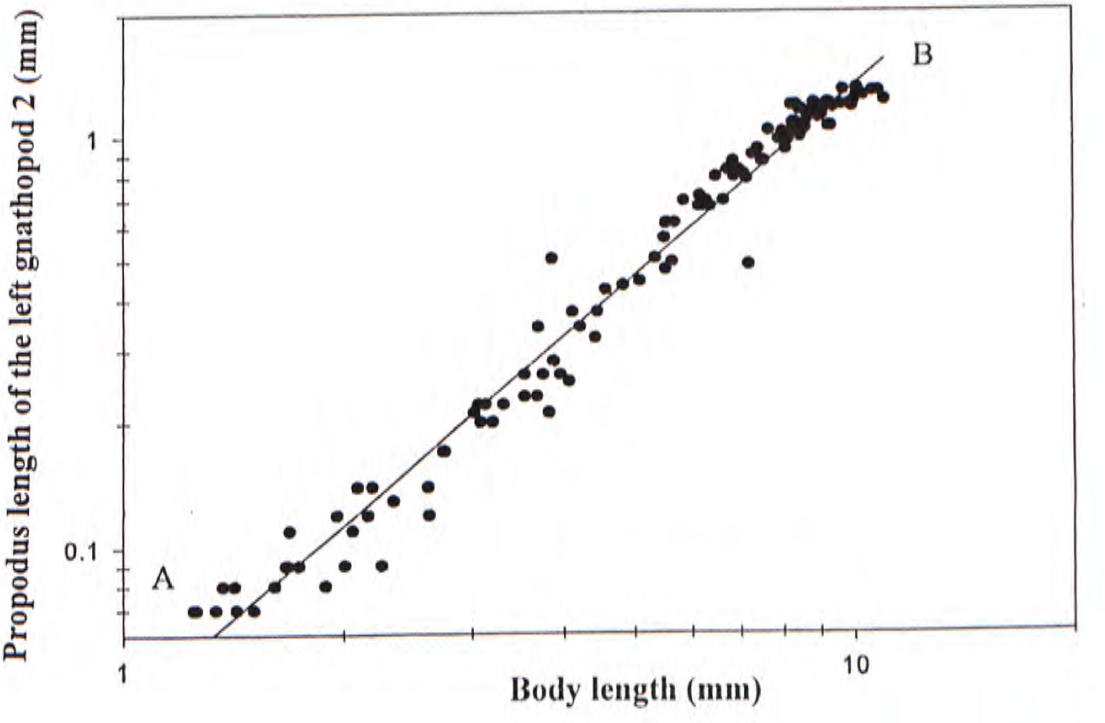

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3.23C

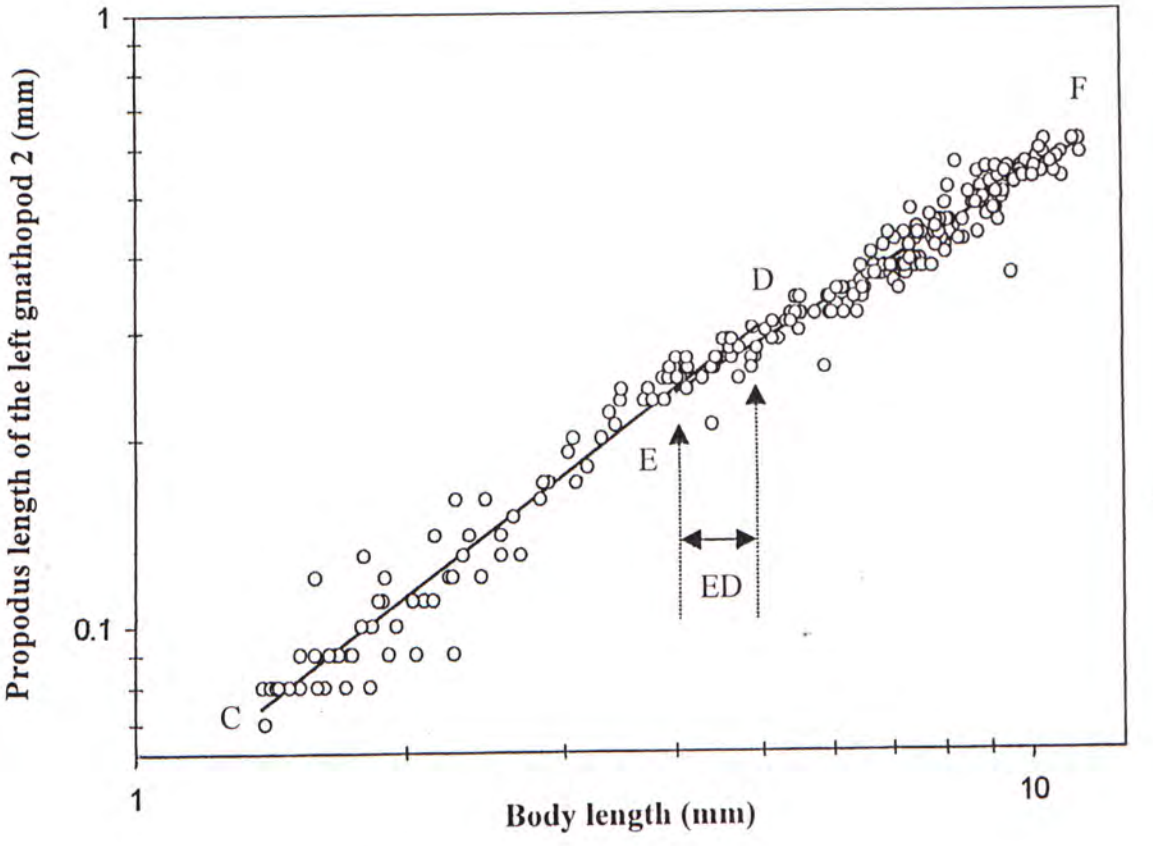


Figure 3.23 The relationships between the propodus length of the left gnathopod 2 (GL2) and the body length (BL) in logarithmic scale. A, the two sexes; B, male, one growth phase AB is marked on the figure, the regression equations of the line is $\log_{10}Y = 1.52 \log_{10}X - 1.40$ ($r^2 = 0.980$); C, female, two growth phases CD and EF are marked on the figure, the regression equations of the lines CD and EF are $\log_{10}Y = 1.10 \log_{10}X - 1.28$ ($r^2 = 0.951$) and $\log_{10}Y = 0.90 \log_{10} X - 1.17$ ($r^2 = 0.931$), respectively. The ED region indicates the body length with overlapping of the two growth phases. In the equations, X represents BL and Y represents GL2. N (male) = 120, and N (female) = 252.

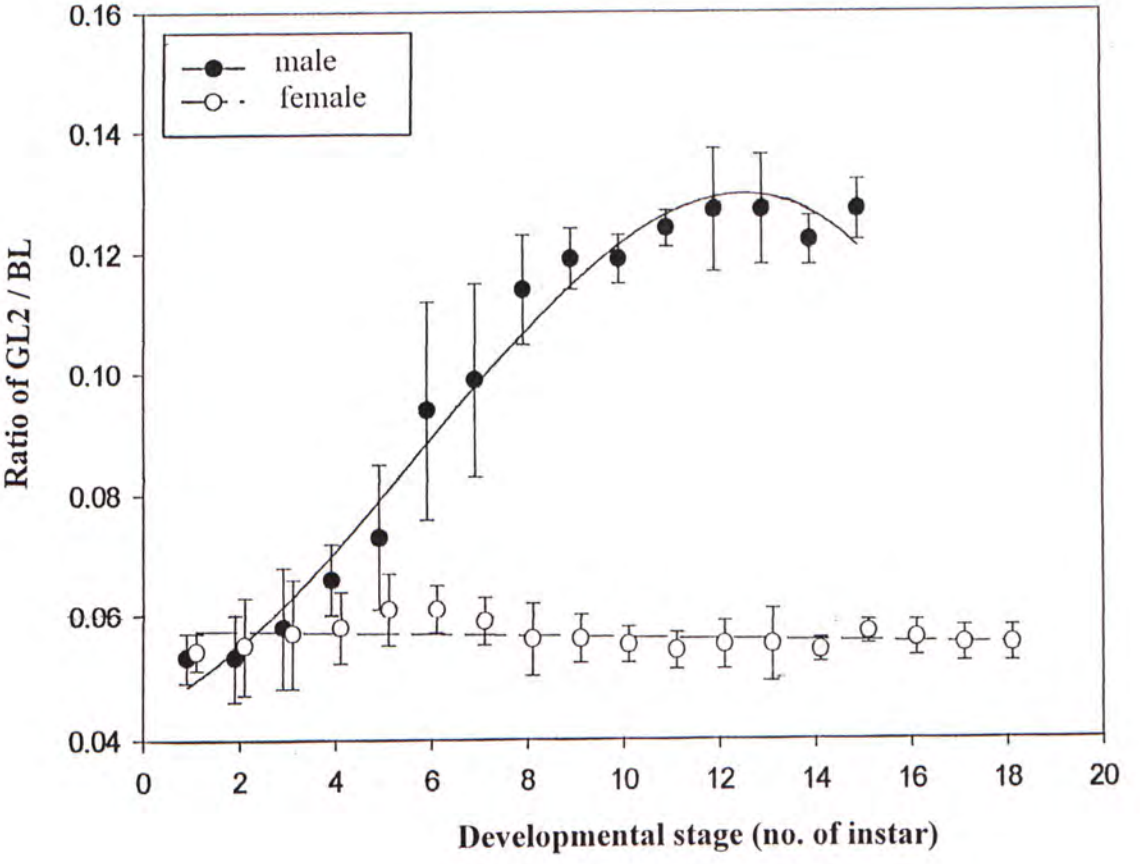


Figure 3.24 The variation on the ratio of the propodus length of the left gnathopod 2 to the body length (GL2 / BL) in different instars. The regression equations of the curves in males and females are $Y = 7 \times 10^{-5}X^3 + 0.001X^2 - 0.003X + 0.044$ ($r^2 = 0.978$) and $Y = 1 \times 10^{-4}X + 0.058$ ($r^2 = 0.122$), respectively. In the equations, X represents number of instar and Y represents GL2 / BL. The error bars represent the standard deviation.

decreased very slowly along the development of females. The analysis showed that the slope of the regression line was significantly different from zero ($P < 0.0001$; Student's *t*-test) and there was a significant correlation between the ratio and the number of instar in females.

The ratio of GL1 to BL and GL2 to BL of males and females were compared independently in Figure 3.25. The relationship between the ratios of males and number of instars is shown in Figure 3.25A. This indicated that the mean ratio GL1 / BL kept in a constant at 0.051 ± 0.002 along development of males. However the growth ratio GL2 / BL increased continuously and stabilized since instar 12. This indicated that GL2 grew in a faster rate than BL. The mean ratio increased continuously and stabilized in the range of 0.129 ± 0.019 at instar 12. The ratio of GL2 / BL was about 2.5 times greater than that of GL1 / BL after instar 12. However the change in the ratio of GL2 / BL and GL1 / BL in females shows similar patterns (Figure 3.25B). The mean ratios of both GL2 and GL1 to BL slightly dropped and finally stabilized at more or less constant ratios.

The relationship between the ratios of GL2 / GL1 and the developmental stages in the two sexes is shown in Figure 3.26. The differentiation was mainly based on the size of GL2. The result showed that the mean ratio of males increased continuously from 1.00 at the first three instars to the peak of 2.60 at instar 12. The maximum ratio of a male was found to be 2.89 at instar 12. However the mean ratio of females only changed within a narrow range. It slightly increased from 1.10 since instar 1 to 1.40 at instar 18. The ratio of the female generally did not exceed 1.50 at any instar. Only two out of 254 data (0.8%) were found to be greater than 1.50. They were 1.89 and 1.71 occurred at instar 3 and instar 2, respectively.

3.3.1.10 Merus Length of Pereopod 7 (MP7)

The growth of MP7 in the two sexes is shown in Figure 3.27. The effects of sex and number of instar on growth of MP7 were analyzed by 2-factor ANOVA. The

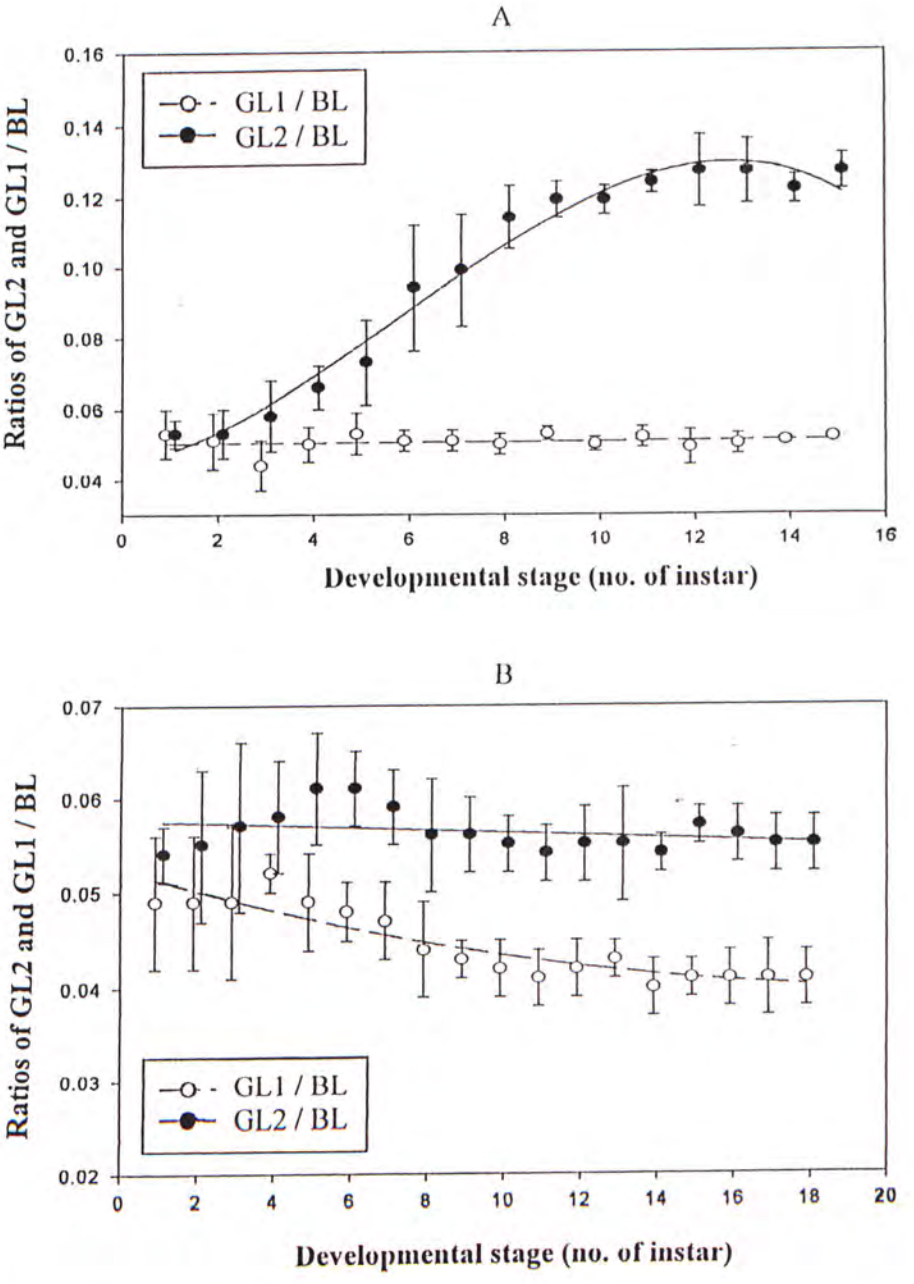


Figure 3.25 The comparison of the ratios between GL1 / BL and GL2 / BL of *Hyale* sp. in different instar. A, male; B, female. The regression equations of the ratio of GL1 / BL and GL2 / BL for males are $Y_1 = 6 \times 10^{-4}X + 0.050$ ($r^2 = 0.013$) and females are $Y_2 = 7 \times 10^{-5}X^3 + 0.001X^2 - 0.003X + 0.044$ ($r^2 = 0.978$), respectively. The regression equations of the ratio of GL1 / BL and GL2 / BL for females are $Y_1 = 3 \times 10^{-5}X^2 - 0.001X + 0.053$ ($r^2 = 0.840$) and $Y_2 = 1 \times 10^{-4}X + 0.058$ ($r^2 = 0.122$), respectively. In the equations, X, Y_1 and Y_2 represent number of instar, GL1 / BL and GL2 / BL, respectively.

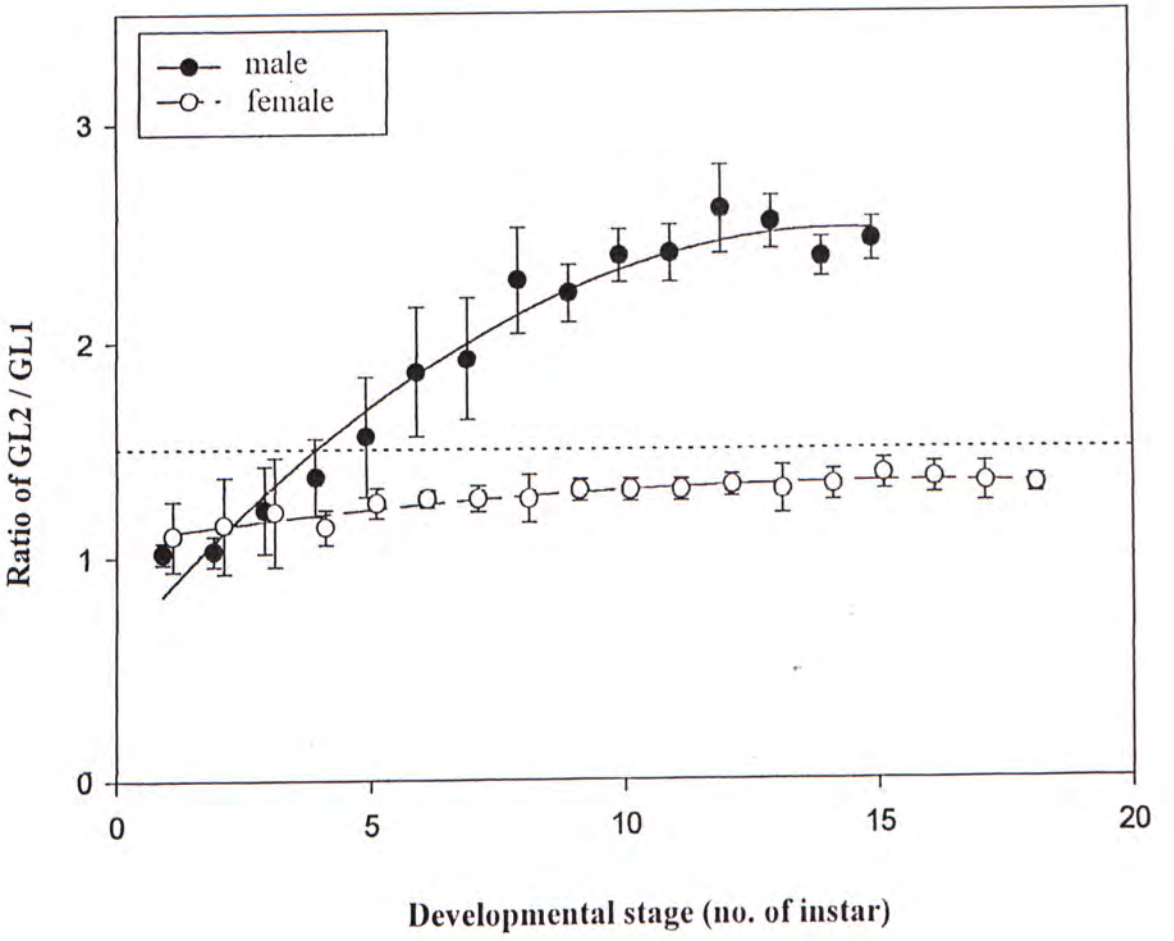


Figure 3.26 The variation on the ratio of the propodus length of the left gnathopod 2 to that of the left gnathopod 1 (GL2 / GL1). The regression equations of the curves in males and females are $Y = -8.33X^2 + 0.25X + 0.61$ ($r^2 = 0.857$) and $Y = -1.06X^2 + 0.03X + 1.09$ ($r^2 = 0.311$), respectively. In the equations, X represents number of instar and Y represents GL2 / GL1. The error bars represent the standard deviation.

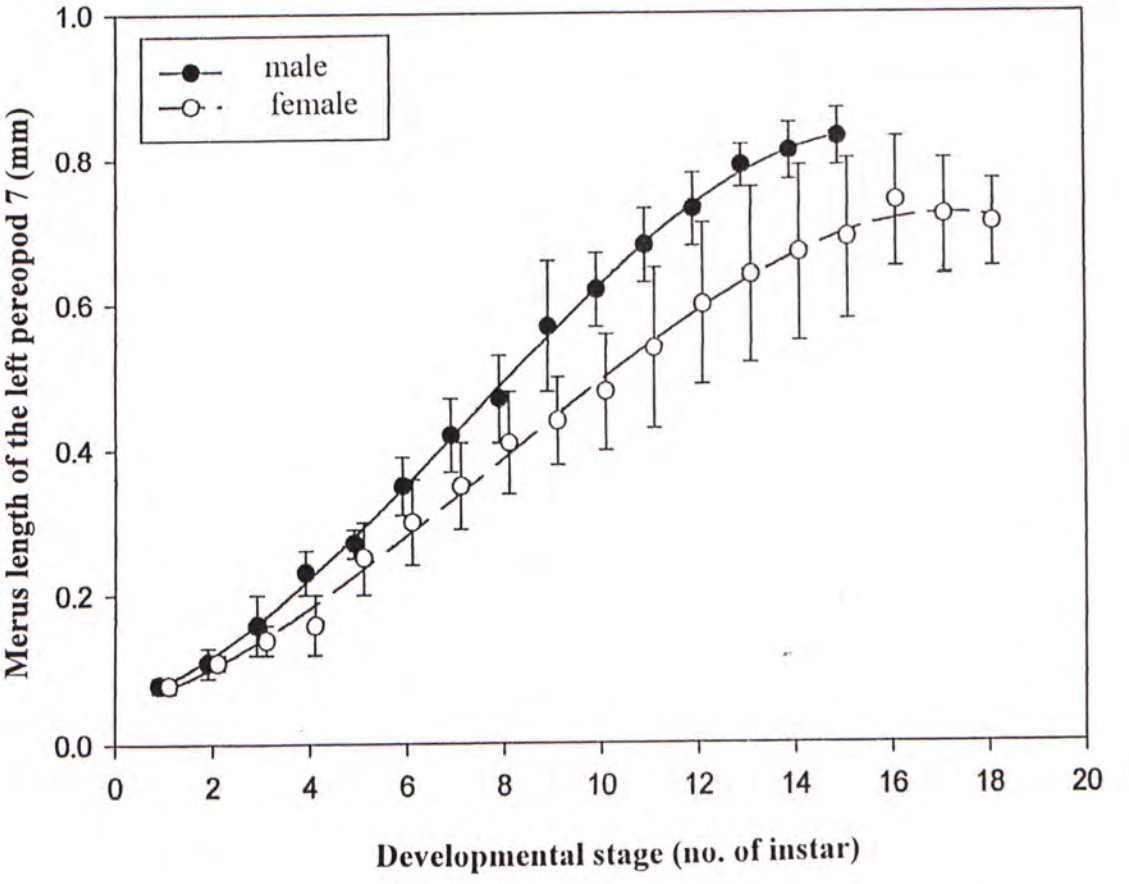


Figure 3.27 Growth curves of *Hyale* sp. in mean merus length of the left pereopod 7 (MP7) at different instars. The regression equations of the growth curves in males and females are $Y = -0.001X^2 + 0.070X - 0.030$ ($r^2 = 0.993$) and $Y = -0.001X^2 + 0.063X - 0.027$ ($r^2 = 0.988$), respectively. In the equations, X represents number of instar and Y represents MP7. The error bars represent the standard deviation.

analysis showed that MP7s were significantly different among instars ($P < 0.001$), and the mean MP7 of males was significantly greater than that of females ($P < 0.001$). A significant deviation of MP7 between the two sexes started at instar 7 ($P = 0.016$; Tukey test after 2-factor ANOVA). At instar 15, the mean MP7 of males and females were 0.83 ± 0.04 mm and 0.69 ± 0.11 mm, respectively. The maximum MP7 recorded was 0.89 mm of a male at instar 16 and 0.86 mm of a female at instar 16, respectively.

The relative growth pattern of logarithmic scale of MP7 to BL in the two sexes is shown in Figure 3.28. Only one growth phase was observed in the relative growth pattern of the two sexes individually. The regression lines of AB and AC represented the growth phases of males and females, respectively. The linear equations of the regression lines of males and females were $\log_{10}Y = 1.19 \log_{10}X - 1.27$ ($r^2 = 0.977$) and $\log_{10}Y = 1.17 \log_{10}X - 1.29$ ($r^2 = 0.964$), respectively. The statistical analysis showed that there was no significant difference in the allometric coefficients of the regression lines between the two sexes ($P = 0.175$; Student's *t*-test). This indicated that MP7 of the two sexes grew in a constant and equal proportion to BL in the whole development.

3.3.2 Growth of Juveniles in Different Salinities

3.3.2.1 Survival Rate

Totally 55 juveniles were successfully followed in the study. The sample size of each batch of salinity 10, 20, 30 and 40‰ was 11, 13, 16 and 15 juveniles, respectively. The sex ratio of juveniles in each experimental salinity is shown in Table 3.4. The rate of juvenile survived for 6 instars in the salinities of 10, 20, 30 and 40‰ were 73.3, 81.3, 36.4 and 40.5%, respectively. The higher survival rate was obtained from the lower salinity exposure (10 & 20‰).

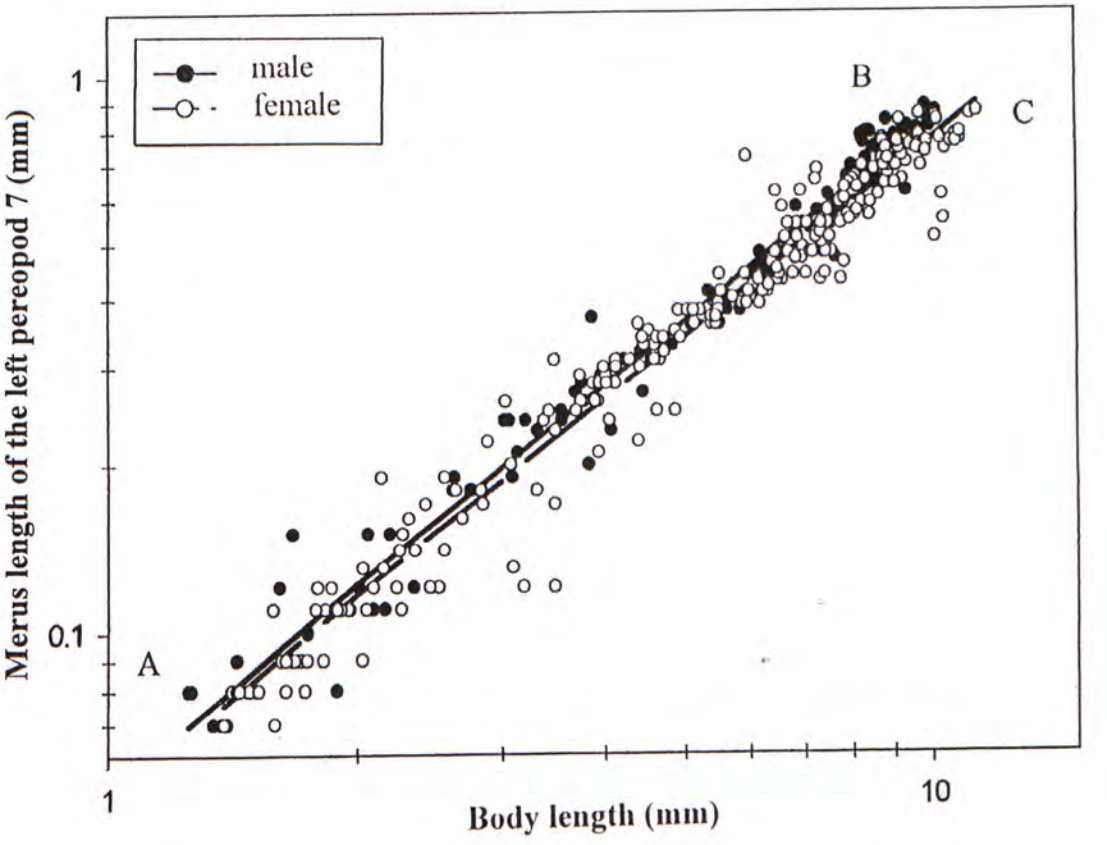


Figure 3.28 The relationships between the merus length of the left pereopod 7 (MP7) and the body length (BL) in logarithmic scale. Only one growth phase is observed in the two sexes. The regression equations of the curves in males and females are $\log_{10}Y = 1.19 \log_{10}X - 1.27$ ($r^2 = 0.977$) and $\log_{10}Y = 1.17 \log_{10}X - 1.29$ ($r^2 = 0.964$), respectively. In the equations, X represents BL and Y represents MP7. N (male) = 120 and N (female) = 253.

Table 3.4. The survival rate of the juvenile *Hyale* sp. reared in different salinities.

	Salinity (%)			
	10	20	30	40
Total no. of juveniles	15	16	44	37
No. of survived juveniles	11	13	16	15
Survival rate	73.3%	81.3%	36.4%	40.5%
Ratio of male:female	5:6	7:6	7:9	8:7

3.3.2.2 Age

The relationship between the mean age and the number of instar in different salinities is shown in Figure 3.29. The effects of salinity and number of instar on mean age of juveniles were analyzed in 2-factor ANOVA. The statistical analysis showed that the mean age was significantly different among instars ($P < 0.001$), and the mean age of juveniles exposed to 10‰ salinity was significantly greater than those exposed to the salinity of 20, 30 and 40‰ ($P < 0.001$). This indicated that juveniles reared in 10‰ salinity took more time to reach the same instar.

3.3.2.3 Intermolt Duration

The effects of salinity and number of instar on intermolt duration were analyzed in 2-factor ANOVA. The analysis showed that the duration were significantly different among instars ($P = < 0.001$), the duration significantly lengthened as the number of instar increased (Figure 3.30). However, the analysis also indicated that but there was no significant difference in the intermolt duration among salinities ($P = 0.443$).

3.3.2.4 Body Length (BL)

The increase in BL of juveniles reared in different salinities is shown in Figure 3.31. The effects of salinity and number of instar on BL were analyzed in 2-factor ANOVA. The analysis showed that BLs were significantly different among instars ($P < 0.001$), and there was a significant effect of salinity on BL increment ($P < 0.001$). The growth rate (BL increment) of juveniles reared in 20‰ was the highest among the salinities studied ($P = < 0.05$; Tukey test after 2-factor ANOVA). And the growth rate of juveniles reared in 30‰ was significantly higher than those in 10 and 40‰. Yet there was no significant difference between the growth rates of juveniles reared in 10 and 40‰ ($P > 0.05$). Thus the decreasing order of the growth rate of the juveniles (body length) in different salinities was 20 > 30 > 40 and 10‰. For example, the mean body length of juveniles reared in 20, 30, 40 and 10‰ at instar 6 were 4.46 ± 0.77 mm,

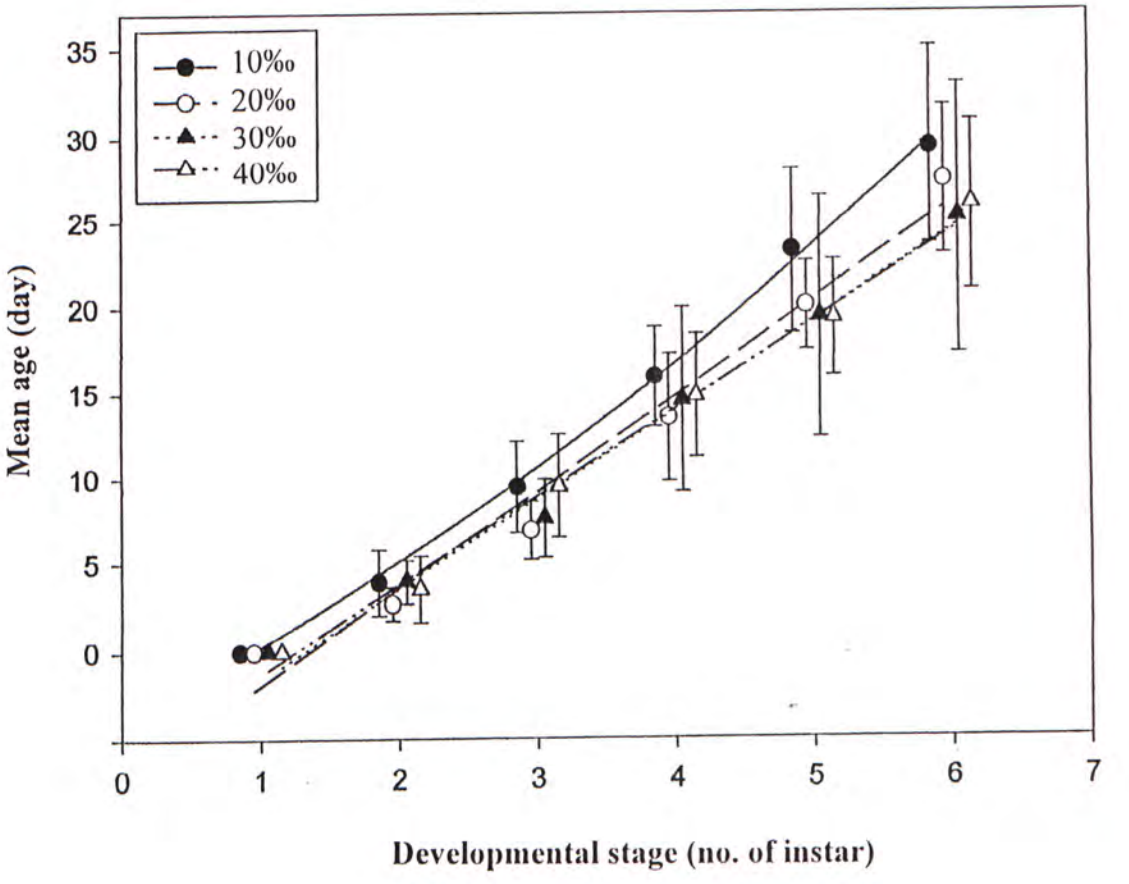


Figure 3.29 Growth curves in mean age of the juvenile *Hyale* sp. reared in different salinities. The regression equations of the growth curves in the salinity of 10, 20, 30 and 40‰ are $Y = 0.32X^2 + 3.79X - 3.87$ ($r^2 = 0.998$), $Y = 0.57X^2 + 1.57X - 2.43$ ($r^2 = 0.998$), $Y = 0.24X^2 + 3.43X - 3.87$ ($r^2 = 0.996$), and $Y = 0.16X^2 + 4.05X - 4.48$ ($r^2 = 0.997$), respectively. In the equations, X represents no. of instar and Y represents mean age. The error bars represent the standard deviation.

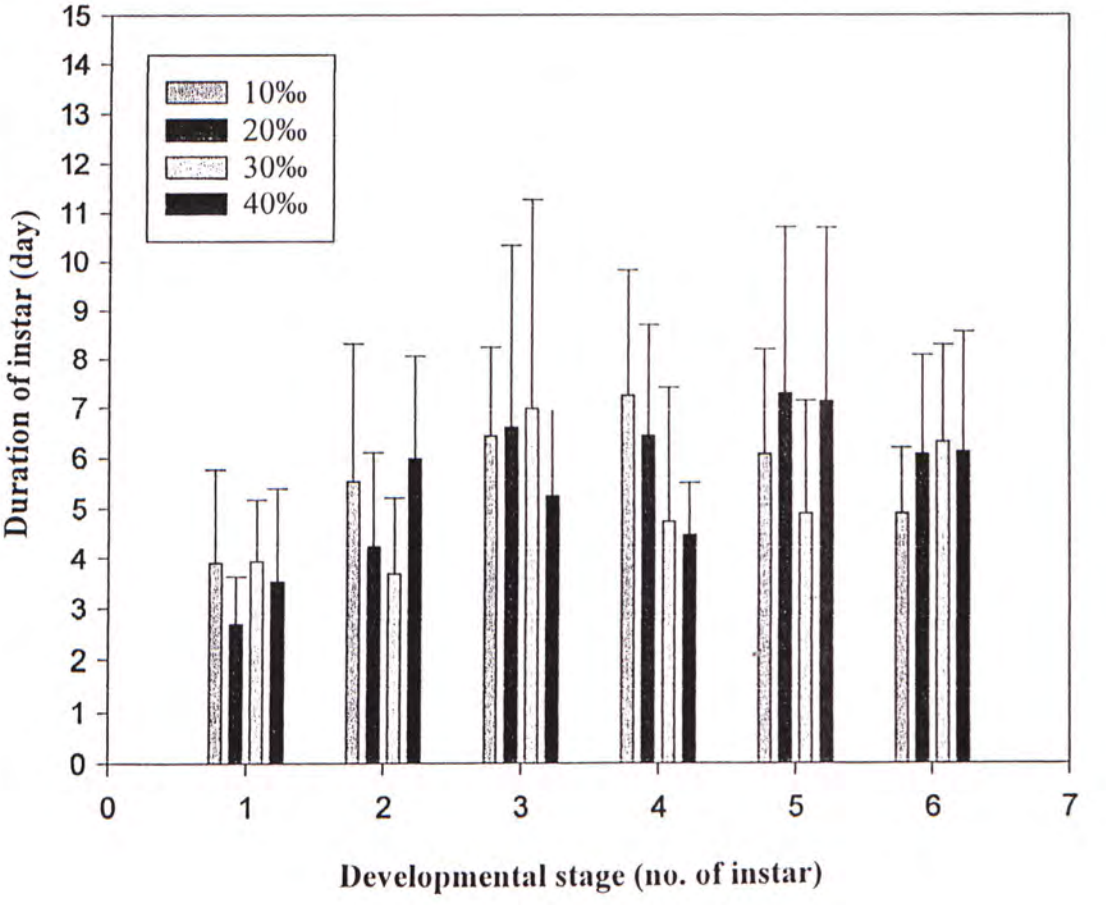


Figure 3.30 Mean intermolt duration of each instar of the juvenile *Hyale* sp. reared in different salinities. The error bars represent the standard deviation.

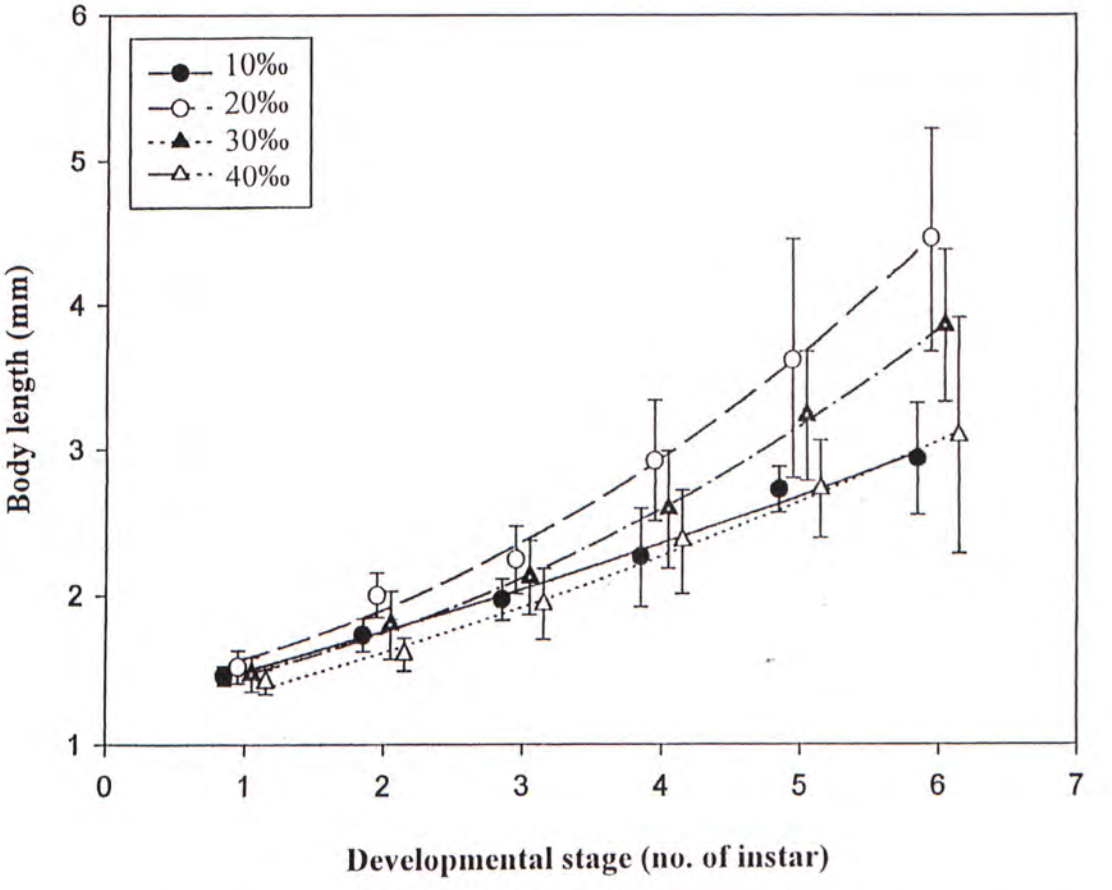


Figure 3.31 Growth curves in mean body length (BL) of the juvenile *Hyale* sp. reared in different salinities. The regression equations of the growth curves in the salinity of 10, 20, 30 and 40‰ are $Y = 0.01X^2 + 0.24X + 1.21$ ($r^2 = 0.998$), $Y = 0.03X^2 + 0.35X + 1.11$ ($r^2 = 0.989$), $Y = 0.04X^2 + 0.20X + 1.22$ ($r^2 = 0.989$) and $Y = 0.04X^2 + 0.12X + 1.26$ ($r^2 = 0.994$), respectively. In the equations, X represents no. of instar and Y represents BL. The error bars represent the standard deviation.

3.86 ± 0.52 mm, 3.10 ± 0.82 mm and 2.94 ± 0.39 mm, respectively. At instar 6, the mean BL of the juveniles reared in 20‰ was found to be 1.50 times greater than those in 10‰.

3.3.2.5 Head Length (HL)

The growth curves in HL of juveniles reared in different salinities is shown in Figure 3.32. The effects of salinity and number of instar on HL were analyzed by 2-factor ANOVA. The analysis showed that HLs were significantly different among instars ($P < 0.001$), and there was a significant effect of salinity on the growth of HL ($P < 0.001$). It also showed that the growth rate (HL increment) of juveniles was significantly the highest when reared in 20‰ than in the other salinities studied ($P < 0.05$; Tukey test after 2-factor ANOVA). The analysis also showed that there was no significant difference in HL increment of juveniles reared in the salinities of 10, 30 and 40‰ ($P > 0.05$). The mean HL of juveniles reared in 20‰ was 0.33 ± 0.04 mm at instar 6, while those reared in 30, 40 and 10‰ were 0.28 ± 0.04 mm, 0.28 ± 0.04 mm and 0.26 ± 0.03 mm, respectively. Thus the decreasing order of the growth rate of juveniles (HL) in different salinities was 20 > 30 and 40 and 10‰. At instar 6, the mean HL of juveniles was 1.27 times greater than in 20‰ than in 10‰.

3.3.2.6 Eye Length (EL)

The growth curves in EL of juveniles reared in different salinities is shown in Figure 3.33. The effects of salinity and number of instar on EL were analyzed by 2-factor ANOVA. The analysis showed that ELs were significantly different among instars ($P < 0.001$), and there was a significant effect of salinity on growth of EL ($P < 0.001$). The statistical analysis showed that EL increment of juveniles reared in 20‰ was the significantly highest among the other salinities studied ($P < 0.05$; Tukey test after 2-factor ANOVA). And the growth rate (EL increment) of juveniles reared in 30‰ was significantly higher than those reared in 10 and 40‰ ($P < 0.05$). However, there was no significant difference in the growth rate of juveniles reared in 10 and 40‰

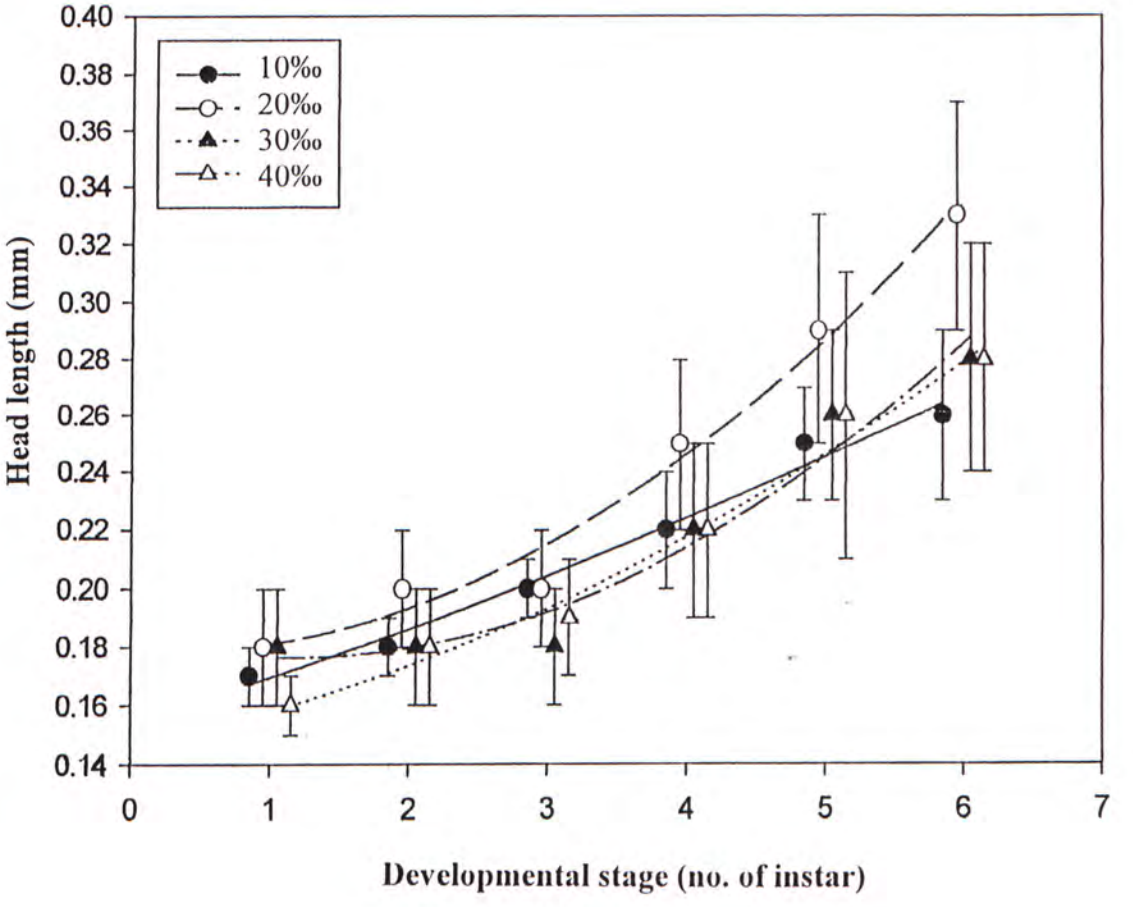


Figure 3.32 Growth curves in mean head length (HL) of the juvenile *Hyale* sp. reared in different salinities. The regression equations of the growth curves in the salinity of 10, 20, 30 and 40‰ are $Y = 0.001X^2 + 0.014X + 0.150$ ($r^2 = 0.984$), $Y = 0.005X^2 - 0.002X + 0.179$ ($r^2 = 0.982$), $Y = 0.005X^2 - 0.010X + 0.182$ ($r^2 = 0.956$), and $Y = 0.002X^2 + 0.010X + 0.148$ ($r^2 = 0.985$), respectively. In the equations, X represents no. of instar and Y represents HL. The error bars represent the standard deviation.

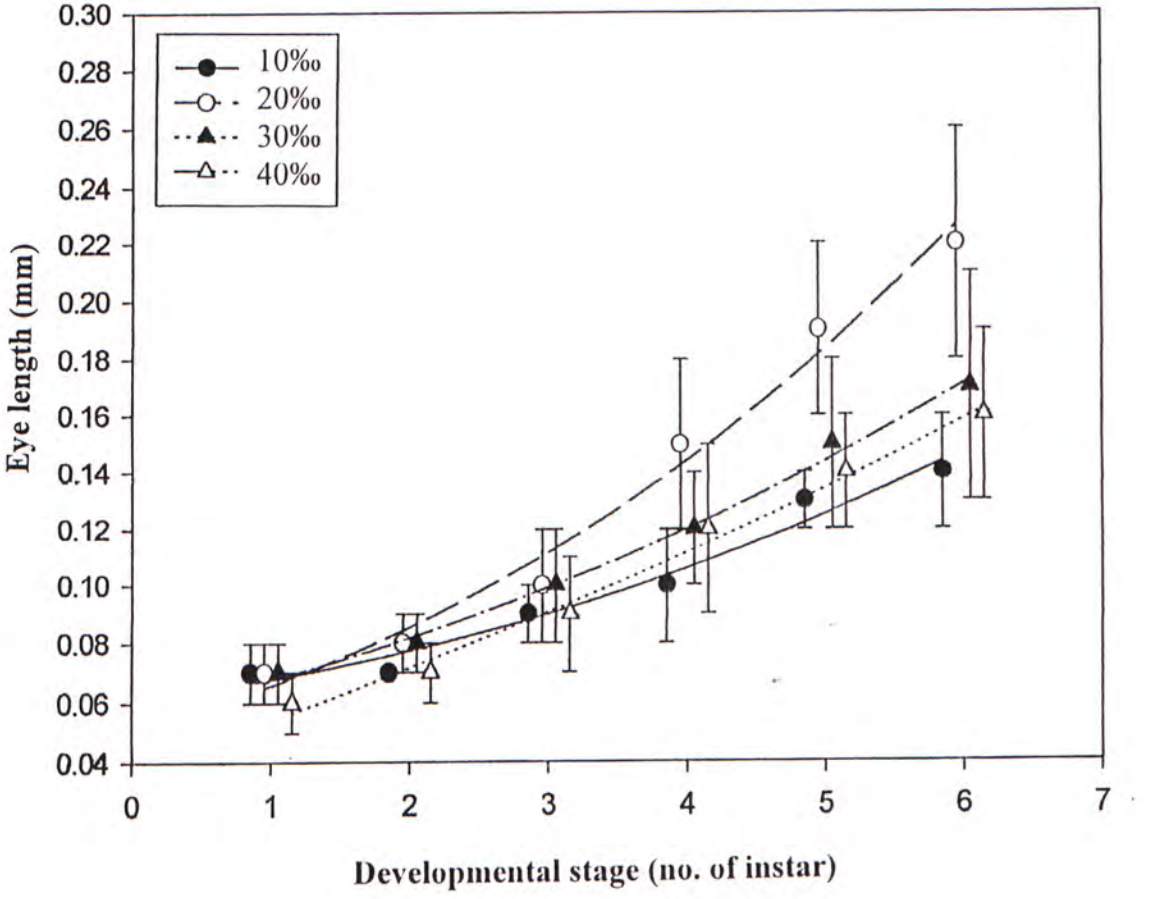


Figure 3.33 Growth curves in mean eye length (EL) of the juvenile *Hyale* sp. reared in different salinities. The regression equations of the growth curves in the salinity of 10, 20, 30 and 40‰ are $Y = 0.002X^2 + 0.004X + 0.061$ ($r^2 = 0.984$), $Y = 0.003X^2 + 0.030X + 0.027$ ($r^2 = 0.967$), $Y = 0.002X^2 + 0.004X + 0.061$ ($r^2 = 0.994$), and $Y = 0.001X^2 + 0.015X + 0.041$ ($r^2 = 0.994$), respectively. In the equations, X represents no. of instar and Y represents EL. The error bars represent the standard deviation.

($P > 0.05$). Thus the decreasing order of the growth rate of the juveniles (EL) in different salinities was 20 > 30 > 40 and 10‰. The mean EL of juveniles reared in 20‰ was 0.22 ± 0.04 mm at instar 6, while those reared in 30, 40 and 10‰ were 0.17 ± 0.04 mm, 0.16 ± 0.03 mm and 0.14 ± 0.02 mm, respectively.

3.3.2.7 Length of Antenna 1 (AL1)

The growth curves in AL1 of juveniles reared in different salinities is shown in Figure 3.34. The effects of salinity and number of instar on AL1 were analyzed in 2-factor ANOVA. The analysis showed that AL1s were significantly different among instars ($P < 0.001$), and there was a significant effect of salinity on growth of AL1 ($P < 0.001$). The analysis showed that the growth rate of juveniles (AL1) reared in 20‰ was the significantly highest among the other salinities studied ($P < 0.05$; Tukey test after 2-factor ANOVA). The statistical analysis also indicated that the growth rates of juveniles reared in 10, 30 and 40‰ were not significantly different among the others ($P > 0.05$). Thus the decreasing order of the growth rate of juveniles (AL1) was 20 > 30 and 40 and 10‰. For example, at instar 6, the mean AL1 of the juveniles reared in salinities 20, 30, 40 and 10‰ were 0.94 ± 0.13 mm , 0.80 ± 017 mm, 0.67 ± 0.11 mm and 0.62 ± 0.10 mm, respectively.

3.3.2.8 Number of Articles on Antenna 1 (AA1)

The growth curves in AA1 of juveniles reared in different salinities is shown in Figure 3.35. The effects of salinity and number of instar on AA1 were analyzed in 2-factor ANOVA. The analysis showed that AA1s were significantly different among instars ($P < 0.001$), and there was a significant effect of salinity on growth of AA1 ($P < 0.001$). Statistical analysis showed that the growth rate of juveniles (AA1 increment) reared in 20‰ was the significantly highest among the salinities studied ($P < 0.05$; Tukey test after 2-factor ANOVA), while those reared in 10‰ was the significantly lowest ($P < 0.05$). There was no significant difference between the growth rates of juveniles reared in 30 and 40‰. Thus the decreasing order of the growth rate of

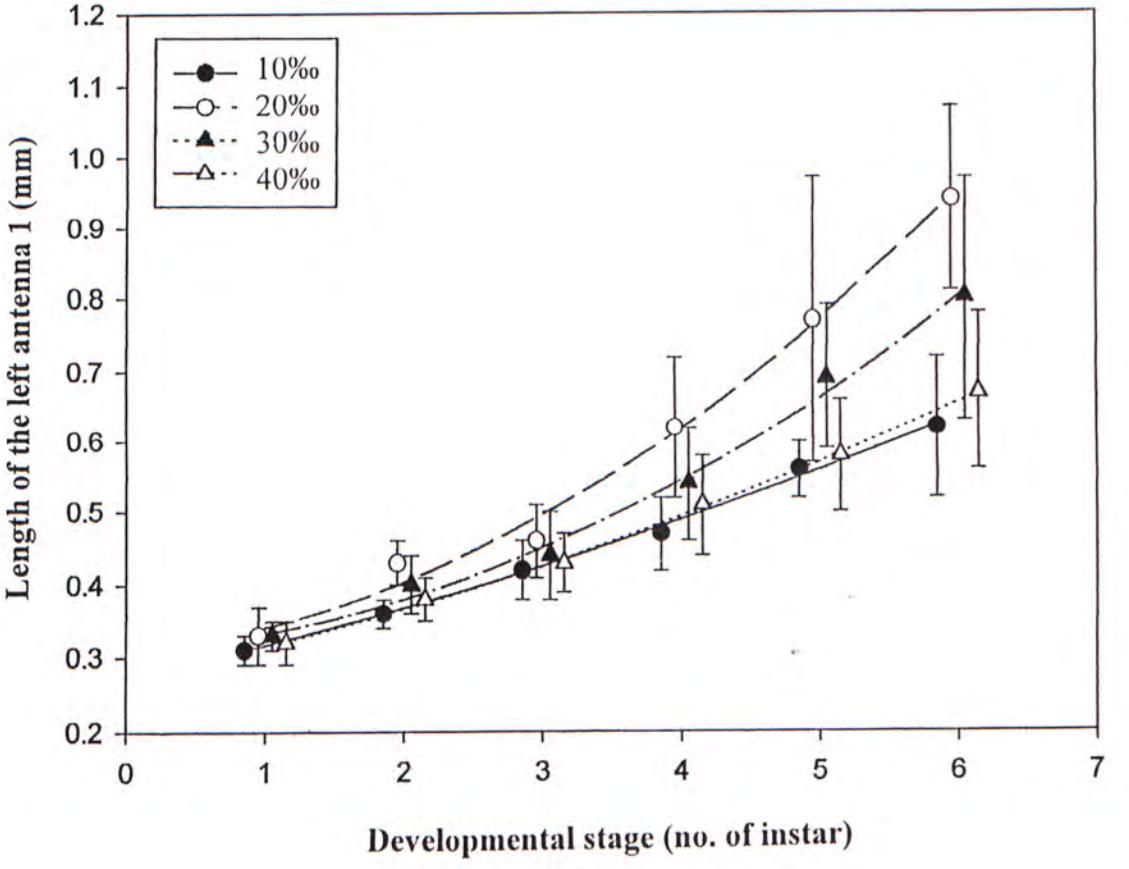


Figure 3.34 Growth curves in mean length of the left antenna 1 (AL1) of the juvenile *Hyale* sp. reared in different salinities. The regression equations of the growth curves in the salinity of 10, 20, 30 and 40‰ are $Y = 0.003X^2 + 0.044X + 0.263$ ($r^2 = 0.998$), $Y = 0.011X^2 + 0.019X + 0.303$ ($r^2 = 0.996$), $Y = 0.007X^2 + 0.066X + 0.250$ ($r^2 = 0.985$), and $Y = 0.005X^2 + 0.034X + 0.284$ ($r^2 = 0.999$), respectively. In the equations, X represents no. of instar and Y represents AL1. The error bars represent the standard deviation.

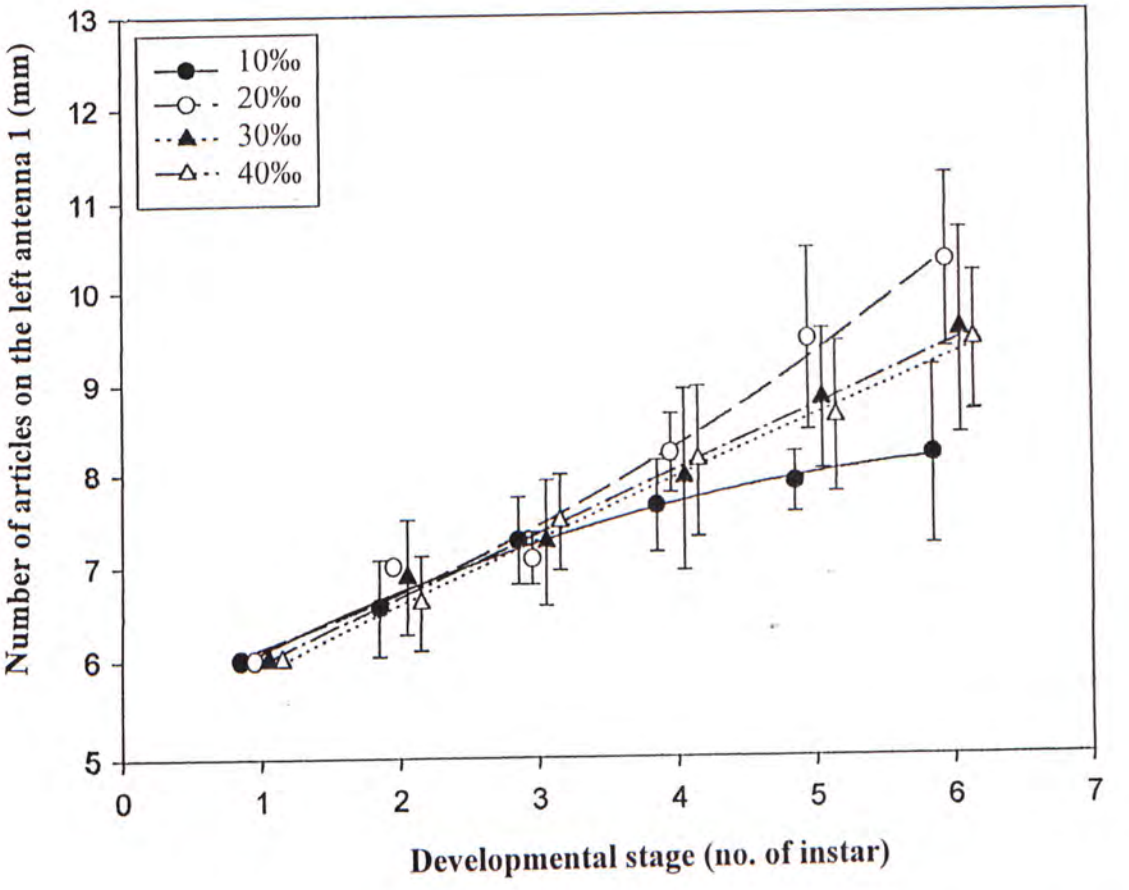


Figure 3.35 Growth curves in mean number of articles on the left antenna 1 (AA1) of the juvenile *Hyale* sp. reared in different salinities. The regression equations of the growth curves in the salinity of 10, 20, 30 and 40‰ are $Y = -0.06X^2 + 0.83X + 5.20$ ($r^2 = 0.995$), $Y = 0.07X^2 + 0.37X + 5.65$ ($r^2 = 0.993$), $Y = 0.02X^2 + 0.53X + 5.53$ ($r^2 = 0.993$), and $Y = -0.01X^2 + 0.74X + 5.25$ ($r^2 = 0.995$), respectively. In the equations, X represents no. of instar and Y represents AA1. The error bars represent the standard deviation.

juveniles in AA1 was 20 > 30 and 40 > 10‰. For example, the mean AA1s measured at instar 6 in salinities 20, 30, 40 and 10‰ were 10.3 ± 1.0, 9.5 ± 1.0, 9.4 ± 0.8 and 8.2 ± 1.0, respectively.

3.3.2.9 Number of Articles on Antenna 2 (AA2)

The growth curves in AA2 of juveniles reared in different salinities is shown in Figure 3.36. The effects of salinity and number of instar on AA2 were analyzed in 2-factor ANOVA. The analysis showed that AA2s were significantly different among instars ($P < 0.001$), and there was a significant effect of salinity on growth of AA2 ($P < 0.001$). The growth rate (AA2 increment) of juveniles reared in 20‰ was the significantly highest among the salinities studied ($P < 0.05$; Tukey test after 2-factor ANOVA), while those reared in 10‰ was the significantly lowest ($P < 0.05$). There was no significant difference between the growth rates of juveniles reared in 30 and 40‰. Thus the decreasing order of the growth rate of juveniles (AA2) was 20 > 30 and 40 > 10‰. For example, the mean AA2s measured at instar 6 in salinities 20, 30, 40 and 10‰ were 14.5 ± 1.1, 13.0 ± 1.2, 12.5 ± 0.9 and 11.5 ± 0.9, respectively. The growth pattern of AA2 was similar to that of AA1.

3.3.2.10 Merus Length of Pereopod 7 (MP7)

The growth curves in MP7 of juveniles reared in different salinities is shown in Figure 3.37. The effects of salinity and number of instar on MP7 were analyzed in 2-factor ANOVA. The analysis showed that MP7s were significantly different among instars ($P < 0.001$), and there was a significant effect of salinity on growth of MP7 ($P < 0.001$). The growth rate (MP7 increment) of juveniles in the mean MP7 was significantly higher in salinities 20 and 30‰ than in 40 and 10‰ ($P < 0.05$; Tukey test after 2-factor ANOVA). However, there was no significant difference between the growth rate of juveniles reared in salinities 20 and 30‰, and between those in 10 and 40‰ ($P > 0.05$). The decreasing order of the growth rate was 20 and 30 > 40 and 10‰.

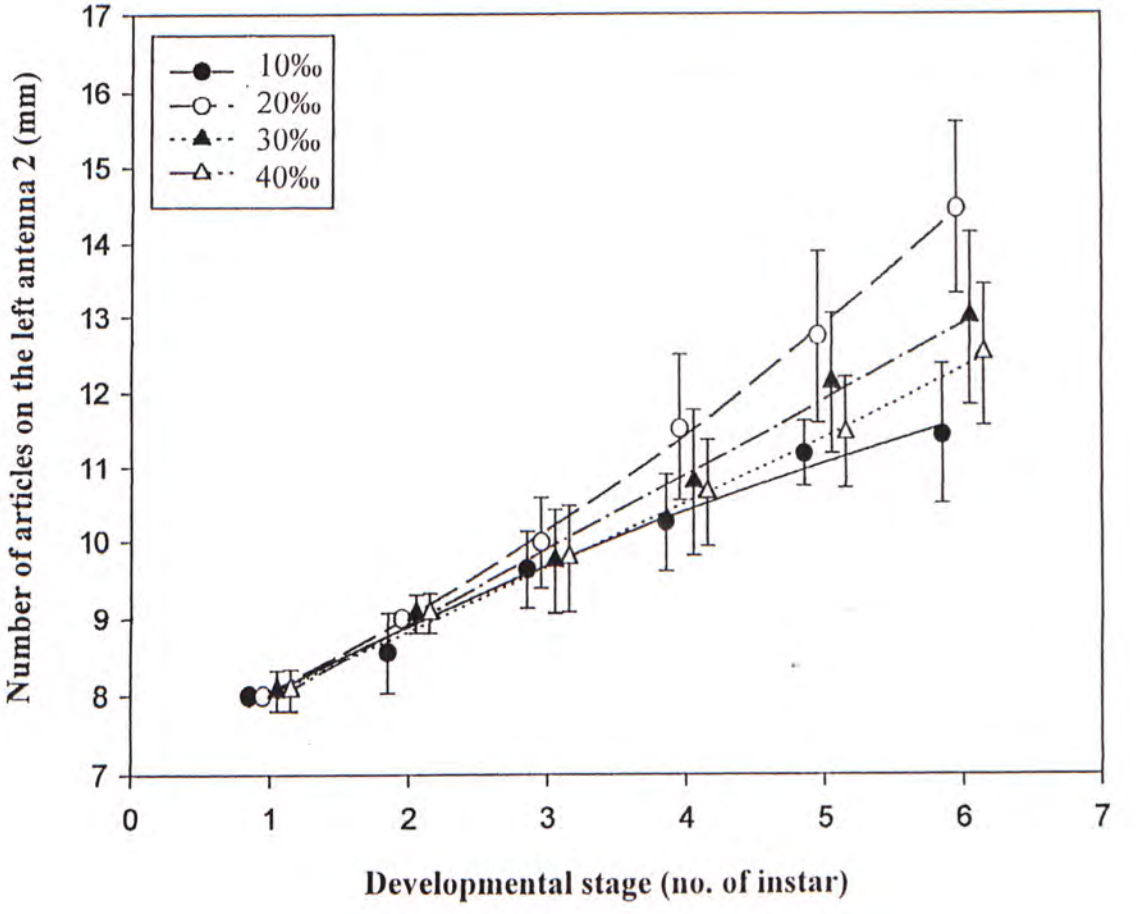


Figure 3.36 Growth curves in mean number of articles on the left antenna 2 (AA2) of the juvenile *Hyale* sp. reared in different salinities. The regression equations of the growth curves in the salinity of 10, 20, 30 and 40‰ are $Y = -0.04X^2 + 1.00X + 6.91$ ($r^2 = 0.987$), $Y = 0.08X^2 + 0.74X + 7.18$ ($r^2 = 0.998$), $Y = 0.03X^2 + 0.77X + 7.28$ ($r^2 = 0.996$), and $Y = 0.01X^2 + 0.80X + 7.33$ ($r^2 = 0.998$), respectively. In the equations, X represents no. of instar and Y represents AA2. The error bars represent the standard deviation.

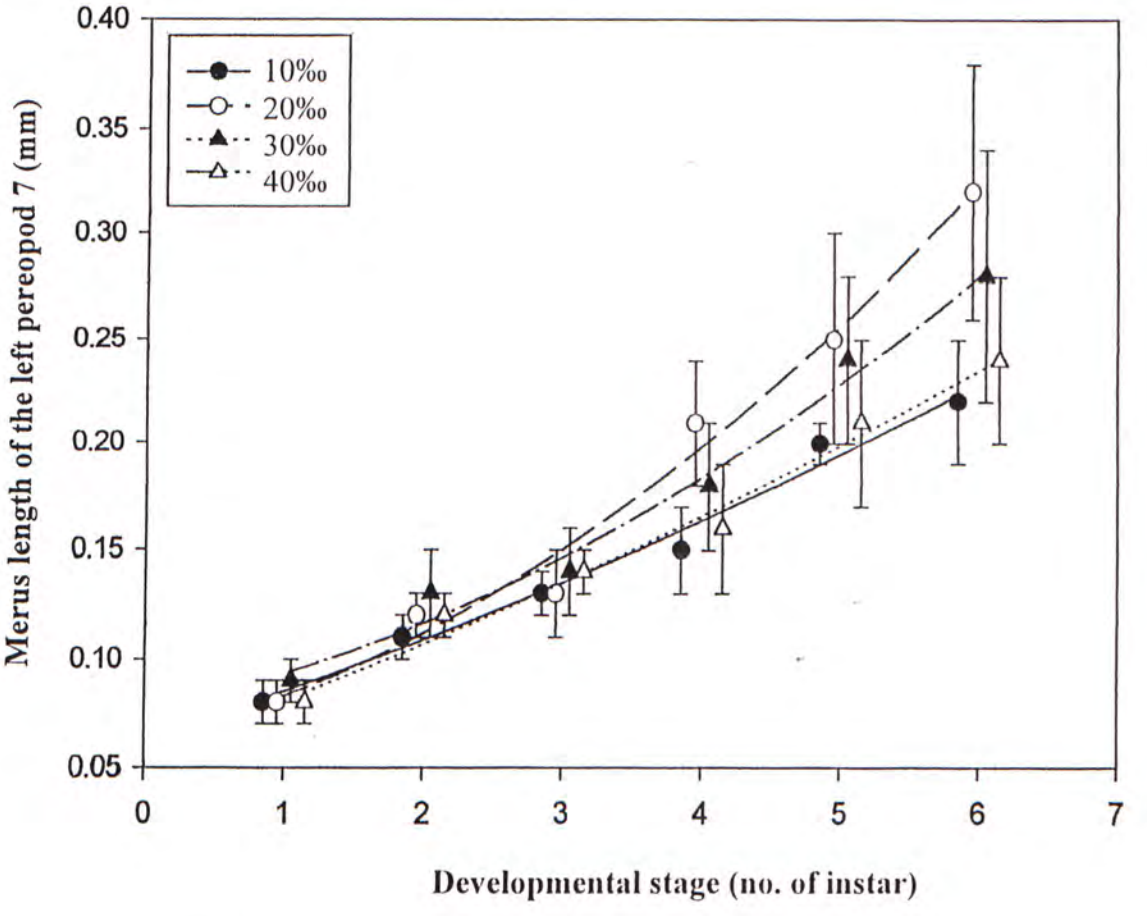


Figure 3.37 Growth curves in mean merus length of the left pereopod 7 (MP7) of the juvenile *Hyale* sp. reared in different salinities. The regression equations of the growth curves in the salinity of 10, 20, 30 and 40‰ are $Y = 0.001X^2 + 0.020X + 0.061$ ($r^2 = 0.984$), $Y = 0.005X^2 + 0.014X + 0.063$ ($r^2 = 0.985$), $Y = 0.004X^2 + 0.013X + 0.078$ ($r^2 = 0.986$), and $Y = 0.001X^2 + 0.022X + 0.061$ ($r^2 = 0.985$), respectively. In the equations, X represents no. of instar and Y represents MP7. The error bars represent the standard deviation.

For example, at instar 6, the mean MP7s in salinities 20, 30, 40 and 10‰ were 0.32 ± 0.06 mm, 0.28 ± 0.06 mm, 0.24 ± 0.04 mm and 0.22 ± 0.03 mm, respectively.

3.3.2.11 Summary on Growth Study in Different Salinities

In this study, the growth of *Hyale* sp. was determined in 10, 20, 30 and 40‰ salinities. The decreasing orders of the growth rate of different body parts in four different salinities are summarized in Table 3.5. It was found that the growth rate of juveniles reared in the salinity of 20‰ was the highest among the salinities studied. All the body parts showed this feature. The growth rate of juveniles reared in 30‰ salinity was significantly greater in the parameters of BL, EL and MP7 than those reared in 40 and 10‰. But there was no significant difference in the effect of salinities on the growth of HL, AL1, AA1 and AA2. All the parameters, except AA1 and AA2, showed no significant difference in growth rate between the salinities 40 and 10‰,. The growth rate of juveniles in the parameters of AA1 and AA2 was significantly greater in 40‰ than in 10‰. In general, the overall order of the growth rate of juveniles reared in different salinities was 20 > 30 > 40 > 10‰.

3.3.3 Morphometric Analysis on Length - Weight Relationship

The relationships between BL (X) and the body weight (Y) of *Hyale* sp. is shown in Figure 3.38. The dry body length and body weight were logarithmically transformed (Figure 3.39), and the regression equation of the curves in males, non-ovigerous females and ovigerous females are $\log_{10}Y = 3.08 \log_{10}X - 2.69$ ($r^2 = 0.921$), $\log_{10}Y = 3.03 \log_{10}X - 2.64$ ($r^2 = 0.867$) and $\log_{10}Y = 3.27 \log_{10}X - 2.81$ ($r^2 = 0.847$), respectively. The analysis showed that only the allometric coefficient of the regression line in ovigerous females was significant greater than 3.00 (P = 0.046; Student's *t*-test). The allometric coefficients of the regression lines of males (P = 0.233) and non-ovigerous females (P = 0409) were not significantly different from 3.00. There was no significant difference between the allometric coefficients of the regression lines of

Table 3.5 The comparison of the growth rate of the juvenile *Hyale* sp. reared in different salinities.

Parameters	Decreasing order
BL	20 > 30 > 40 & 10‰
EL	20 > 30 > 40 & 10‰
MP7	20 > 30 > 40 & 10‰
HL	20 > 30 & 40 & 10‰
AL1	20 > 30 & 40 & 10‰
AA1	20 > 30 & 40 > 10‰
AA2	20 > 30 & 40 > 10‰

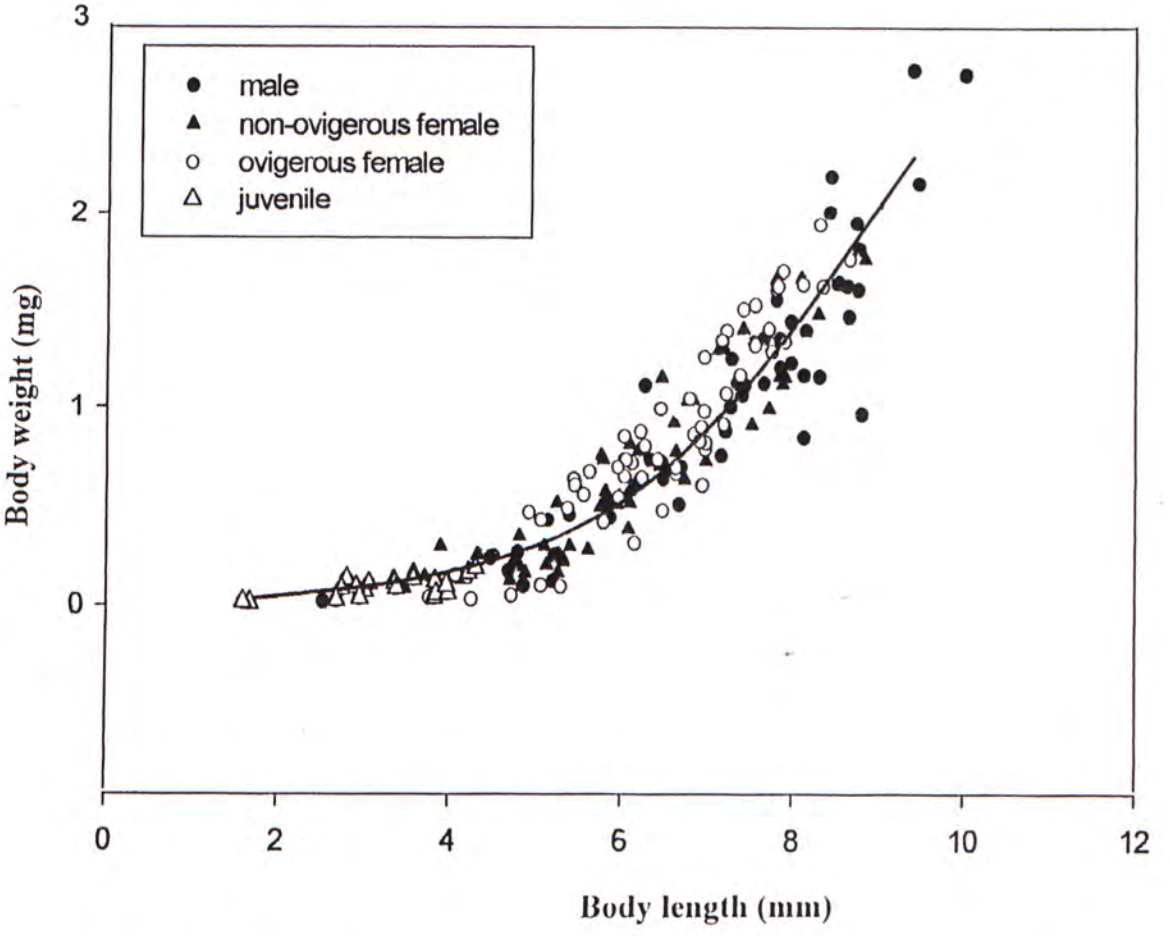


Figure 3.38 The relationships between the body weight and the body length of all aged groups and sexes in *Hyale* sp.

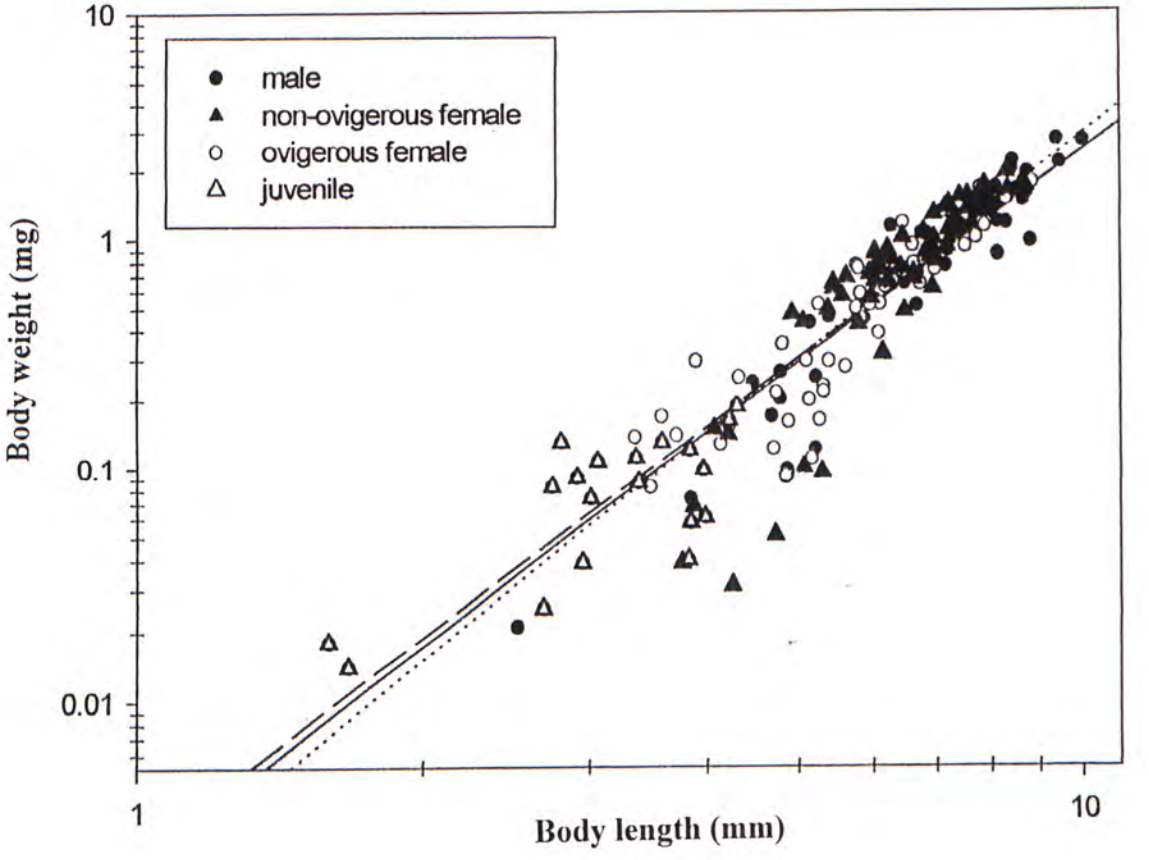


Figure 3.39 The relationships between the body weight (BW) and the body length (BL) of *Hyale* sp. in logarithmic scale. The regression equations of the curves in males, non-ovigerous females and ovigerous females are $\log_{10}Y = 3.08 \log_{10}X - 2.69$ ($r^2 = 0.921$), $\log_{10}Y = 3.03 \log_{10}X - 2.64$ ($r^2 = 0.867$) and $\log_{10}Y = 3.27 \log_{10}X - 2.805$ ($r^2 = 0.847$), respectively. In the equations, X represents BL and Y represents BW.

males and non-ovigerous females (P = 0.386), males and ovigerous females (P = 0.161), and also ovigerous females and non-ovigerous females (P = 0.125).

3.4 Discussion

Growth of many amphipods has been studied (Charniaux-Cotton 1957; Steele & Steele 1969; Dexter 1971; Myers 1971; Takeuchi & Hirano 1991, 1992; Delong *et al.* 1993; Leite 1996; Moore & Farrar 1996). Yet only Leite (1996) focused on the growth of a member of the genus *Hyale, Hyale media* in Brazil. Information on the growth of the local amphipod *Hyale* sp. is provided in the present study.

3.4.1 Discontinuous Growth

The developmental stage of amphipods is divided into successive instars due to its discontinuous nature in growth. Based on the results shown in Table 3.1, the mean number of instars over the life span of males and females were 14 (range, from 12 to 19 instars) and 17 (range, from 11 to 21 instars), respectively. The variation in numbers of instar indicated that ecdyses continued in the two sexes of *Hyale* sp. until their death. There was no evidence of any terminal ecdysis or final instar in *Hyale* sp. Thus the species carried out indeterminate growth process. Indeterminate growth is proposed to be a primitive phenomenon in the evolutionary development of crustaceans (Hartnoll 1982). Most amphipods undergo indeterminate growth process, such as *Gammarus duebeni* (Steele & Steele 1969), *Microdeutopus gryllotalpa* (Myers 1971), *Neohaustorius schmitzi* (Dexter 1971), *Orchestia gammarella* (Charniaux-Cotton 1957), *Tryphosella kergueleni* (Bregazzi 1972), and *Hyalella azteca* (Hogg & Williams 1996). In some species, the number of instars preceding the sexually mature stage of a female is constant even they have variable numbers of instar in their life span. In this study, sexually maturity in the female *Hyale* sp. was found to be falling into a range of instar 6 to 8. The reproductive biology of the species is discussed in details in Chapter 4 (Section 4.3.1). In the other species, the female *Gammarus duebeni* reaches its sexually mature stage at instar 13 (Steele and Steele 1969). Takeuchi and Hirano

(1991) reported that all female *Caprella danilevskii* reaches the sexually mature stage at instar 7. *Microdeutopus gryllotalpa* has 9 pre-puberty molts in females (Myers 1971). The female *Orchestia gammarella* has 11 or 12 pre-puberty molts (Charniaux-Cotton 1957).

3.4.2 Growth Pattern

The discontinuous nature of growth can be broken down into two components (Hartnoll 1982). The first component is the molt duration, which represents the duration between two successive molts. The second one is the molt increment or growth factor, which indicates the size increase during molting.

3.4.2.1 Molt Cycle – Molt Frequency and Molt Duration

Figure 3.2 shows that the mean age is significantly greater in males than in females at the same instar. This indicates that the molt frequency of females is higher than that of males. In this study, the male and female *Hyale* sp. had molted an average of 15 times and 18 times, respectively at 170 days of age. The mean molt frequency of males and females are 0.09 and 0.11 molt day^{-1}, respectively. Based on Figure 3.2, the difference in the molt frequency between the two sexes was greater in older amphipods.

A clear difference between the intermolt duration of the two sexes was often observed in crustaceans (Hartnoll 1982). The intermolt duration of the sexually mature male and female *Hyale* sp. varied from 6 to 20 days, and 3 to 22 days, respectively (Table 3.3). No significant difference was observed between the mean intermolt duration of the two sexes in most of the instars. In the caprellidean *Caprella okadai*, the intermolt duration of the sexually differentiated stage varied within the range from 3 to 8 days in males and 3 to 7 days in females (Takeuchi & Hirano 1992).

3.4.2.2 Body Length

Two sexes show similar growth pattern in body length increment (Figure 3.5). The growth of the body length stabilizes in the range of 9 to 10 mm in males and 8 to 10 mm in females. The body length of *Hyale* sp. in the two sexes might reach 11.38 mm. In general, the male amphipod is larger in the body size than the female (Lewbel 1978; Takeuchi & Hirano 1991; Moore & Wong 1996). Based on Figure 3.5, the mean body length of *Hyale* sp. was significantly longer in males than in females at the same instar. The significant deviation between the two sexes started at instar 7. This sexually dimorphic character is caused by the higher growth rate in males (Lewbel 1978; Takeuchi & Hirano 1991). For example, the male *Caprella danilevskii* increased its body length exponentially, while the female grew in a sigmoidal pattern (Takeuchi & Hirano 1991).

The growth in body length can also be indicated by the relationship between the percentage molt increment the pre-molt body length (Mauchline 1976). In the present study, two linear curves were obtained from the relationship (Figure 3.7). The result showed that the percentage molt increment of the two sexes decreased steadily with size. The allometric coefficients of the regression lines of males and females are –0.133 and –0.115, respectively. The negative values indicated that the rate of the percentage increment declined in logarithmic scale with body length. However, the body length increment remained constant in the whole life span of *Pseudoprotella phasma* (Harrison 1940). In *Gammarus duebeni,* the increment only decreased slightly during the development (Kinne 1959). Hartnoll (1982) stated that species with a steeper regression line could never grow to a larger size. The maximum body lengths of the male and female *Hyale* sp. were 11.02 and 11.38 mm, respectively. Yet the allometric coefficients of the regression lines of *Gammarus duebeni* and *Pseudoprotella phasma* were -0.0023 (Kinne 1959) and -3.5×10^{-4} (Harrison 1940), respectively. The maximum body length of *G. duebeni* and *P. phasma* were found to be 15 and 13 mm, respectively (Table 3.6). The allometric coefficient was more negative in *Hyale* sp. than in *G. duebeni* and *P. phasma*. According to Hartnoll (1982),

Table 3.6 The allometric coefficients of the regression lines of the percentage molt increment in logarithmic scale versus body length of different amphipods

Species	Reference length	Maximum size	Slope	References
Hyale sp. male	Body length	11.02 mm	-0.133	This study
Hyale sp. female	Body length	11.38 mm	-0.115	This study
Gammarus duebeni	Body length	15.00 mm	-0.023	Kinne 1959
Pseudoprotella phasma	Body length	13.00 mm	-0.00035	Harrison 1940

Hyale sp. has the steepest regression line among the three amphipod species and so its maximum body size is comparatively smallest.

3.4.2.3 Head Length and Merus Length of Pereopod 7

Body length is not the only parameter to indicate growth in amphipods. Lengths of the other body parts can also be employed for the growth study. In the growth study of *Hyale* sp., head length and merus length of the left pereopod 7 were regarded as the growth parameters. And the parameters also effectively indicated the growth of *Hyale* sp. In other studies, head length (Bell & Fish 1996; Delong *et al.* 1993) and merus length of pereopod 7 (Charniaux-Cotton 1957) were also used to determine growth patterns of amphipods. The application was based on the linear correlation between head length and body length of *Pectenogammarus planicrurus* (Bell & Fish 1996). According to Cooper (1965), the head length was proven to be an effective parameter to determine the body size of amphipod.

3.4.3 Morphometric Analysis on Length - Weight Relationship

Growth can be expressed as the increase of either wet or dry body weight with time (Hartnoll 1982). Body weight increment was a growth indicator commonly adopted in crustaceans (Cheng & Chen 1990; Chen *et al.* 1992; Wyban *et al.* 1995; Nipper & Roper 1995; Chen *et al.* 1996; Hogg & Williams 1996; Moore & Farrar 1996). Growth patterns of the amphipods *Hyalella azteca* (Hogg & Williams 1996; Moore & Farrar 1996), and *Chaetocorophium* cf. *lucasi* (Nipper & Roper 1995) were investigated by dry body weight measurement. In this study, the relationship between the body length and dry body weight was determined.

An equation of $W \propto L^3$ is proposed, where W and L represented body weight and body length, respectively (Thompson 1942). However, the growth of body weight is usually allometric to body length, so that body weight is rarely proportional to the cube of the body length as it will be if growth is isometric (Hartnoll 1982). A

regression line can be obtained by plotting the dry body weight versus body length in logarithmic scale. In the present study, four groups of *Hyale* sp. were separately analyzed. They were sexually undifferentiated juveniles, non-ovigerous females, ovigerous females and males. Figures 3.38 & 3.39 show the relationships between the body length and the body weight of the four groups. The regression equations of males, non-ovigerous females and ovigerous females were found to be $W = 0.002L^{3.08}$ ($R^2 = 0.921$), $W = 0.0023L^{3.03}$ ($R^2 = 0.867$) and $W = 0.0015L^{3.27}$ ($R^2 = 0.847$), respectively. However, only the allometric coefficient of the regression line of ovigerous females was significantly greater than 3, this indicated that the relative growth of the body weight to body length was positively allometric in the ovigerous female *Hyale* sp., while those of males and non-ovigerous females were generally isometric. In *Gammarus duebeni*, the allometric coefficients of the regression lines of males and females were 2.74 and 2.79, respectively (Steele & Steele 1969). The growth was negatively allometric. The allometric coefficient of regression lines in males of the lobster *Homarus* sp. and the crab *Cancer* sp. usually exceeded 3, which was due to the positive allometry of the enlarged chelae (Hartnoll 1982). In ovigerous females of *Hyale* sp., the positively allometric growth pattern might be due to the development of embryos in the marsupium. In ovigerous females of the amphipod *Gammarus insensibilis*, the egg volume was about 2.9 times greater at stage V (the stage prior to hatchling) than at stage I (early eggs) (Sheader 1996).

3.4.4 Sexual Dimorphism

Sexual dimorphism is often strong in amphipods (Barnard 1975). The sexes of gammaridean can usually be distinguished by the differences in body size and gnathopods 2 (Moore & Wong 1996). Eye size and length of antennae are generally greater in males than in females (Barnard 1971; Mclaughlin 1980). For example, the male *Orchomene nanus* generally has larger eyes and longer flagella of the second antennae 2 (Moore & Wong 1996). Not only the gammarideans, the caprellideans also have obvious sexual dimorphism in body size (Lawbel 1978). Other than the body length, the additions of flagellar articles to antennae 1 were found to be different

between two sexes in *Caprella danilevskii* and *Caprella okadaii* (Takeuchi & Hirano 1991, 1992). The study of sexual dimorphism in *Hyale* sp. mainly focused on these characters.

3.4.4.1 Body Length

The mean body length of the male *Hyale* sp. was significantly greater than that of the female after instar 7 (Figure 3.5). Body length is thus a sexually dimorphic character. The male *Caprella danilevskii* also had a significantly larger body size than the female (Takeuchi & Hirano 1991). A male-male competition was observed in *Eogammarus oclairi,* larger males might either outcomplete or take over the females of smaller males, so that larger males had a higher mating chance and hence the selective advantage (Iribarne *et al.* 1996). Also, the body length was significantly smaller in the female *Caprella danilevskii* than in the male because of energy conversion from growth to embryos production (Takeuchi & Hirano 1991).

3.4.4.2 Antennae 1 and 2

Some species of gammarideans have longer antennae in males than in females (Barnard 1971). In the present study, the length of the left antenna 1 and 2 (AL1 & AL2) and their respective articles (AA1 & AA2) were investigated. Figures 3.11 & 3.15 show the growth curves in AL1 and AL2, respectively. In general, both AL1 and AL2 of males grew faster than those of females. The significant deviation between the two sexes started at instar 6 in AL2, and instar 7 in AL1, AA1 and AA2. The results indicated that *Hyale* sp. had longer antennae 1 and 2 in the males than in the females because the increment on the number of articles of the antennae 1 and 2 was faster in males than in females (Figures 3.13 & 3.17). Moore & Wong (1996) reported that the flagellum of antenna 2 of *Orchomene nanus* became more elongated after sexual differentiation. The elongation was due to the increase in number of articles at greater rate on the stage of post-maturation. The antenna 2 of the female *Gammarus cheuteuxi* also lengthened in a faster rate at the sexually undifferentiated stage, but not at the

sexual differentiation stage (Kunkel & Robertson 1928). In the present study, the distal articles (> 16 articles) were found to be longer in length than the basal articles on antennae 2 of males (Section 3.3.1.7), yet the length of the articles were similar in females.

3.4.4.3 Gnathopods 1 and 2

Gnathopod 2 is one of the most prominent sexually dimorphic characters in amphipods (Barnard 1971). It is generally larger, more complex, and more strongly ornamented in males (Barnard 1971; Hayward 1995). The enlarged gnathopods 2 of males are used to clasp the thoracic region on the coxal plates of the female during the precopulatory pairing (Ruppert & Barnes 1994). Sexual dimorphism is obvious in the propodus length of the left gnathopods 1 & 2 (GL1 & GL2) of *Hyale* sp. (Figures 3.19 & 3.22). The maximum GL1 of males recorded was 0.49 mm, while that of females was 0.36 mm. The maximum GL1 was 1.36 times greater in males than in females. The mean GL2 of males and females at instar 15 were 1.22 mm and 0.50 mm, respectively. The mean GL2 was 2.44 times greater in males than in females. Such large difference displayed the obvious sexual dimorphism in *Hyale* sp. In other species, the male *Caprella gorgonia* possesses a poison spine on its large gnathopods 2 to combat with other males for mating (Lewbel 1978). Kunkel and Robertson (1928) reported that the length of gnathopods 2 in *Gammarus chevreuxi* showed a marked increase at sexually mature stage. The left gnathopod 2 of the female *G. chevreuxi* grew in a faster rate than that of the left pereopod 3 before sexual maturity. The relative growth ratio of the left gnathopod 2 to the left pereopod 3 of females became constant (97%) at sexual maturity. However, the relative growth ratio of males rapidly raised from 100% to 164% shortly during the stage of sexual maturity.

3.4.5 Growth Phase

Allometry is any study of size and its consequence (Hartnoll 1982). In allometric or relative growth studies, the relationship of two independent variables is compared.

One variable is usually employed for a reference dimension, which represents the general size of the animal. Another variable is the dimension to be studied (Hartnoll 1982). Two dimensions may change in different proportions during the development of the animal. The change may occur gradually over a series of instars, or may occur abruptly at a single molt. Growth phase transition can be revealed by these changes. The development of sexual dimorphism is highly correlated to the different patterns of relative growth. In the present study, body length was commonly used to be a reference dimension.

The relative growth of two dimensions Y and X, is expressed as the allometric equation, $Y= aX^b$ (Hartnoll 1982). The equation is transformed into the logarithmic scale, an equation $\log Y = b \log X + \log a$ is obtained. The allometric coefficient (b value) indicates the type of relative growth (see Section 3.1). Thus any phase change or transition can be indicated by level change in allometry or dimensions. In *Gammarus chevreuxi*, an immature phase and a mature phase were differentiated by the relative growth of the second peduncular article length of the antenna 1 and the propodus length of gnathopod 2 to the propodus length of pereopod 1 (Kunkel & Robertson 1928). In the present study, the relative growth patterns of merus length of pereopod 7, eye length, propodus length of gnathopod 1 and 2, length of antennae 1 and 2, to body length were investigated.

3.4.5.1 Merus Length of Pereopod 7

Figure 3.28 shows that there was no growth phase transition in the allometric relationship between the MP7 and the body length (BL) of *Hyale* sp. The mean MP7 of the two sexes grew in an isometric allometry with BL. In *Orchestia gammarella*, the mature phase could be differentiated by the relative growth of MP7 (Charniaux-Cotton 1957). Yet the change in growth pattern of MP7 during sexual maturity was regarded to be a species-specific character in *O. gammarella*.

3.4.5.2 Eye Length (EL)

Eye size is one of the sexually dimorphic characters in gammarideans (Barnard 1971; McLaughlin 1980; Moore & Wong 1996). Yet the mean EL is not a good parameter to indicate sexual dimorphism in *Hyale* sp. (Section 3.3.1.5). However, the allometric relationship between EL and BL provides an evidence of growth phase transition in *Hyale* sp. Figure 3.10 shows a change in the level of allometry of the two sexes. The change occurred as BL of the two sexes reached the range of 4 to 5 mm. The body length of 4 to 5 mm of the amphipod is equivalent to instar 5 to 6 in males and instar 5 to 8 in females (Figure 3.5). The instar 5 to 8 and the instar 5 to 7 are the beginning stages of sexual maturation of the female and male *Hyale* sp., respectively (Section 4.3.1 & Table 4.1). At the instars, females of *Hyale* sp. is observed to have well-developed oostegites and brood setae. Point B in the figure 3.10 represents the puberty molt, where AB and BC represent the sexually immature phase and sexually mature phase, respectively. Change in the level of allometry at point B indicates the transition from the sexually immature phase to the sexually mature phase. According to Hartnoll (1982), the transition from immature phase to mature phase (puberty molt) is usually indicated by the change in dimension of the variables together with level of allometry, because of the marked morphological change in the sexually mature phases transition.

3.4.5.3 Length of Antennae 1 and 2

The different growth phases of amphipods can also be determined by the change in relative growth patterns of the antennae 1 and 2. Two growth phases were differentiated by the allometric relationship of AL1 and AL2 to BL in *Hyale* sp. Figures 3.12 and 3.16 show the allometric relationships in the two sexes. The phases AB and CD were separated by a change in the level of allometry of AL1 in males (Figure 3.12B). The change occurred as males reached the body length in the range of 3.5 to 4.5 mm. Such body size was equivalent to instar 4 to 6 in males. Instar 5 to 7 was found to be the beginning stage of sexual maturation in the male *Hyale* sp. Thus

the change involved the transition from the immature phase to the mature phase and indicated the occurrence of the puberty molt. The situation in AL2 was different from that of AL1. There was only one growth phase observed in allometric growth of AL2 in the male *Hyale* sp. (Figure 3.16B). However, the two growth phases were differentiated in females (Figure 3.16C). Growth phases CD and EF were separated by a change of the level of allometry. The change occurred as females reached the body length in the range of 3.5 to 4.5 mm. Such body size was equivalent to instar 4 to 8 in females. The range of instar 5 to 8 was found to be the beginning stage of sexual maturation of the female *Hyale* sp. (Section 4.3.1) and thus the change involved the transition from the immature phase to the mature phase and indicated the puberty molt of the female. In other studies, the allometric growth of the second peduncular articles on antenna 2 was also used to separate the immature and mature phases in *Gammarus cheureuxi* (Kunkel & Robertson 1928). In *Corophium volutator*, the allometric growth of the antenna 2 showed a pre-puberty change in females, and a puberty change in males (Chevais 1937).

3.4.5.4 Propodus Length of Gnathopods 1 and 2

Enlarged gnathopod commonly displays sexual dimorphism in amphipods. For example, *Caprella gorgonia* (Lewbel 1978), *Gammarus chevreuxi* (Kunkel & Robertson 1928), and *Orchestia gammarella* (Charniaux-Cotton 1957). The allometry of GL1 and GL2 of *Hyale* sp. were studied. The results showed that the allometry of GL1 in males kept in the same level throughout the development (Figure 3.20B). There was only one growth phase observed in the male *Hyale* sp. In the growth of GL1 of females, the level of allometry changed when the body length of the female reached 4 to 5 mm (Figure 3.20C). This indicated that the morphological change of GL1 occurred at instar 5 to 8 in females. The range of instar 5 to 8 were found to be the beginning stage of sexual maturation of the female *Hyale* sp. (Section 4.3.1) and thus the change indicated the puberty molt and involved the transition from the immature phase to the mature phase in females. In the allometric growth of GL2 (Figure 3.23), there was only one growth phase recognized in males and two different growth phases

were differentiated in females. A changes in the level of allometry of GL2 was observed when the body length of females reached the range of 4 to 5 mm (Figure 3.23C), which was equivalent to instar 5 to 8. The transitional instars were the beginning stage of sexual maturation in the female *Hyale* sp. Thus the change involved the transition from the immature phase to the mature phase and indicated the puberty molt.

3.4.5.5 Summary on Growth Phase

Generally, two immature phases and one mature phase can be differentiated in the development of crustaceans (Hartnoll 1982). The first phase is sexually undifferentiated phase. There is no difference in the allometric growth between the two sexes. The second phase is termed as juvenile phase, which is characterized by morphological difference between the two sexes, but the animal is not sexually mature yet. The third phase is the sexually mature phase. In the present study, only two growth phases were differentiated in the allometric growth of sexually dimorphic characters in *Hyale* sp. The change of allometry level only occurred during the transition from the sexually immature phase to the sexually mature phase. The transition is termed as puberty molt. The puberty molt occurred in the range of instar 4 to 6 in males and instar 4 to 8 in females. Based on the results obtained from Section 4.3, instar 5 to 8 and instar 5 to 7 were the ranges of the beginning stages of sexual maturation in the male and the female *Hyale* sp., respectively (Table 4.1). The allometric growth pattern of sexually dimorphic characters was effective to recognize the sexually immature and mature phases in life history of *Hyale* sp.

3.4.6 Optimal Salinity of Growth

Hyale sp. is found to have a high tolerance to wide range of salinity (Section 5.3.2, Chapter 5). The species could have more than 80% survival in salinities within the range of 10 to 50‰. Some marine species also showed a high salinity tolerance. For example, the survival rate of *Leptocheirus plumulosa* and *Eohaustorius estuarius*

exceeded 90% under the range of salinities from 2 to 32‰ (ASTM 1992). The test salinity of *Grandidierella japonica* is standardized in the range of 30 to 35‰, yet high survival rate (more than 85%) was observed when the amphipod was exposed to 34‰ (ASTM 1992). The amphipod *Traskorchestia traskiana* was reported to survive in the salinity ranged from 2.5 to 50‰ for at least one week at 10°C (Koch 1991). Its high salinity tolerance is due to the presence of highly efficient osmoregulatory ability. *Orchestia scutgerula* is a strong hyper- or hypo-osmoregulator, which regulates its haemolymph osmolality at around 635 mmol kg^{-1} over a wide range of environmental salinity (5-34%) (Moore *et al.* 1995).

Since *Hyale* sp. could tolerate in such a wide range salinity, a question was addressed on whether which was the most optimal salinity for the growth and reproduction of the species. Thus the growth of the juvenile *Hyale* sp. reared in different salinities was studied. According to Hartnoll (1982), salinity only exerts minimal effect on molt increment of crustaceans. In post-larvae of the crab *Rhithropanopeus harrisii*, no great difference in increments of carapace width was observed when the larvae were reared between the salinities of 7.5 and 20‰ (Hartnoll 1978). Chakraborti *et al.* (1986) reported that there was no significant difference in the growth rate of the giant tiger prawn *Penaeus monodon* reared in salinities within the range of 5 to 31‰. The growth of juveniles of the blue crab *Callinectes similis* in carapace width was unaffected by the salinity (Guerin & Stickle 1997).

However, the growth rate of some crustaceans is significantly affected by salinity. For example, juveniles of the fleshy prawn *Penaeus chinensis* grew in the best at the isosmotic point (480 $mOsmkg^{-1}$, which was equivalent to 16.5‰) or slightly higher salinity (20 - 30‰), and the worst in the hypoosmotic environment (35 - 40‰) at 12-30°C (Chen *et al.* 1992, 1996). Salinity could affect scope for growth in the blue crab juvenile *Callinectes similis* (Guerin & Stickle 1992, 1997). Juveniles of *C. similis* reared in 5‰ salinity was half of dry weight as those reared in 10‰. *C. similis* exhibited the highest growth performance in 35‰ salinity. However, the salinity did not exert any significant effect on growth of carapace width in *C. similis*. Thus salinity

might exert different effects on growth of different parameters in measured. The results of this study showed that salinity exerted significant effects on the growth of *Hyale* sp. The descending order on the growth rate of the juvenile *Hyale sp.* was 20 > 30 > 40 > 10‰ (Section 3.3.2.11). The juvenile *Hyale* sp. exhibited the highest growth rate in 20‰ salinity. It might be explained by the scope for growth. That was the energy accumulated for growth and reproduction (Guerin & Stickle 1997). The energy spent on osmoregulation increased if the osmotic stress was higher (Einarson 1993), so that the energy expenditure left less energy available for growth. For example, the amphipod *Orchestia scutigerula* maintained its haemolymph osmolality actively at more or less 635 mmol kg^{-1} (equivalent to 22‰) during the exposure to the environmental salinity ranged from 0 - 34‰ (Moore *et al.* 1995). Less energy was spent on osmoregulation if the environmental salinity was close to the isosmotic point, and thus more energy was spare for growth. The scope for growth of the juvenile blue crab *Callinectes similis* was highest when the animal was exposed to 35‰ (Guerin & Stickle 1997). But the scope for growth of *C. similis* was lowered when the crab was exposed to the salinity below 35‰. It indicated that the rate of energetic expenditure for osmoregulation increased as the osmotic stress increased. Therefore further investigation is required to determine the isomostic point of *Hyale* sp. through the relationship between the change of haemolymph osmolality and the environmental salinity.

Intermolt duration (intermolt period) is generally unaffected by salinity (Hartnoll 1982). For example, there was little effect on the intermolt duration in the crab *Rhithropanopeus harrisii* exposed to the salinities ranged from 7.5 to 32.5‰. Similar result was found in the growth of larvae of the stone crab *Menippe mercenaria* (Brown *et al.* 1992) and juveniles of the shrimp *Penaeus chinensis* (Chen *et al.* 1992) in salinities within the range of 5 to 40‰. In the present study, no significant difference was found in the intermolt duration when the juvenile *Hyale* sp. was reared in salinities ranged from 10 to 40‰. Only the extreme salinity exerted variable effects on both molt increment and intermolt duration to certain crustaceans (Hartnoll 1982). For example, the intermolt duration of postlarvae of the decapod *Rhithropanopeus harrisii*

lengthened in the salinities of 2.5 and 40‰ (Costlow *et al.* 1966). The intermolt duration in juveniles of the grapsid crab *Armases miersii* (Anger 1996) only prolonged in the extremely low and high salinities (5, 45 and 55‰). Guerin & Stickle (1992) reported that no significant difference was found in the intermolt duration when juveniles of the blue crab *Callinectes sapidus* were reared in salinities between 2.5 and 35‰. Yet the intermolt duration significantly lengthened when the juvenile was reared in salinities ranged from 35 to 50‰. However, Hartnoll (1982) suggested that it is hard to obtain reliable data on intermolt duration of crustaceans as any small variation of intrinsic or extrinsic factors other than the salinity can exert influence on the duration.

Chapter 4 Reproductive Biology of *Hyale* sp.

4.1 Introduction

Amphipods are dioecious with strong sexual dimorphism. The development of marsupium is a good sexually dimorphic character to exhibit sexual differentiation and the maturity of females. The marsupium is a unique characteristic of the superorder Peracarida to which amphipods belonging to (Borowsky 1990). The developing embryos are retained in the marsupium formed by the oostegites in females (Ruppert & Barnes 1994). This brooding feature is readily accessible for study of reproductive biology and egg production of amphipods (Borowsky 1990; Sainte-Marie 1990; Steele & Steele 1991a; Takeuchi & Hirano 1991, 1992; Jones & Wigham 1993; Morritt & Stevenson 1993; Bell & Fish 1996; Morritt & Spicer 1996; Moore & Wong 1996; Sheader 1996). It is observed that egg size of amphipods is dependent upon the structure of the marsupium (Steele & Steele 1991a) and different season (Bell & Fish 1996; Moore & Wong 1996; Sheader 1996).

Life history of amphipods involves the sequential change in morphological structures under different developmental stages. Sexual differentiation and maturity are two major transitions in the life cycle of amphipods. The transitions can be identified by the morphological variations in sexually dimorphic characters and the development of sex organs. For example, sexual differentiation of *Orchomene nanus* is identified at instar 5 by the increase of the increment on flagellar articles of antenna 2 (Moore & Wong 1996). On the other hand, sexual differentiation and maturation can also be determined by the relative growth patterns of two body dimensions. For example, the phase transition can be recognized from the change in allometric level of the relative growth pattern of basis length of pereopod 3 to propodus length of gnathopod 2 in males of *Orchestia gammarella* (Charniaux-Cotton 1957). According to Borowsky (1990), female hormones drive the reproductive behaviors once sexual maturity is attained. Thus reproductive behavior can also be an indication of sexual maturation of

the amphipods. As amphipods reach sexually mature stage, a series of pheromonal stimulations and receptions would act on both sexes (Borowsky 1990; Schmitz 1992; Ruppert & Barnes 1994). Pairing is consequently induced. The male accompanies with the female until ovulation occurs, and thus copulation and fertilization can take place simultaneously during pairing. Thus the pairing behavior indicates the beginning of the sexual maturation.

Most temperate freshwater amphipods commonly produce only one brood of 15 to 50 eggs annually (Ruppert & Barnes 1994). However marine species generally produce more than one brood per year (iteroparous), with 2 to 750 eggs in a brood. For example, the marine caprellidean *Caprella okadai* produces an average of 5.8 clutches at 5-6 days intervals, and totally 36.1 offspring during a female life (Takeuchi & Hirano 1991, 1992). Females of *Orchomene nanus* generally produce 27 to 52 eggs in an average of four broods throughout their life span (Moore & Wong 1996). Jones & Wigham (1993) observed that the larger female *Orchestia gammarellus* carried more eggs than the smaller one. Linear relationships between the brood size and the body length were reported in the following female amphipods, *Caprella danilevski* and *Caprella okadai* (Takeuchi & Hirano 1991), *Orchestia gammarellus* (Jones & Wigham 1993), *Orchomene nanus* (Moore & Wong 1996), and *Pectenogammarus planicrurus* (Bell & Fish 1996). Bell & Fish (1996) found that the brood size of *Pectenogammarus planicrurus* was strongly correlated to female head length. The small females with 0.5 mm head length generally produced mean brood size ranged from 1.4 to 5.9 eggs. Yet a larger female with head length of 0.7 mm was recorded to produce 14 eggs in a single brood.

In the present study on the reproductive biology of *Hyale* sp., sexual maturation was identified by the occurrence of pre-copulatory pairing behavior and the full development of sex organs. The number of juveniles released from each brood and the total number of offspring generated was obtained by making an observation on the

whole life span of females. The morphometric relationship between the fecundity and the body length of females was also determined in the study.

4.2 Materials and Methods

4.2.1 Fecundity

Ovigerous females were randomly collected from outdoor concrete tanks in MSL and isolated individually in a 100-ml beaker in October, 1998. They were reared under standardized laboratory conditions (see Section 3.2.1). All feeding and water exchange procedure were the same as described in the growth study in standardized conditions (Section 3.2.1.2). Two juveniles just released from the marsupium were randomly selected from each female. Totally 24 juveniles, including 11 males and 13 females were observed daily until sexual maturation occurred.

As a female amphipod in the group of the 24 juveniles reached 20 days of age or instar 4, a sexually mature male collected from outdoor tank (not from the group) was introduced into the beaker and vice versa for a male amphipod. The pair of amphipods was reared together. The physical parameters of the culture conditions were monitored every two days (see Section 3.2.1.2). Each pair of couple was fed with two pieces of 1 mm^3 krill flesh. A small piece (10 cm^3) of thallus of the green alga *Ulva lactuca* was provided as food source and substratum. All water exchange and cleansing procedures followed steps shown in Section 3.2.1.

Each couple was examined daily for any molting, precopulatory pairing, egg bearing and juvenile release. Any morphological change of the female was also recorded in its life cycle. The female amphipod was reared and followed throughout its life span in order to determine the lifetime fecundity. The observation on the male amphipod was made until the first occurrence of pre-copulatory pairing. Sexual maturation was recognized so that the observation made on the male was ceased. Other

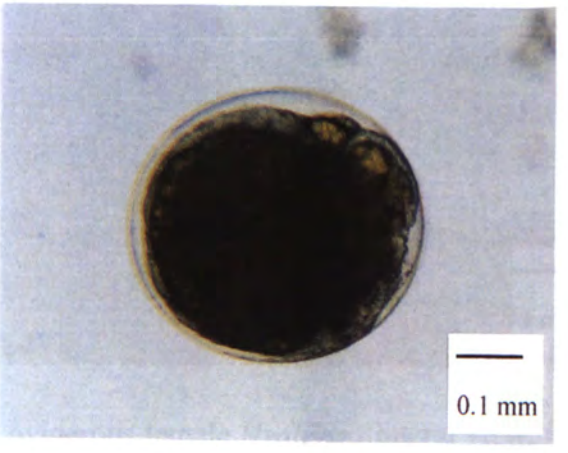
than the number of juveniles released from the female, the incubation duration and recuperative period were also recorded. Incubation period was regarded as the duration between the first day of oviposition (occurrence of early eggs) and the first juvenile released from the marsupium (Takeuchi & Hirano 1991). Recuperative period was defined as the interval between the liberation of the last juvenile from the marsupium and the appearance of the next oviposition (Morritt & Stevenson 1993). A preliminary study showed that the counting error was unavoidable on the inspection of the egg number in the marsupium of a live female, especially for those carrying a large brood size (number of eggs >20). Thus the determination of fecundity was based on the number of juveniles released from the same clutch instead of the number of eggs in marsupium (Takeuchi & Hirano 1991, 1992).

4.2.2 Morphometric Relationship between Brood Size and Body Length

Totally 102 ovigerous females carrying stage I (early) eggs were analyzed in this part of the study. A study showed that the number of stage I egg carried by a female amphipod was proportional to its body length (Beare & Moore 1996). Thus the fecundity was regarded as the number of stage I (early) eggs (Figure 4.1A) counted in the marsupium. According to Sainte-Marie *et al.* (1990), the development of embryos (eggs) was composed of five stages. The stage I egg was identified by an orange-color yolk cell occupied the most volume of the egg, and the absence of any differentiated tissue (Sainte-Marie *et al.* 1990; Jones & Wigham 1993; Morritt & Spicer 1996). The egg was found in the marsupium of the female within the first 24 hours of oviposition. Stage IV egg was characterized by the developed appendages and pigmented eyes (the stage prior to hatch) (Figure 4.1B), and stage V embryo was the hatched embryo.

The body length of the ovigerous females carrying stage I eggs (Figure 4.2) were measured under a compound microscope (Nikon model SE) and a color camera (Teli ® CCD CS 5110) connected to a television set (Sharp VT-1428) as described in Section 3.2.1.3. After the measurement, the females were anesthetized with lignocaine HCl

A



B

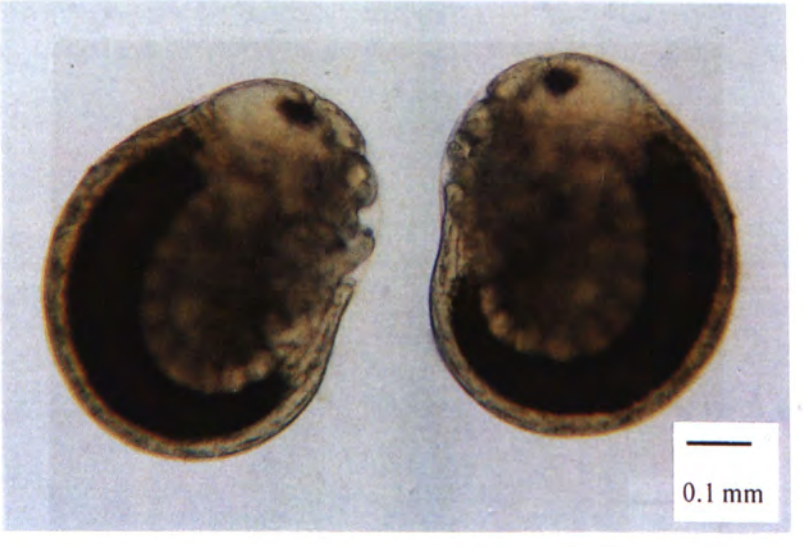


Figure 4.1 Eggs (embryos) of *Hyale* sp., whole specimen. A, stage I egg (undiffentiated yolk cell); B, stage IV egg (appendages complete and eyes pigmented).

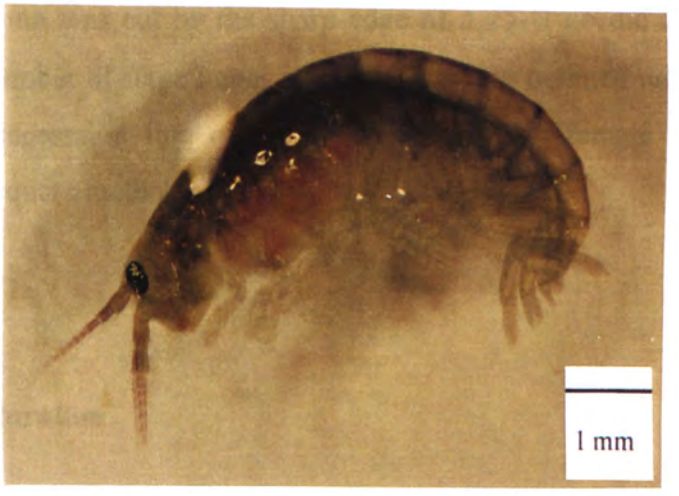


Figure 4.2 An ovigerous female *Hyale* sp., lateral view, whole animal.

Figure 4.3 Precopulatory pairing of a male (upper position) and a female (lower position) *Hyale* sp.

1%. The marsupium was cut by the sharp edge of a 25-G needle and all eggs were evacuated. The number of stage I eggs in marsupium was counted under the dissecting microscope. A regression line of the brood size versus female body length was obtained by least square method.

4.3 Results

4.3.1 Sexual Maturation

Only 13 female and 11 male individuals were successfully observed to the sexually mature stage. Thus totally 24 pairs of amphipods were analyzed. Table 4.1 shows the first appearance of pre-copulatory pairing (Figure 4.3) of the couples. The behavior started to occur at instar 5 to 8 in females, and the mean instar number was 6.6 ± 1.0 (mean ± SD). The mean age at which the pairing behavior occurred was 43 ± 4 days. The youngest female attaining sexual maturity was recorded at instar 5 in 38 days of age. And the oldest female sexually matured at instar 6 in 53 days of age. The occurrence of sexual maturation of females varied from instar 5 to 8. Three out of the 13 females (23%) started their first pairing at instar 8, which was equivalent to 40 to 46 days of age. In the male *Hyale* sp., the pre-copulatory pairing behavior started to occur at instar 5 to 7. The youngest male started its first pairing at instar 5 in 34 day-old, while the oldest male was recorded at instar 7 in 44 days of age. Males matured significantly earlier than females in terms of age ($P = 0.018$; Student's *t*-test). There was no significant difference between the two sexes in sexual maturity in terms of the number of instar ($P = 0.443$; Student's *t*-test).

All sexually mature females were found to have fully developed marsupium. The marsupium was formed by overlapping of a pair of bilateral and spoon-like oostegites with entangled brood setae (Figure 4.4). This morphological characteristic was obvious as the female reached instar 6 on the average. Oostegites were found in the ventral region of pereomeres 2 to 5 in females at about instars 3-4. At this stage,

Table 4.1 The first appearance of pre-copulatory pairing of couples of amphipods.

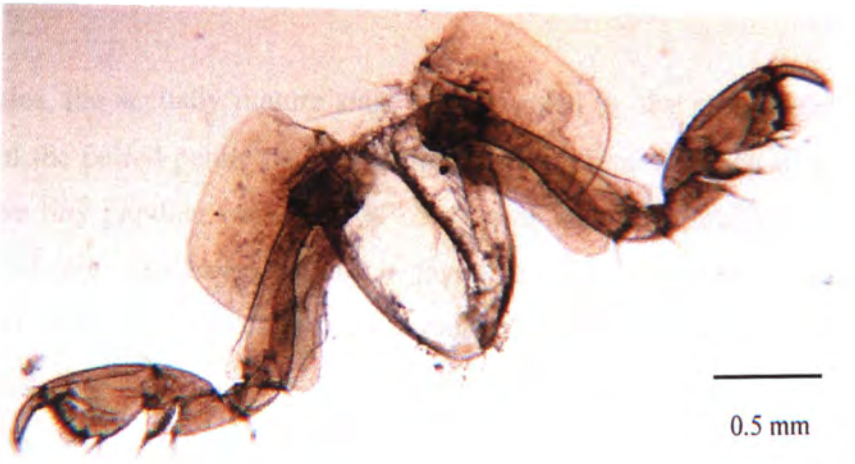
A. Female

Individual number	1	2	3	4	5	6	7	8	9	10	11	12	13	Mean	SD
No. of instar	8	7	6	6	6	6	6	6	8	5	8	7	7	6.6	1.0
Age (day)	46	40	45	53	38	43	41	45	40	38	41	45	45	43.1	4.1

B. Male

Individual number	1	2	3	4	5	6	7	8	9	10	11	Mean	SD
No. of instar	6	7	6	6	7	7	6	6	7	6	6	6.4	0.5
Age (day)	42	44	37	34	38	43	38	39	40	35	41	39.2	3.2

A



B

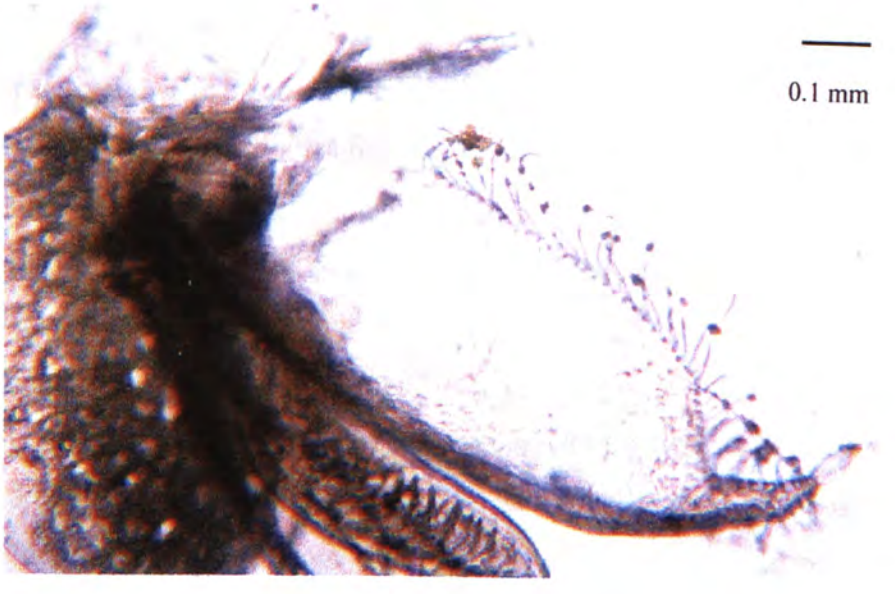


Figure 4.4 Oostegites of *Hyale* sp., female, anterior view. A, spoon-shaped oostegites forming a brood pouch; B, oostegite with curl-tipped fringing setae.

the developing buds of oostegites were small, non-overlapped and absence of entangled brood setae. The developing oostegites were small and difficult to observe. The oostegites became obvious as instars 6-8 was reached.

In males, the sexually mature stage was revealed by the pre-copulatory pairing behavior and the paired penile papillae projected from ventral region of pereomere 7. However, the tiny papillae were hard to observe on live male amphipods. Thus the appearance of pre-copulatory pairing behavior provided an indirect but effective alternative to identify the sexually mature stage. The sexual differentiation of males was obvious in the development of secondary sexual character, such as enlargement of gnathopod 2.

4.3.2 Fecundity

The total number of brood in a lifetime of females varied from 2 to 17 with an average of 6.2 ± 5.1 broods (Table 4.2). The median and mode values were 4 and 3 broods, respectively. The mean number of juveniles released from each brood was 13.2 ± 6.1 (range, 3–33). The highest and the lowest brood size were 33 and 3 juveniles, respectively. The mode and median values of the brood size were 10 and 12, respectively (Figure 4.5A). The total number of offspring generated in the lifespan of females were recorded in the range between 16 and 290 juveniles. The mean and median fecundity of a female during its life span were 81 ± 81 and 54 (Figure 4.5B), respectively in 13 females. The large standard deviation indicated the great variation in fecundity among females. The present study showed that three females died after 2 broods from which only an average of 17 juveniles were generated from each female. But another two long-lived females produced 15 and 17 broods throughout their lifetime. One of them produced 290 juveniles during its life span. Thus the fecundity of the female was highly dependent upon its life span.

Table 4.2 The fecundity of the female *Hyale* sp. The fecundity is expressed as the number of juveniles released from a single brood of an ovigerous female in different instars.

	No. of juveniles												
Individual number No. of instar	1	2	3	4	5	6	7	8	9	10	11	12	13
5	#	#	#	#	#	#	#	#	#	8	#	#	#
6	#	#	12	20	6	6	10	4	#	14	#	#	#
7	#	3	15	18	9	10	10	7	#	16	#	6	10
8	10	9	16	16	12		14	8	5		6	13	13
9	15	14	12		12		13	7	12		11		12
10	15	9			4		12	9	10				15
11		7			7		8	10	13				
12		11			11		14	19	10				
13		10			17		18		5				
14		12			20		22		#				
15					13		24		18				
16					18		27		19				
17					14		33		23				
18					13		29		20				
19					25		3						
20					18		17						
21							24						
22							12						
Lifetime fecundity	40	75	55	54	199	16	290	64	135	38	17	19	50

No juvenile production in the instar

Mean brood size = 13.2 + 6.1 juveniles

Mean lifetime fecundity = 80.9 + 81.1 juveniles

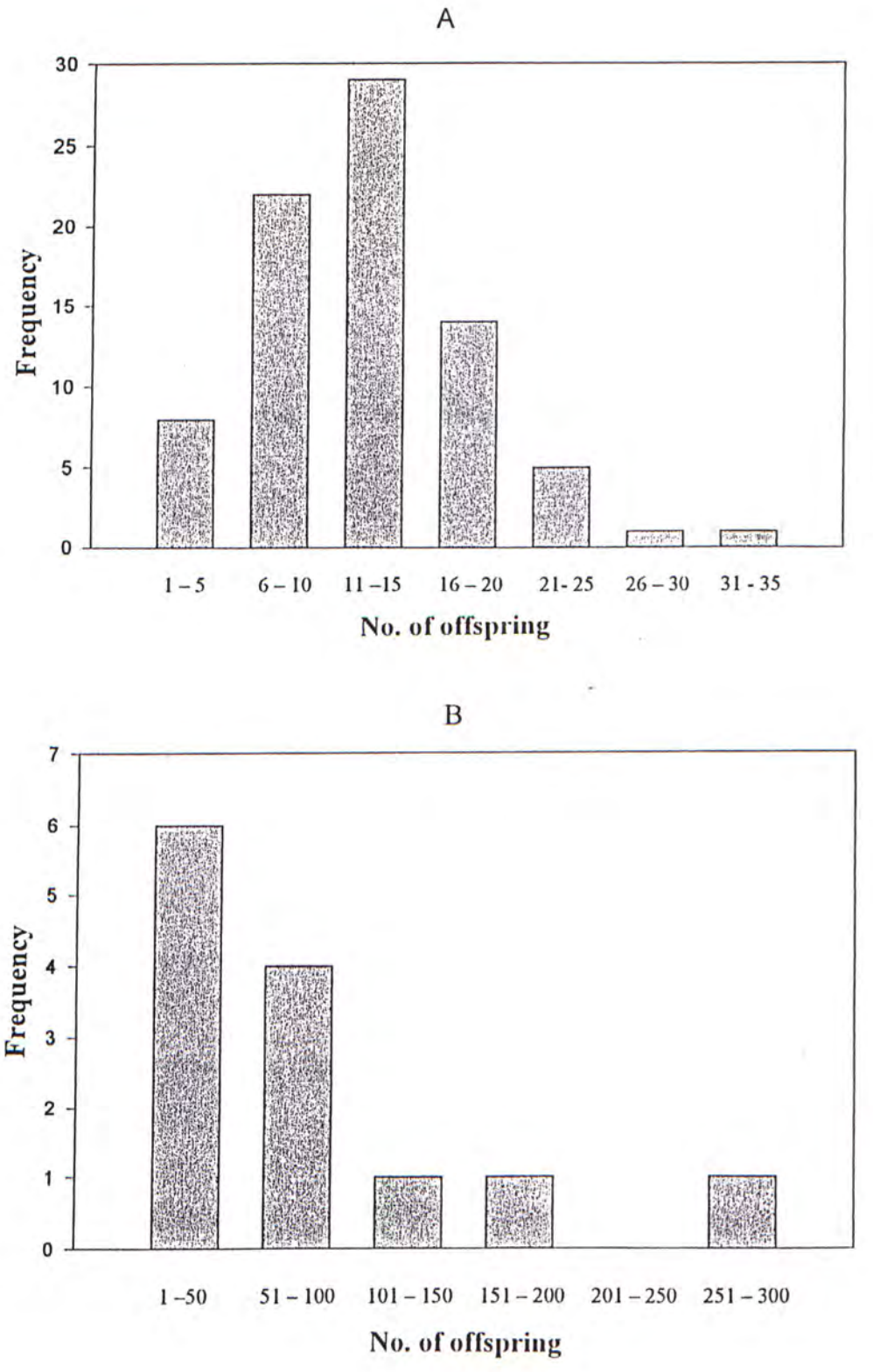


Figure 4.5 The frequency distribution in fecundity of *Hyale* sp. A, the no. of offspring produced in each single brood of a female pairing with a male; B, the total no. of offspring produced in the life span of a female.

The correlation between the brood size and the number of brood was determined (Table 4.3 & Figure 4.6). The statistical analysis showed that the brood size and the number of brood was significantly correlated in the population ($P < 0.0001$; Student's *t*-test). The results indicated that the brood size generally increased as the number of brood increased.

4.3.3 Duration of Recuperative Period and Incubation Period

The mean incubation period was 7 ± 2 days (Table 4.4). The development of embryos inside the marsupium required at least 4 days and at most 11 days. The mode and median values of incubation periods were 5 and 7 days, respectively. The mean recuperative period was 3 ± 3 days (Table 4.5). The period ranged from 0 to a maximum of 18 days. The mode and median values of recuperative period were both 1 day. In an individual (no. 9), a long recuperative period (18 days) was observed between the 6^{th} and 7^{th} brood. Two subsequently broods were separated by a complete instar (instar 14), during which no pairing was observed.

4.3.4 Morphometric Relationship between Brood Size and Body Length

The statistical analysis showed that there was a significant correlation between the brood size and the body length of females ($P < 0.0001$; Student's *t*-test). The brood size was linearly correlated to the body length of the ovigerous females (Figure 4.7). The regression equation was $Y = 3.68X - 15.91$ ($r^2 = 0.532$), where X represents the body length of the female individuals and Y represents the number of stage I eggs.

4.4 Discussion

The results presented in Chapter 3 showed that the sexually mature phase started at the range of instar 4 to 6 in males and instar 4 to 8 in females (Chapter 3, Sections 3.4.5.2-4). The sexually immature phase and sexually mature phase was indirectly

Table 4.3 The fecundity of the female *Hyale* sp. The fecundity is expressed as the number of juveniles released from a Single brood of an ovigerous female in different broods.

	No. of juveniles													Mean brood fecundity	SD*	Sample size (N)
Individual number	1	2	3	4	5	6	7	8	9	10	11	12	13			
No. of brood																
1	10	3	12	20	6	6	10	4	5	8	6	6	10	8.2	4.5	13
2	15	9	15	18	9	10	10	7	12	14	11	13	13	12.0	3.1	13
3	15	14	16	16	12		14	8	10	16			12	13.3	2.8	10
4		9	12		12		13	7	13				15	11.6	2.7	7
5		7			4		12	9	10					8.4	3.0	5
6		11			7		8	10	5					8.2	2.4	5
7		10			11		14	19	18					14.4	4.0	5
8		12			17		18		19					16.5	3.1	4
9					20		22		23					21.7	1.5	3
10					13		24		20					19.0	5.6	3
11					18		27							22.5	6.4	2
12					14		33							23.5	13.4	2
13					13		29							21.0	11.3	2
14					25		3							14.0	15.6	2
15					18		17							17.5	0.7	2
16							24							24.0	\	1
17							12							12.0	\	1
Lifetime fecundity	40	75	55	54	199	16	290	64	135	38	17	19	50			

* SD represents standard deviation

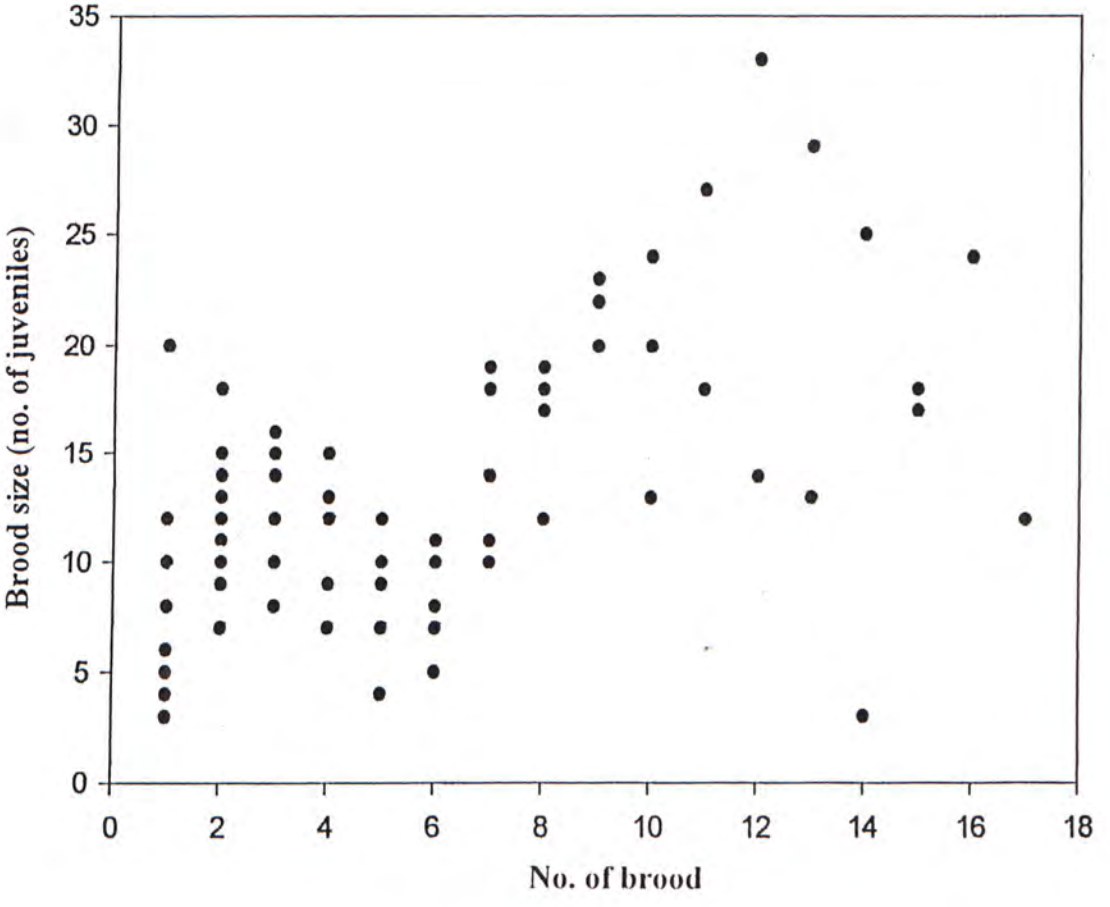


Figure 4.6 The variation of brood size of the female *Hyale* sp. in different number of instar (correlation coefficient r = 0.543). N = 64.

Table 4.4 Duration of incubation (day) period in each brood.

	Duration (day)												
Individual number No. of instar	1	2	3	4	5	6	7	8	9	10	11	12	13
5	#	#	#	#	#	#	#	#	#	6	#	#	#
6	#	#	8	9	11	6	4	5	#	5	#	#	#
7	#	5	7	5	7	5	5	10	#		#	5	6
8	5	8	7	10	6		8	11	7		6	8	5
9	5	8	8		10		5	9	7				8
10		5			7		9	6	7				7
11		6			4		10	7	5				
12		5			4		8	6	7				
13		8			7		7		#				
14		6			9		9		4				
15					9		9		9				
16					9		9		4				
17					7		7		6				
18					5		7						
19					5		4						
20							5						
21							6						
22							6						

Mean = 6.8 SD = 1.8

No spawning in the instar

Mean incubation period = 6.8 + 1.8 days

Table 4.5 Duration of recuperative period between two successive broods.

	Duration (day)												
Individual number	1	2	3	4	5	6	7	8	9	10	11	12	13
No. of instar													
5	#	#	#	#	#	#	#	#	#	#	#	#	#
6	#	#	#	#	#	#	#	#	#	1	#	#	#
7	#	#	1	6	1	3	0	0	#	9	#	#	#
8	#	0	7	1	1		4	9	#		#	2	1
9	1	0	3		4		6	0	0		7		5
10	5	7			1		1	2	3				1
11		5			2		2	1	4				
12		7			5		0	3	1				
13		2			2		1		4				
14		3			1		1		#				
15					0		0		18				
16					1		1		1				
17					1		0		1				
18					3		1		5				
19					0		1						
20							0						
21							4						
22							1						

No spawning production in the instar

Mean recuperative period = 2.6 ± 3.0 days

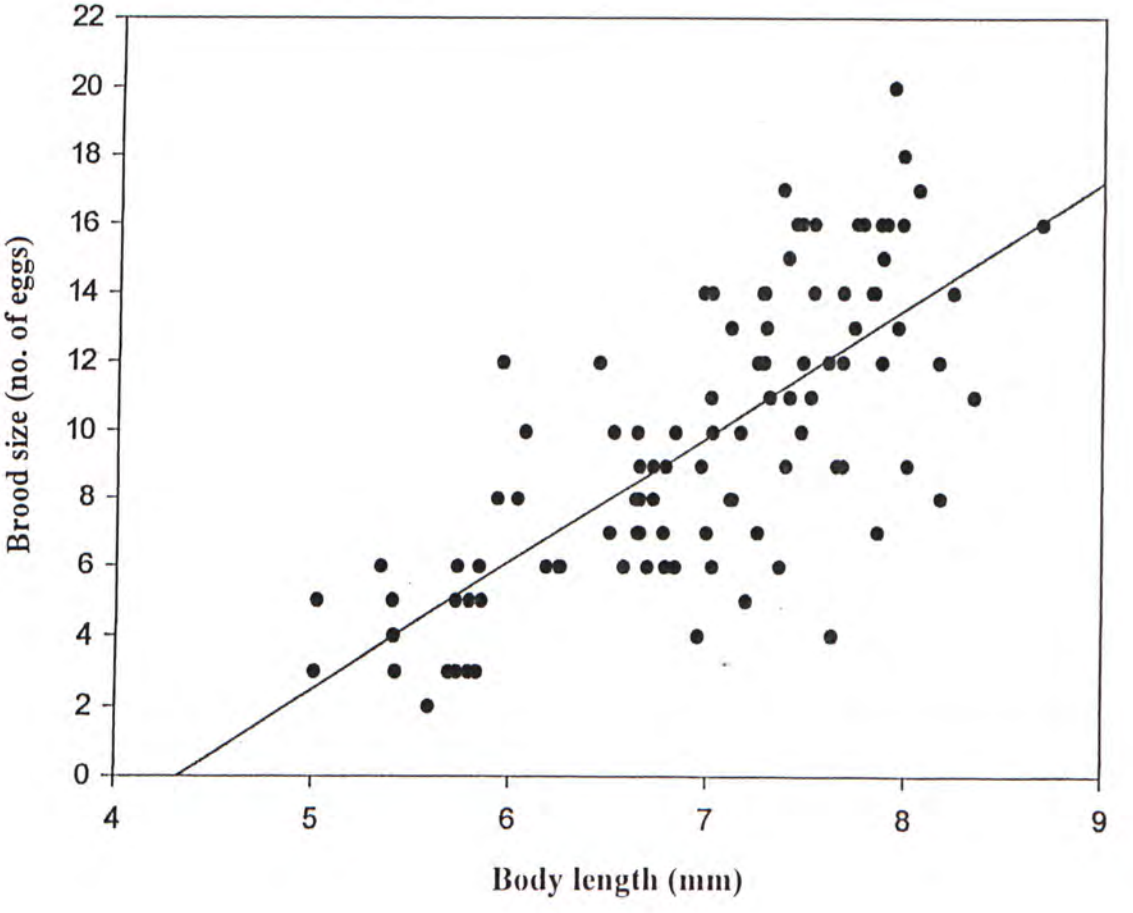


Figure 4.7 The relationships between the brood size and body length of the ovigerous *Hyale* sp. The regression equation of the best-fit line is Y = 3.68X – 15.91 (r^2 = 0.532). N = 102.

differentiated by the change in the allometric relationship between two growth dimensions. In the present chapter, sexual maturity was recognized by the observation on the development of fully setose oostegites (Section 4.3.1) and the appearance of the reproductive behavior (pre-copulatory pairing) in the two sexes (Section 4.3.1). The present study shows that males reached sexual maturity between instar 5 to 7 in an average of 43 ± 4 days of age. The females reached sexual maturity between instar 5 to 8 in an average of 39 ± 3 days of age. The study indicated that the timings of sexual maturation of the two sexes obtained from this chapter matched to the results obtained from the allometric growth patterns in Chapter 3. The life cycle of *Hyale* sp. is similar to that of the other amphipods. For example, sexual maturation of the female *Orchomene nanus* occurs at instar 8 (Moore & Wong 1996). Takeuchi & Hirano (1991) reported that the caprellidean *Caprella danilevskii* sexually matured at instar 7. Based on the results obtained from the above studies, different amphipods may have similar life cycle. Sexual maturation in amphipods commonly occurs in the range of instar 5 to 8.

The maximum number of offspring was found to be 33 juveniles in a single brood of the female *Hyale* sp. reared in laboratory culture (Section 4.3.2). But the maximum number of stage I egg carried by the females collected from outdoor tanks was 20 only (Figure 4.7). The great variation in the maximum brood size of the females recorded between two sources might be due to the difference in environmental conditions. The laboratory culture was maintained in a standardized and stable environment with continuous food supply. The conditions favored the higher scope for growth and reproduction of amphipods (Einarson 1993; Guerin & Stickle 1997). In contrast, food limitation and relatively unstable environment is a common cause to lower the potential fecundity and reproductive effort of amphipods living in field site (Sainte-Marie *et al.* 1990).

The total number of offspring produced by the female *Hyale* sp. was found in the range of 16 to 290. The average value was 81 ± 81 juveniles with a median of 54

juveniles. The total number of brood in the life span of the female varied from 2 to 17, with an average value of 6.2 ± 5.0 broods. The reproductive output of *Hyale* sp. was compared to the other species. *Orchomene nanus* can produce four broods at most in a life span of the female (Moore and Wong 1996). The total number of offspring produced by the females *O. nanus* was found in the range between 27 – 52 eggs. The female caprellidean *Caprella danilevskii* produced an average of 5.4 ± 1.8 broods and totally 69.0 ± 21.4 offspring during its lifetime (Takeuchi & Hirano 1991).

The mean incubation period (Table 4.4) and recuperative period (Table 4.5) in *Hyale* sp. were 6.8 ± 1.8 (range, 4 –11) and 3 ± 3 days (range, 0 – 18), respectively. However, some females molted immediately after the termination of the last brood and released unfertilized eggs just after molting (i.e. no recuperative period). The absence of recuperative period was also reported in a caprellidean *Caprella danilevskii* (Takeuchi & Hirano 1991). Some females of *C. danilevskii* molted and released eggs immediately after the liberation of all juveniles of the last brood from the marsupium. In the present study, a female (individual no. 9) stopped oviposition in the whole instar 14 and started its subsequent brood after 18 days of recuperative period. The situation was also reported in *Caprella okadai* that two successive broods were separated by a complete instar (Takeuchi & Hirano 1992).

In the caprellidean *Caprella danilevskii*, the brood size increased from 6.3 ± 1.3 (N = 8) at instar 7 to 22.0 at instar 12 (Takeuchi & Hirano 1991). A similar correlation also occurred in *Hyale* sp. that the brood size increased as the number of brood increased (Figure 4.6). The female has generally larger body size at later instars (with later broods). The ovary length and hence the capacity of carrying eggs is proportional to the body length (Jones & Wigham 1993). As the female is getting older, its body size, ovary size and hence embryo-carrying capacity also become greater.

The relationship between the brood size and the body length of females was therefore studied. A positive linear correlation was observed. The linear correlation is

commonly found in amphipods, for example, *Caprella okadai* (Takeuchi & Hirano 1992), *Orchestia gammerallus* (Jones & Wigham 1993), *Gammarus balcanicus* (Zieliñaki 1995), *Hyalella azteca* (Hogg & Williams 1996), *Orchomene nanus* (Moore & Wong 1996), and *Pectenogammarus planicrurus* (Bell & Fish 1996). Larger females generally carry more eggs than the smaller ones because the length and hence the embryo-carrying capacity of ovary are proportional to their body length (Jones & Wigham 1993). The linear function between the brood size and the body length of *Ampelisca tenuicornis* is explained by the tubular structure of the ovaries (Sheader 1977a). Each tubular structure of the ovary is lying dorsolaterally to the midgut (Schmitz 1992) and a single linear row of oocytes is found inside each tubular ovary. A single and linear row of oocytes lying inside each tubular ovary was also found in the female *Hyale* sp. and thus the linear correlation between the brood size and body length was obtained. However, a non-linear correlation is obtained if the ovary is not a tubular structure. For example, the brood size of the hyperiidean *Parathemisto gaudichaudi* varies with approximately the square of the body length of the females because the ovaries of the species are flattened plates instead of tubular strands (Sheader 1977b).

Chapter 5 Tolerance of *Hyale* sp. to Temperature and Salinity

5.1 Introduction

The physical parameters to which a species exposed have a significant impact on its physiology (Brown & Bert 1993). The most important environmental factors affecting the survival and growth of marine invertebrates are temperature and salinity. Ferraris *et al.* (1986) and Chen *et al.* (1995) stated that the survival of penaeids was determined by the adaptability to change in environmental salinity and temperature. Brown & Bert (1993) showed that the annual differences in temperatures and salinities could greatly affect the overall survival of the juvenile stone crab *Menippe adina.* The understanding of the effects of temperature and salinity on the survival and development of marine invertebrates is also essential for interpreting their temporal and spatial distribution (Mills & Fish 1980; Sulkin & McKeen 1989; Steele & Steele 1991b; Brown *et al.* 1992; Brown & Bert 1993).

Temperature is recognized as one of the major environmental determinants of the metabolism of poikilothermic organisms, such as crustaceans. The assessment of the tolerance limit to temperature is the first step towards the understanding of the animal and the development of proper methods of maintaining the culture stock (Hardy *et al.* 1994). For example, the Sub-Antarctic beach hopper *Orchestia sertigerula* was able to survive in air temperatures ranged from –2 to 19°C (Moore *et al.* 1995). The temperature tolerance is not only the basic information for the maintaining the culture stock, but also a useful index to evaluate the deleterious effects of pollutants in the ambient environment (Rosas & Ramirez 1993; Alcaraz *et al.* 1997). For example, Alcaraz *et al.* (1997) reported that exposing postlarvae of the white shrimp *Penaeus setiferus* to nitrogenous compound, such as ammonia and nitrite, could reduce its ability to tolerate thermal stress (critical thermal tolerance).

Salinity in the intertidal and supralittoral zone varies abruptly and thus many shore invertebrates have physiological adaptation to the environment of highly fluctuated salinity (Kinne 1971). Supralittoral amphipods of the superfamily

Talitroidea are particularly tolerant of extreme salinity (Marsden 1980; Moore & Francis 1986; Morritt 1988). The talitroid *Orchestia sertigerula* is a strong hyper- and hypo-osmoregulator (Moore *et al.* 1995). The talitroid regulated its haemolymph osmolarity in a constant level 635 mmol kg^{-1} to a wide range of environmental salinities (5–34‰). The euryhaline amphipod *Traskorchestia traskiana* tolerated to salinities of 2.5 to 50‰ for at least a week at 10°C (Koch 1991). Similar results have been reported for the other marine talitroids, such as *Orchestia gammarellus.* The amphipod was able to survive within the salinity range from 1 to 60‰ (Moore & Francis 1986). *Orchestia chiliensis* tolerated to the salinities between the range from 0.3 to 51‰ at 10°C in winter (Marsden 1980).

Temperature and salinity factors may affect organisms in different ways. For example, salinity exerted greater effect on survival of the mysid *Mysidopsis bahia* than temperature did (McKenney 1994). However, survival and development of the juvenile stone crab *Menippe mercenaria* was primarily affected by temperature, but not by salinity (Brown *et al.* 1992). In nature, temperature and salinity frequently interact together to affect organisms. Brown & Bert (1993) showed that the annual differences in both temperatures and salinities influenced the overall survival on the juvenile stone crab *Menippe adina.* Salinity tolerance of the species was strongly modified by temperature and the interaction of these two factors (Kinne 1971).

The objective of this study is to determine the resistance patterns of *Hyale* sp. to a certain range of salinity and temperature, with the view to determine the optimal salinity and temperature conditions for the survival of the species. Many marine invertebrates require specific temperature and salinity at different developmental stages (Kinne 1970, 1971; Thomas & Rice 1992; Charmantier & Charmantier-Daures 1994; Guerin & Stickle 1997), the temperature and salinity tolerance of both adults and juveniles of *Hyale* sp. were thus determined in the study. Furthermore, the understanding of the survival response of the amphipod to standardized conditions (temperature, 25°C; salinity, 30‰; pH value, 7.8; photoperiod, 14L:8D) provides

fundamental information to the development of environmental bioassays (Alcaraz *et al.* 1997).

5.2 Materials and methods

The tolerance test was divided into 2 main parts. In the first part, the amphipods were held at temperatures ranged from 0 to 35°C at a constant salinity of 30‰. In the second part of the study, the amphipods were held in artificial seawater of different salinities ranged from 0 to 64‰ at a constant temperature of 25°C.

5.2.1 Sampling

The experimental amphipods were collected from outdoor concrete tanks in Marine Science Laboratory (MSL), the Chinese University of Hong Kong (Section 2.2.1). Adults and juveniles of *Hyale* sp. were inspected for any physical damage under a dissecting microscope (Nikon SMZ-2T). Any amphipods with incomplete body parts, egg-bearing marsupium or any abnormalities were removed. The experimental amphipods were separated into adults and juveniles according to their body size. Based on the previous study in Sections 3.4.5 and 4.3, the body length of adults was standardized in the range of 5 to 8 mm. Amphipods with the body length falling in the range which showed the evidence of sexual dimorphism characters were regarded as adults. The body length of juveniles was limited within the range of 2 to 3 mm. No sexually dimorphic character was fully developed in those juveniles.

5.2.2 Acclimation

Adults and juveniles were acclimated in the standardized laboratory conditions for at least one week. Fully aerated artificial seawater was used in the acclimation and the experiments. Artificial seawater of 30‰ salinity was made by mixing 33 g of commercial sea salt (Instant Ocean®) into 1 liter of distilled water. The pH value of the

artificial seawater was adjusted to 7.8 as necessary with the addition of either 1M hydrochloric acid or 1M sodium hydroxide.

During the first five days of the acclimation period, the amphipods were held in a 4-L plastic container that contained 3.5 L aerated artificial seawater. The density of amphipods was kept at below 50 adults L^{-1} and 80 juveniles L^{-1}. Acclimation was carried out at 25°C under a photoperiod of 14L:8D in an environmental chamber (Shel-lab model 2015). The adults were fed on chopped flesh of two frozen-dried krills (about 2 cm long in body length) and provided with 2 thalli (size, 100 cm^2) of the green alga *Ulva lactuca*. The juveniles were fed on chopped pieces of one frozen-dried krill body and provided with two pieces of the thallus (size, 60 cm^2). The acclimation was carried out with a static and renewal technique. Half volume of aerated artificial seawater was refreshed every two days. Any excreta, dead body, detritus, food residue and any undesirable algal colonies were removed every day.

During the last two days of the acclimation, the amphipods were transferred into glass beakers instead of plastic containers. Twelve individuals of the same group (adults or juveniles) were acclimated in a 500-ml glass beaker. Each beaker was covered with a sheet of perforated plastic film to minimize evaporation. No food or algal thallus was provided within this period.

5.2.3 Tolerance Tests

The high and low tolerance limits of the adult and juvenile *Hyale* sp. to temperature and salinity were determined. The lethal end-point was monitored in the study. Ten amphipods were randomly selected and placed in a 500-ml beaker. The experimental amphipods were exposed to the respective conditions for 96 hours. The percentage mortality of amphipods was determined at the end of the experiment. The acute tolerance limits were expressed as median lethal salinity (96h LS_{50}) and median lethal temperature (96h $LTemp_{50}$). The median mortality is used to express the

tolerance limit to salinity or temperature in crustaceans (Thomas & Rice 1992; Hardy *et al.* 1994; Charmantier & Charmantier-Daures 1994; Guerin & Stickle 1997).

5.2.3.1 Temperature Tolerance Tests

The study was made up of two major steps. The preliminary study was to determine the tolerance within the board range of temperatures of 0 to 35°C at 5°C interval. The temperature increment (5°C) used in this preliminary study were unable to precisely determine the median lethal values by probit analysis. The second phase of the study was performed to determine the median lethal temperatures from a narrower range of increment. The lower and higher experimental temperatures used were in the range of 0 to 14°C and 30 to 35°C, respectively. The increment between two successive temperatures was set at 2°C in the study. The temperature was adjusted to the selected level from 25°C (the standard condition) in the rate of 2–5°C h^{-1}.

Five replicates were performed in each experimental temperature. The experimental vessels were held at a selected temperature. High range temperatures (> 25°C) were achieved by immersing the experimental vessels in water baths under the regulation of thermal circulators (Haake DC 10). Low range temperatures (< 25°C) were achieved by keeping the experimental vessels in the environmental chamber (Shel-lab model 2015). The salinity, pH value and photoperiod were standardized at 30‰, 7.8 and 14L:10D, respectively.

During the experimental period, no food or algal thallus was provided. Any dead amphipod and exuivae were removed from the beakers during the daily inspection. Half volume (250 ml) of water content in experimental vessels was exchanged every 48 hours by newly prepared, fully aerated and temperature adjusted artificial seawater at 30‰ salinity. The physical parameters of the environmental conditions were monitored daily during the experimental period. The temperature was measured with a digital long stem thermometer (model NEW 61220-416). The salinity of artificial

seawater was measured by means of an optical refractometer (Sper Scientific, model W/ATc 300011). The pH value was monitored by a pH meter (pH Testr. 3 model 59000-30). The dissolved oxygen (DO) concentration was monitored by a D.O. meter (YSI model 5000). All temperatures were recorded to vary within the range of ± 0.5°C. The salinity and pH value was measured in 30 ± 2‰ (mean ± SD) and 7.8 ± 0.4, respectively. The light intensity and dissolved oxygen content varied within the ranges of 968 – 1325 lux and 5.2 – 8.8 mg L^{-1}, respectively. After 96 hours of exposure, the number of live amphipods was counted in each experimental vessel.

5.2.3.2 Salinity Tolerance Tests

The study was also made up of two major steps. The preliminary study was to determine the salinity tolerance within the board range of 0 to 65‰ at 5‰ interval. The second step of the study was to determine the median lethal salinity in a narrower range that provided better resolution. The range of lower and higher experimental salinities used were 0 to 10‰ and 50 to 65‰, respectively. The increment between two successive levels of salinity level was set at 2-3‰. The salinity was adjusted to the respective level from the standardized condition 30‰ at the rate of 2-5‰ h^{-1}.

The experimental amphipods were exposed in a selected salinity. Five replicates were conducted in each experimental salinity. The temperature, pH value and photoperiod were standardized at 25°C, 7.8 and 14L:10D, respectively. During the experimental period, the procedures of seawater exchange, the physical parameters in daily monitoring and the instruments used in the measurement were the same as discussed in section 5.2.3.1. The salinity was recorded to vary within the range of ± 2‰. The temperature and pH value was measured in 25 ± 0.6°C and 7.8 ± 0.9, respectively. The dissolved oxygen content and light intensity varied within the ranges of 5.0 – 8.5 mg L^{-1} and 1128 – 1563 lux, respectively.

5.2.4 Data Analysis

Results from acute survival tests were analyzed by probit analysis. Probit transformation was applied to the cumulative mortality data. The values of the median lethal (50% mortality) salinity and temperature were consequently obtained. The computer software USEPA Probit Analysis program Version 1.5 was employed for calculating the median lethal values. The data obtained from adults and juveniles were analyzed by linear regression equations and Student's *t*-test (Zar 1996).

5.3 Results

5.3.1 Temperature Tolerance Tests

The percentage survival of both adults and juveniles in temperatures ranged from 0 to 36°C is shown in Figure 5.1. Within the range of 6 to 30°C, more than 90% of the amphipod survived for 96 hours. At the temperatures 8, 10, 12 & 14°C, 100% of juveniles survived during the experimental period. For adults, 100% survival was found at the temperatures 10 and 12°C. As the temperatures reached below 6°C and above 30°C, the survival rate fell below 90%. No *Hyale* sp. survived at 0 or 35°C.

The median (50%) lethal temperatures to adults and juveniles *Hyale* sp. were determined by probit analysis. The 95% confidence limit of the value was expressed in the parentheses below. The values of low range 96h median lethal temperatures (96h $LTemp_{50}$) in adults and juveniles were 3.2°C (2.6 to 3.6°C) and 4.2°C (3.9 to 4.3°C), respectively (Figure 5.2). The two values were not significantly different from each other (P = 0.242; Student's *t*-test). The regression equations of the linear curves in adults and juveniles were $Y = -5.07 \log_{10}X + 7.41$ ($r^2 = 0.974$) and $Y = -10.77 \log_{10}X + 11.65$ ($r^2 = 0.999$), respectively. In the equations, X represented the ambient temperature and Y represented the percentage mortality expressed as probits. The slopes of the regression lines for adults and juveniles were compared by Student's *t*-

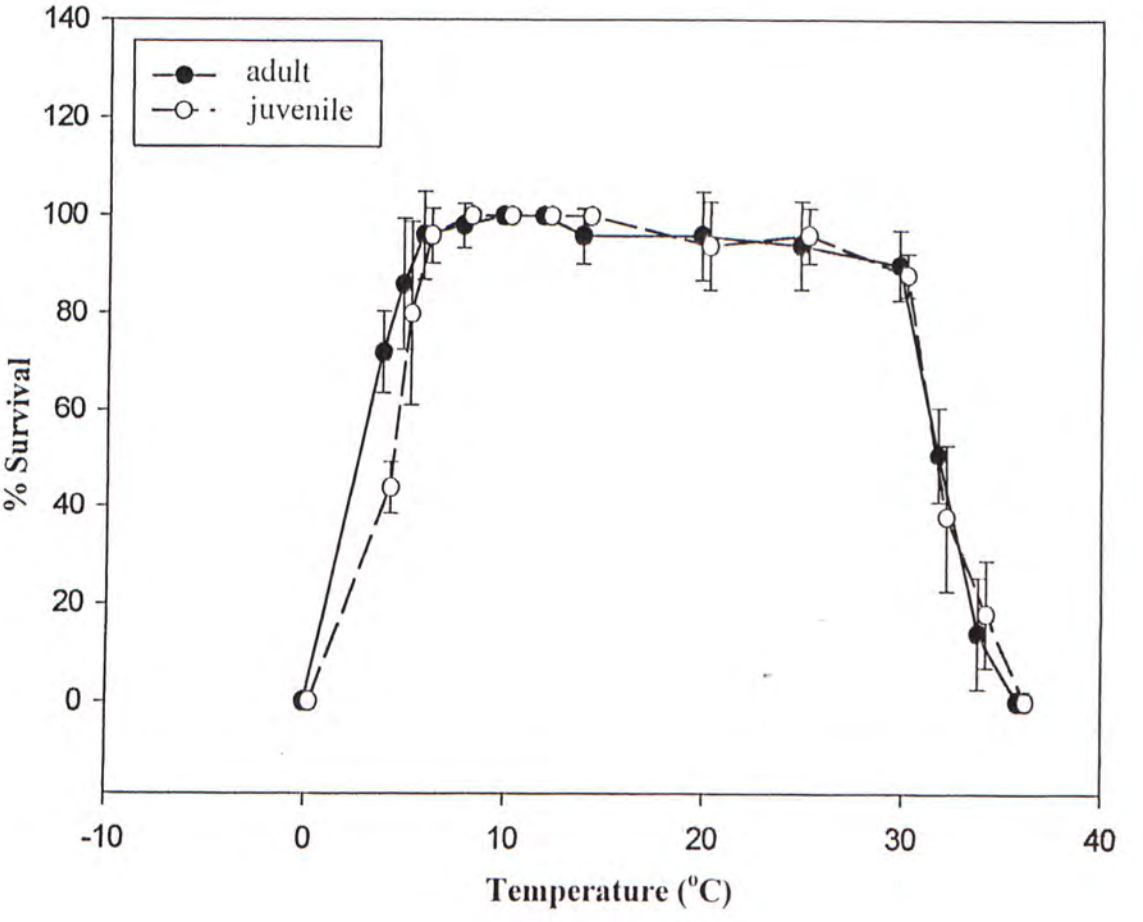


Figure 5.1 Mean survival of the adult and the juvenile of *Hyale* sp. after 96 hours of exposure to different temperatures at a salinity of 30‰. The error bars represent the standard deviations of 5 replicates (N = 10 in each replicate).

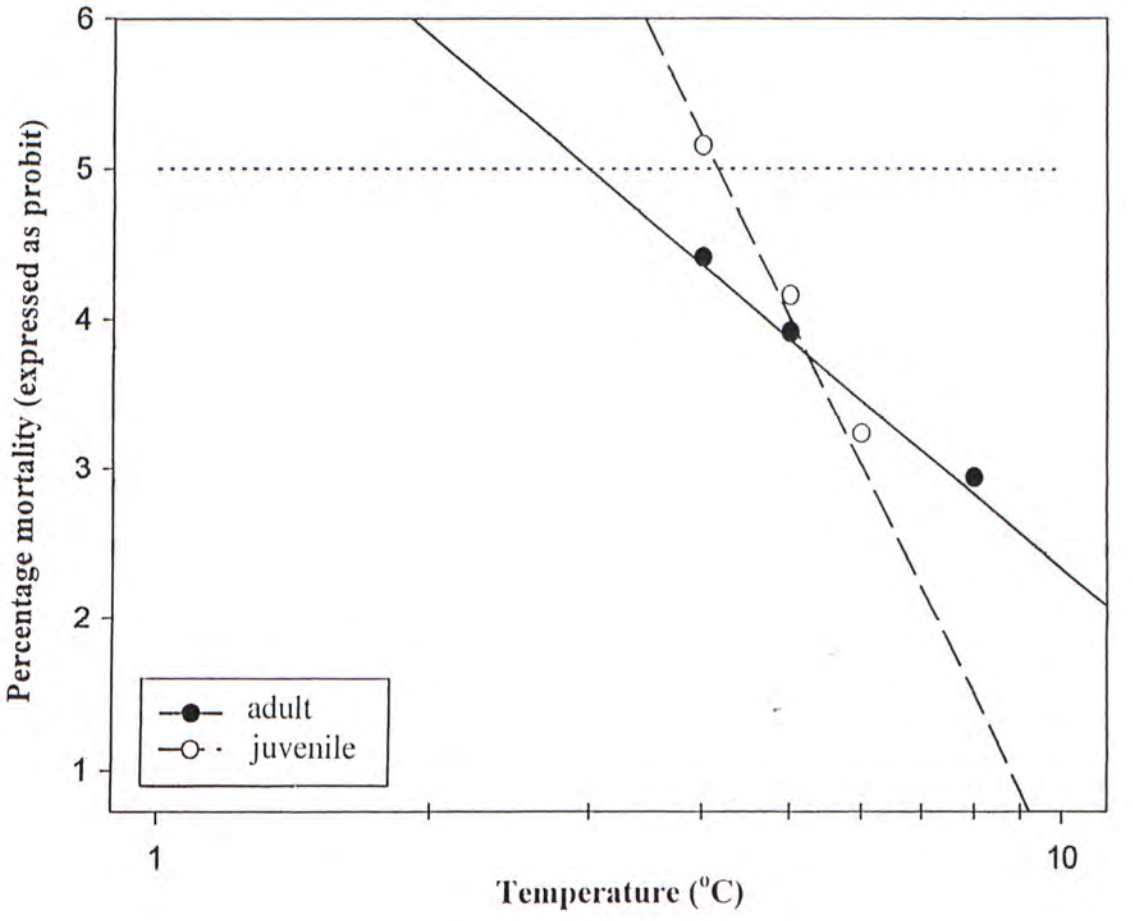


Figure 5.2 Probit analysis of the percentage mortality of the adult and the juvenile *Hyale* sp. to the low range temperature in logarithmic scale. The solid and dash lines represent the best-fit linear regression curves for the adult and the juvenile, respectively. The regression equations of the linear curves in adults and juveniles were $Y = -5.07 \log_{10}X + 7.41$ ($r^2 = 0.974$) and $Y = -10.77 \log_{10}X + 11.65$ ($r^2 = 0.999$), respectively. In the equations, X represents the temperature and Y represents the probit value. The dotted line represents the median mortality (50%) with the equation of $Y = 5$.

test. The statistical analysis showed that the slope of the regression line was significantly greater in juveniles than in adults (P = < 0.0001; Student's *t*-test). This indicated that adults of *Hyale* sp. had significantly higher tolerance to lower temperatures than juveniles. It was observed that no amphipod remained active after 96 hours of exposure to the temperatures of 4 and 6°C. However, live amphipods regained their activity within 15 minutes when the amphipods were placed at 25°C (room temperature), and still survived after one day of acclimation at room temperature.

The values of high range 96h median lethal temperatures in adults and juveniles were 31.7°C (31.1 to 32.3°C) and 31.5°C (29.7 to 32.5°C), respectively (Figure 5.3). The two values were not significantly different form each other (P = > 0.5; Student's *t*-test). The regression equations of the linear curves in adults and juveniles were $Y = 41.61 \log_{10}X - 57.53$ ($r^2 = 0.971$) and $Y = 36.99 \log_{10}X - 50.40$ ($r^2 = 0.704$), respectively. Two slopes of the regression lines in adults and juveniles were not significantly different from each other (P = 0.443; Student's *t*-test).

5.3.2 Salinity Tolerance Tests

Within the range of 10 to 30‰, more than 90% of the amphipod survived for 96 hours (Figure 5.4). The survival rate was lower beyond this salinity range. Two percent of adults and 8% of juveniles could still survive at the salinity of 62‰ after 96 hours. At the salinity of 2‰, the percentage survival of adults and juveniles were 48% and 64%, respectively. No amphipod was observed to survive for more than one day at 0‰ (distilled water) and more than two days at 65‰.

The values of low range 96h median lethal salinity (96h LS_{50}) in adults and juveniles were 2.4‰ (1.7 to 2.9‰) and 1.6‰ (0.9 to 2.2), respectively (Figure 5.5). No significant difference in the values of median lethal salinity was observed between adults and juveniles (P = 0.097; Student's *t*-test). The regression equations of the linear curves in adults and juveniles were $Y = -1.95 \log_{10}X + 5.74$ ($r^2 = 0.955$) and $Y = -1.73$

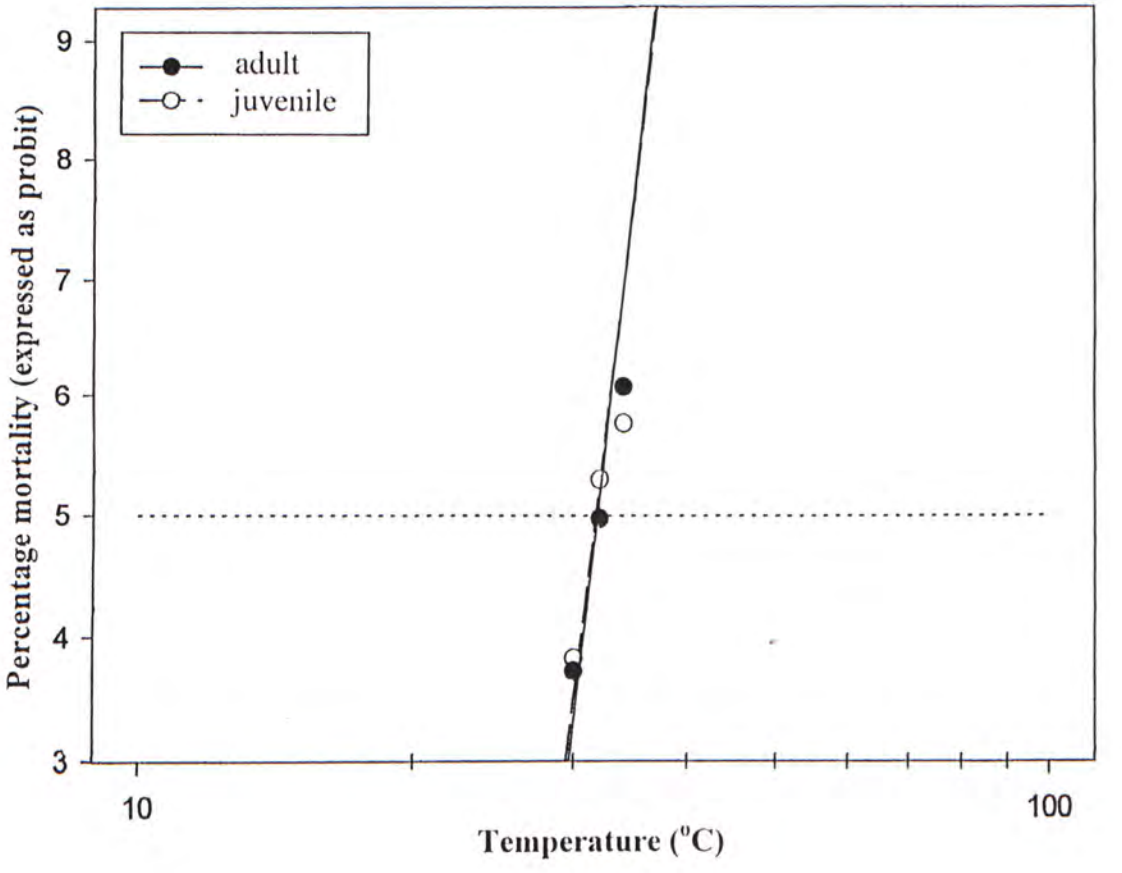


Figure 5.3 Probit analysis of the percentage mortality of the adult and the juvenile *Hyale* sp. to the high range temperature in logarithmic scale. The regression equations of the best-fit linear curves in adults and juveniles were $Y = 41.61 \log_{10}X - 57.53$ ($r^2 = 0.971$) and $Y = 36.99 \log_{10}X - 50.40$ ($r^2 = 0.704$), respectively.

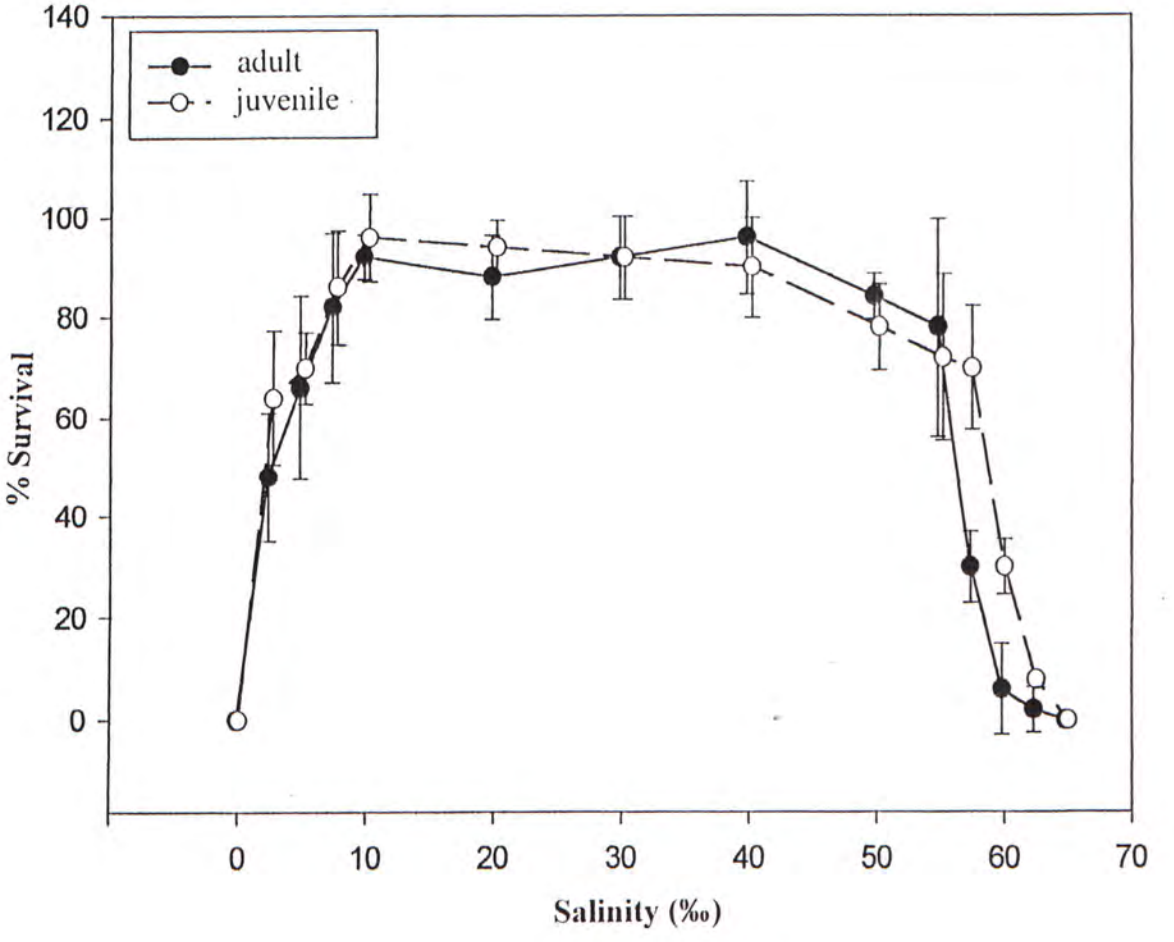


Figure 5.4 Mean survival of the adult and the juvenile of *Hyale* sp. after 96 hours of exposure to different salinities at a temperature of 25°C. The error bars represent the standard deviations of 5 replicates (N = 10 in each replicate).

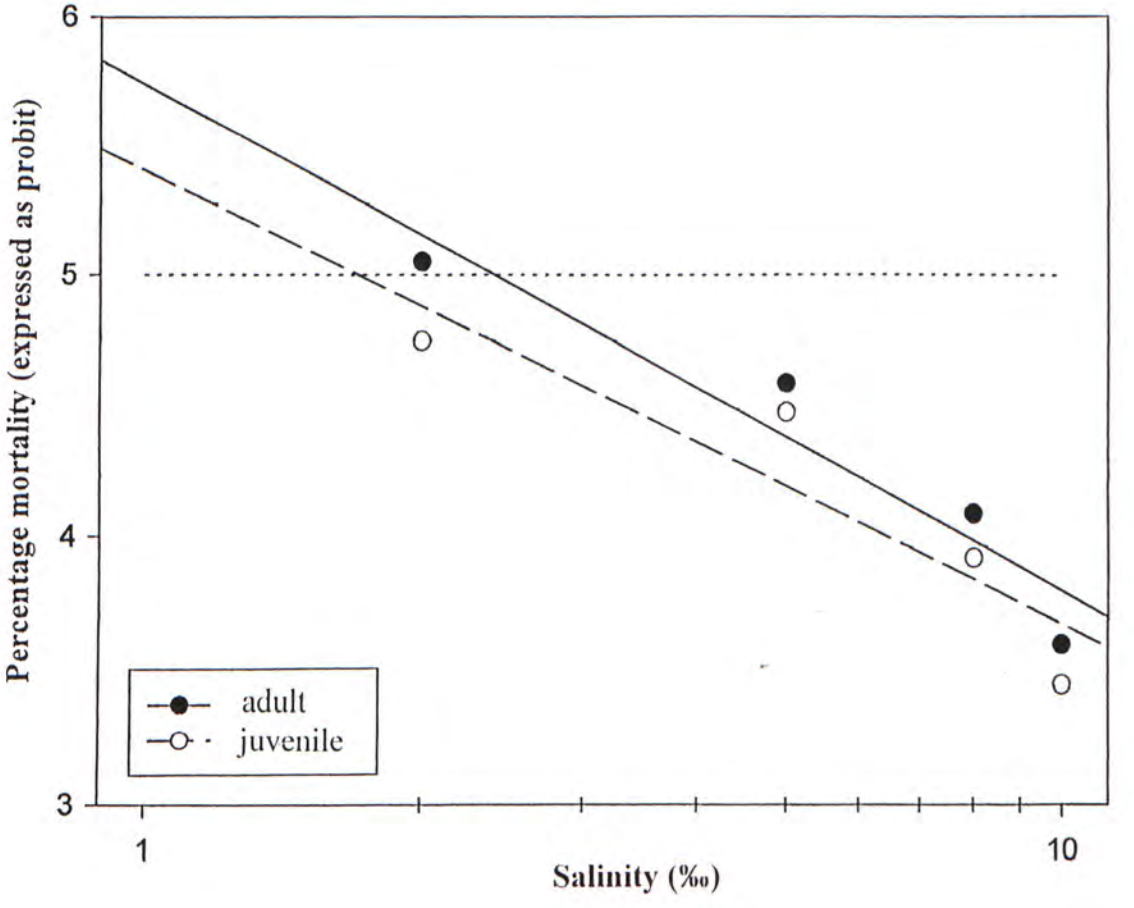


Figure 5.5 Probit analysis of the percentage mortality of the adult and the juvenile *Hyale* sp. to the low range salinity in logarithmic scale. The solid and dash lines represent the best-fit linear regression curves for the adult and the juvenile, respectively. The regression equations of the linear curves in adults and juveniles were $Y = -1.95 \log_{10}X + 5.74$ ($r^2 = 0.955$) and $Y = -1.73 \log_{10}X + 5.40$ ($r^2 = 0.920$), respectively. In the equations, X represents the salinity and Y represents the probit value. The dotted line represents the median mortality (50%) with the equation of $Y = 5$.

$\log_{10}X + 5.40$ ($r^2 = 0.920$), respectively. In the equations, X and Y represented the ambient salinity and the percentage mortality expressed as probits, respectively. The statistical analysis showed that two slopes of the regression lines between adults and juveniles were not significantly different from each other (P = 0.371; Student's *t*-test).

The values of high range 96h median lethal salinity in adults and juveniles were 55.5‰ (50.9 to 58.1‰) and 56.9‰ (42.1 to 62.4‰), respectively (Figure 5.6). The two values were not significantly different from each other (P = 0.488; Student's *t*-test). The regression equations of the linear curves in adults and juveniles were $Y = 34.43 \log_{10}X - 54.93$ ($r^2 = 0.931$) and $Y = 20.91 \log_{10}X - 31.68$ ($r^2 = 0.832$), respectively. No significant difference in the slopes of the regression lines was observed between adults and juveniles (P = 0.119; Student's *t*-test).

5.4 Discussion

Based on the 96h tolerance test, adults and juveniles of *Hyale* sp. can tolerate the temperature range between 6°C and 30°C (Section 5.3.1). Within the range, an average of 90% of the amphipod could survive for 4 days. The temperature tolerance of *Hyale* sp. can be compared to the Sub-Antarctic beach-hopper *Orchestia scutigerula* of the superfamily Talitroidea (Moore *et al.* 1995). The temperatures below -4°C and above 28°C are lethal to *O. scutigerula.* Yet the temperatures below 0°C and above 34°C are lethal to *Hyale* sp.

The adaptability of *O. scutigerula* to sudden change in temperature is great (Moore *et al.* 1995). The amphipod still survived by changing the temperature from 26 to 0°C in half an hour. The amphipod also survived when the animal was immediately put into humid air at 28°C from the acclimation temperatures of 5 to 10°C. The high adaptability to sudden change in temperature was also observed in *Hyale* sp. The inactivated amphipods regained their activity within 15 minutes as being put into 25°C from 4 to 6°C (Section 5.3.1). These amphipods still survived for one day after

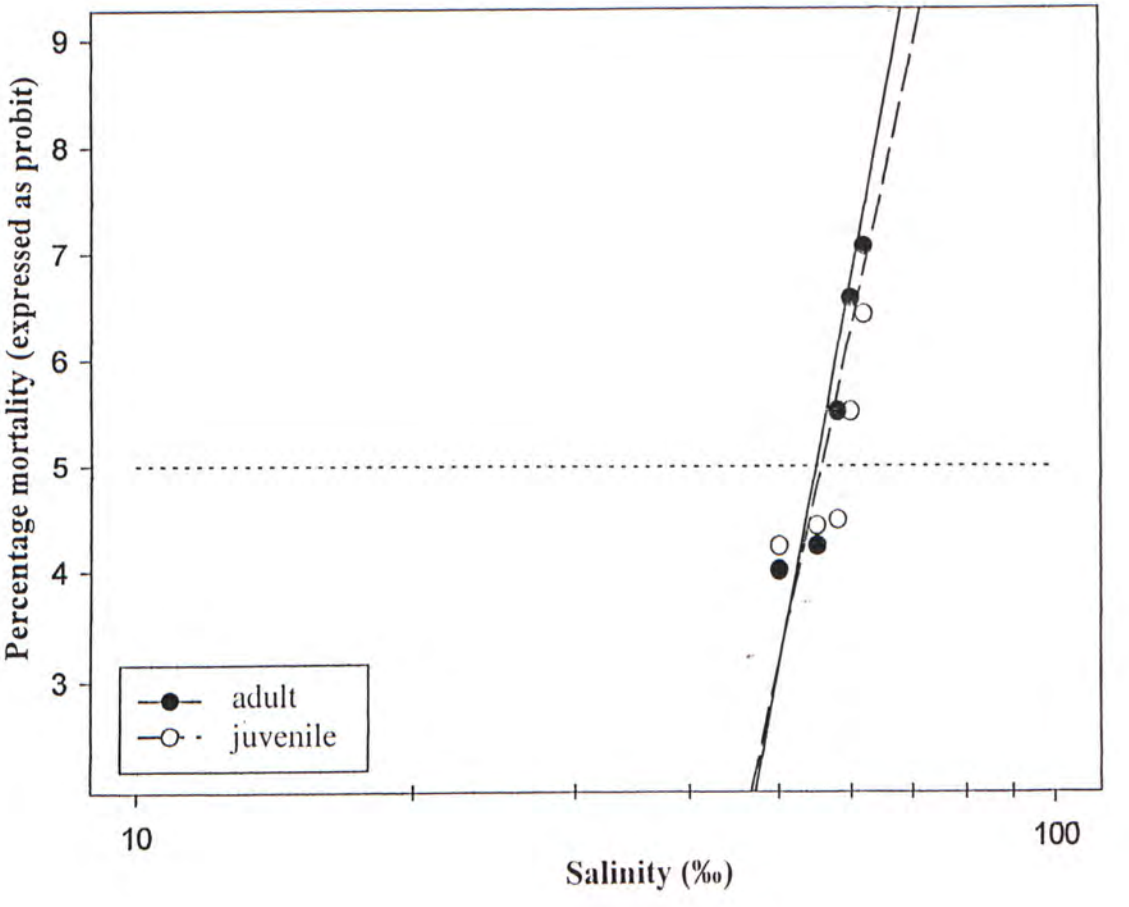


Figure 5.6 Probit analysis of the percentage mortality of the adult and the juvenile *Hyale* sp. to the high range salinity in logarithmic scale. The regression equations of the linear curves in adults and juveniles were $Y = 34.43 \log_{10}X - 54.93$ ($r^2 = 0.931$) and $Y = 20.91 \log_{10}X - 31.68$ ($r^2 = 0.832$), respectively.

changing the temperature abruptly. According to Brown and Bert (1993), the difference of temperature tolerance in crustaceans may be resulted from the adaptation to the different environmental conditions. *O. scutigerula* was found beneath supralittoral stones at Kanin point, South Georgia (Moore *et al.* 1995). The environmental temperature change of the Sub-Antarctic site is dramatic. The wide range of temperature tolerance is an adaptation to the habitat. The temperature tolerance of *Hyale* sp. may also be resulted from the adaptation to the fluctuation of environmental temperature. The highest and lowest water surface temperatures of Tolo Harbor, Hong Kong recorded since 1998 were 31.8°C (air temperature, 30°C) in August 1998 and 16°C (air temperature, 12°C) in January 1999, respectively (Records of Marine Science Laboratory, The Chinese University of Hong Kong). The mean water surface temperature in the Cape d'Aguilar, Hong Kong changed between 16.6 and 28.8°C within the period from February to September 1989 (Morton & Harper 1995).

At the temperatures higher than 30°C, the survival rate of *Hyale* sp. decreased. The uppermost temperature limit for the survival of *Hyale* sp. is 34°C. Only 14 and 18% survival was observed in adults and juveniles, respectively after 96 hours of 34°C exposure. Yet no amphipod survived at 35°C. According to Spicer & Taylor (1987a), oxygen uptake is a temperature-dependent process in *Talitrus saltator*. The reduction of tolerance to high water temperature in amphipods may be caused by lowering the capability to transport sufficient oxygen into the body (Moore *et al.* 1995). Moreover, higher temperature increases the overall metabolic rate, which may overload the oxygen delivery capabilities in amphipods (Moore *et al.* 1995). Death eventually occurs due to hypoxia at high temperature.

Based on the 96h tolerance test, *Hyale* sp. was tolerant to wide range of salinity from 10 to 30‰ (Section 5.3.2). The survival limits of the highest and lowest salinity were 2‰ and 62‰, respectively. Members of the superfamily Talitroidea to which *Hyale* sp. belongs to commonly have high tolerance to wide range of external salinity

(Marsden 1980; Moore & Francis 1986; Morritt 1988). For example, the talitroid *Traskorchestia traskiana* tolerated the salinity in the range of 2.5 to 50‰ for at least a week at 10°C (Koch 1991). Spicer and Taylor (1987b) reported that haemolymph sodium was actively regulated within a nearly constant value between 204 ± 31 to 443 ± 30 mmol kg^{-1} when the talitroid *Orchestia gammarellus* was exposed to the external salinity from 5‰ (≈ 135 mmol kg^{-1}) to 40‰ (≈ 1160 mmol kg^{-1}). Thus *O. gammarellus* is a good osmoregulator in wide range of salinity. Moreover, the Talitroidea is the only family in Amphipoda to successfully invade into terrestrial habitats (Wildish 1988; Koch 1991). The transition from marine shores to terrestrial leaf litter habitats is essentially based on the osmotic and ionic regulatory mechanisms to permit survival of the amphipods at reduced environmental salinity (Koch 1991).

Chapter 6 Acute Toxicity of Cadmium to *Hyale* sp.

6.1 Introduction

Ecotoxicological tests are desirable in water pollution evaluations as the effects of chemical interactions and the influence of complex matrices on toxicity cannot be determined from chemical tests alone (APHA, AWWA, WPCF, 1985). According to Burridge *et al.* (1995), traditional acute lethal tests are still commonly employed as a standard ecotoxicological procedure by environmental agencies to assess the potential impact of chemical interactions on ecological systems. Information obtained from bioassay also indicates the capacity of the habitat to support aquatic life (Bhat & Vamsee 1993). In ecological study, static bioassay is commonly used to determine the acute toxicity of pollutants (Zanders & Rojas 1992; Bhat & Vamsee 1993; Collyard *et al.* 1994; Burridge *et al.* 1995).

Heavy metals in aquatic environment pose a potential threat to the ecosystem (Bhat & Vamsee 1993). Cadmium is a heavy metal known to be toxic to marine invertebrates. It exerts toxic effect most probably on homeostatic ion regulation through disruption of different osmoregulatory pathways (Wood 1992). Its toxicity is relatively lower than mercury but higher than copper (Bhat & Vamsee 1993). Among various metals, cadmium concentration is commonly high in marine invertebrates (Rainbow 1989; Bhat & Vamsee 1993). Ahsanullah & Williams (1991) found that cadmium ion was accumulated inside the body of the amphipod *Allorchestes compressa* without any regulation until the amphipod died. Its tissue content was in simple proportion to the ambient concentration of cadmium. A similar result was found in the burrowing amphipod *Corophium volutator* living in Californian sewage outfall (Hershelman *et al.* 1981). The body cadmium level of *C. volutator* was highly correlated to the metal contents in the seabed around the sewage outfalls. Thus the measurement of cadmium concentrations of the deposit-feeder *C. volutator* indicated the level of sewage contamination around the outfalls (Hershelman *et al.* 1981). Most amphipods potentially accumulate cadmium from the environment and reflect the

cadmium loading at the site of deposition (Rainbow 1995b). The ecologically important and widespread amphipods may attenuate the biomagnification effects to upper trophic levels in the food chain. For example, the hyperiid amphipods *Themisto* spp. were reported to be the most probably dietary source of high cadmium contents to pelagic seabirds (Rainbow 1989). In general, talitroid amphipods were commonly used to monitor cadmium pollution (Hershelman *et al.* 1981; Ahsanullah & Williams 1991; Rainbow 1992; Zanders & Rojas 1992; Bhat & Vamsee 1993; Rainbow *et al.* 1993; Weeks & Rainbow 1993; Caparis & Rainbow 1994; Rainbow 1995a, b). Certain amphipods are highly sensitive to cadmium toxicity, such as, *Hyalella azteca* (Collyard *et al.* 1994; Phipps *et al.* 1995), *Allorchestes compressa* (Ahsanullah & Williams 1991) and *Elasmopus rapax* (Zanders & Rojas 1992). An acute 96h median lethal test to the metal cadmium is standardized to be a reference test in conducting static sediment toxicity tests using amphipods (ASTM 1992).

Cadmium is a major concerning pollutant in bioassays because of its toxicity, mobility, efficiency of deposition and long half-life in animal tissue (Caparis & Rainbow 1994). The biological half-life of cadmium in gammarideans ranges from 6 to 12 days (Zauke *et al.* 1995). Therefore cadmium is selected to be a reference toxicant to determine on whether the species is suitable for local environmental bioassays. The present study is to determine the cadmium toxicity to the amphipod *Hyale* sp. in terms of 24h, 48h, 72h and 96h median lethal concentrations (LC_{50}). The results may provide reference indices for the further bioassays using the local amphipod *Hyale* sp.

6.2 Materials and Methods

6.2.1 Sampling

The experimental amphipods were collected from outdoor concrete tanks in Marine Science Laboratory (MSL), The Chinese University of Hong Kong. The procedures of sampling and the separation of adults and juveniles basically followed the steps described in Sections 2.2.1 and 5.2.1.

6.2.2 Acclimation

The amphipods were acclimated under the standardized laboratory conditions for one week as described in Section 5.2.2. The procedures of the acclimation followed the steps discussed in the same section. During the last two days, a group of ten amphipods was starved and acclimated in a 500-mL glass beaker.

6.2.3 Cadmium Toxicity Tests

The acute toxicity of cadmium to *Hyale* sp. was studied in four different duration of exposure, 24h, 48h, 72h and 96h. A preliminary study was performed to identify the mortality of *Hyale* sp. exposed to various concentrations of cadmium for 24 hours. The preliminary results indicated that the appropriate concentrations of cadmium ion employed for the study were ranged from 0.0 (negative control) to 24.0 mg L^{-1}.

Ten amphipods were exposed to a 500-mL beaker containing one of the concentrations of 0.0, 3.0, 6.0, 9.0, 12.0, 15.0, 18.0, 21.0 and 24.0 mg L^{-1} at interval of 3.0 mg L^{-1} for 24 hour study. In 48 hours study, the interval between two successive concentrations was lowered to 2.0 mg L^{-1} because of a longer duration of exposure, and thus the concentrations of 0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg L^{-1} were employed for the study. The concentrations employed for 72 hours study were basically the same as those used in the 48 hours study, but two more concentrations of 0.5 and 1.0 mg L^{-1} were added. In 96 hours study, the interval between two successive concentrations was lowered to 1.0 mg L^{-1} and two more concentrations of 0.2 and 0.5 mg L^{-1} were added for better resolution. The concentrations used for the 96 hours study were 0.0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mg L^{-1}. All concentrations of cadmium solution were prepared by the addition of the respective volume of stock solution (10.0 mg mL^{-1}) into 500.0 mL artificial seawater. The stock solution was made by dissolving 203.0 mg of cadmium chloride salt $CdCl_2 \cdot 2½ H_2O$ into 10.0 mL distilled water.

Each beaker was covered with a sheet of perforated plastic film to minimize evaporation. Five replicates were performed for each concentration. The amphipods were starved during the experimental period. For the 72 and 96 hours studies, half volume (250 mL) of solution in each experimental vessel was exchanged by newly prepared and aerated artificial seawater with respective concentration of cadmium after 48 hours of exposure. Any dead amphipod and exuvium were removed from the vessels during the daily check. All the physical parameters of the laboratory conditions were standardized and maintained as described in Section 5.2.2. The physical parameters of the environmental conditions were monitored daily during the experimental period. The equipment used to monitor the parameters were the same as described in Section 5.2.3.1. During the experimental period, the salinity of cadmium solutions were recorded within the range of 30 ± 2‰ (mean ± SD). The water temperatures and the pH value of the solutions were 25 ± 2°C and 7.8 ± 0.8, respectively. The dissolved oxygen (DO) concentration and light intensity of the solution surface were recorded in the range of 4.8 – 8.4 mgL^{-1}.and 1014 to 1373 lux, respectively.

At the end of the experiment, the number of live amphipods was counted. The percentage mortality of adults and juveniles *Hyale* sp. was obtained from five replicates. The 24h, 48h, 72h and 96h median lethal concentrations were determined.

6.2.4 Data Analysis

Results from acute survival tests were analyzed by probit analysis. Probit transformation was applied to the cumulative mortality data. The computer software USEPA Probit Analysis program Version 1.5 was employed for calculating the median lethal values. The data obtained from adults and juveniles were analyzed by Student's *t*-test and linear regression equations (Zar 1996).

6.3 Results

The percentage mortality of the adult and juvenile *Hyale* sp. exposed to a series of cadmium solutions for different duration are shown in Figures 6.1 and 6.2. In the figures, different curves represent the best-fit regression lines in different duration.

The median (50%) lethal concentrations LC_{50} of cadmium to adults and juveniles of *Hyale* sp. were determined by the probit analysis. The range of 95% confidence limit was expressed in the parentheses followed. The percentage mortality expressed as probit was potted against the external cadmium concentration in $\log_{10}$ scale in Figures 6.3 to 6.6.

The 24h LC_{50} values of adults and juveniles were 8.91 (5.65 to 12.44) mg L^{-1} and 8.80 (6.90 to 10.53) mg L^{-1}, respectively (Figure 6.3). The two values were not significantly different form each other (P = 0.432; Student's *t*-test). When percentage mortality expressed as probit was regressed against the concentration of cadmium in $\log_{10}$ scale, the slope of the regression line was significantly steeper in juveniles than in adults (P = 0.0013; Student's *t*-test). The equations of the best-fit regression lines in adults and juveniles are $Y = 2.00 \log_{10}X + 3.09$ ($r^2 = 0.943$) and $Y = 3.89 \log_{10}X + 1.42$ ($r^2 = 0.915$), respectively. In the equations, X represents the concentration of cadmium solution and Y represents the probit value.

The 48h LC_{50} values of adults and juveniles were 4.47 (2.08 to 7.85) mg L^{-1} and 5.83 (3.43 to 8.24) mg L^{-1}, respectively (Figure 6.4). The two values were not significantly different from each other (P = 0.312; Student's *t*-test). When percentage mortality expressed as probit was regressed against the concentration of cadmium in $\log_{10}$ scale, the slopes of the regression lines in adults and juveniles were not significantly different from each other (P = 0.227; Student's *t*-test). The equations of the best-fit regression lines in adults and juveniles are $Y = 1.95 \log_{10}X + 3.72$ ($r^2 = 0.900$) and $Y = 2.72 \log_{10}X + 2.87$ ($r^2 = 0.806$), respectively. The analysis indicated

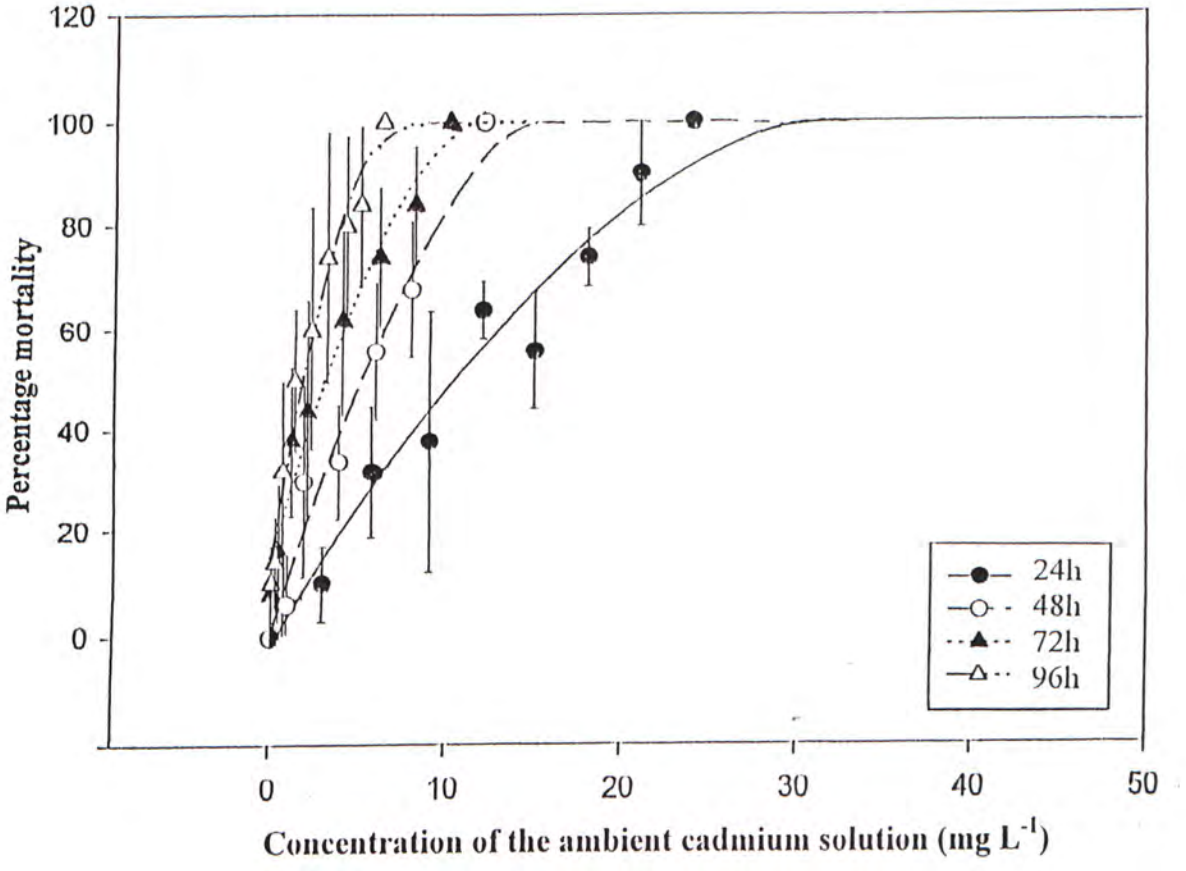


Figure 6.1 The percentage mortality of the adult *Hyale* sp. exposed to various concentrations of the cadmium under different duration. The regression equations of different duration are $Y = -0.08X^2 + 5.86X - 1.73$ ($r^2 = 0.976$) for 24h, $Y = -0.35X^2 + 12.49X - 1.22$ ($r^2 = 0.972$) for 48h, $Y = -0.51X^2 + 13.34X + 14.78$ ($r^2 = 0.980$) for 72h, and $Y = -1.60X^2 + 23.31X + 14.49$ ($r^2 = 0.979$) for 96h. The error bars represent the standard deviation.

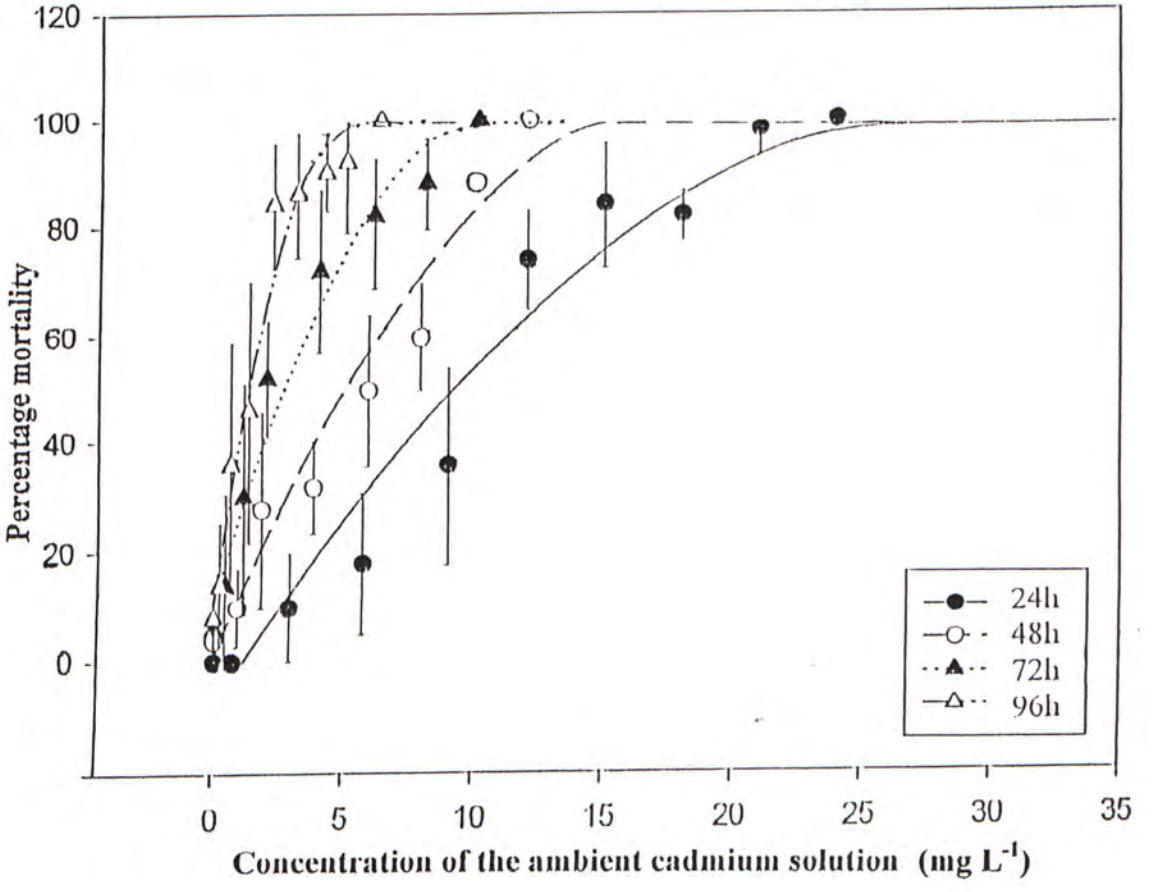


Figure 6.2 The percentage mortality of the juvenile *Hyale* sp. exposed to various concentrations of the cadmium under different duration. The regression equations of different duration are $Y = -0.13X^2 + 7.54X - 8.23$ ($r^2 = 0.972$) for 24h, $Y = -0.32X^2 + 11.35X + 1.34$ ($r^2 = 0.961$) for 48h, $Y = -0.78X^2 + 16.73X + 11.48$ ($r^2 = 0.980$) for 72h, and $Y = -3.44X^2 + 34.87X + 10.83$ ($r^2 = 0.964$) for 96h. The error bars represent the standard deviation.

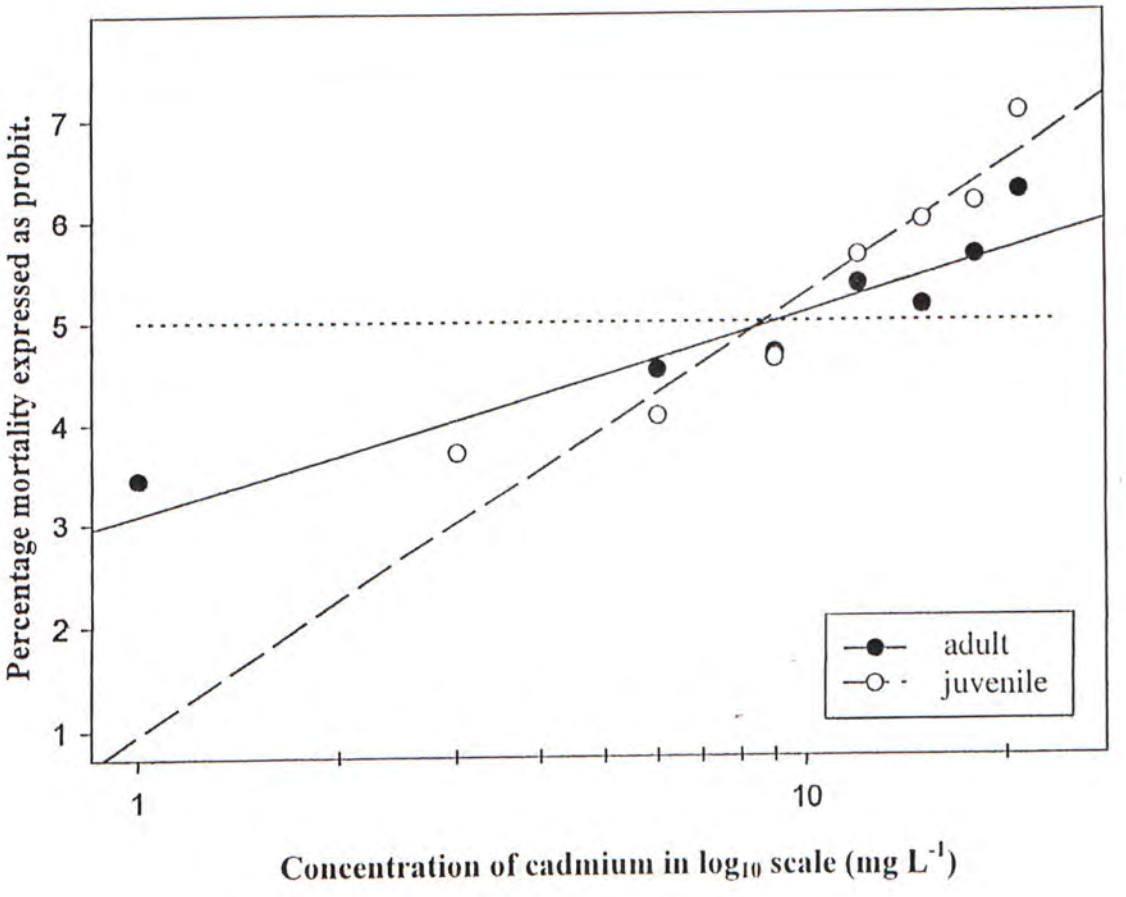


Figure 6.3 Effects of cadmium concentrations (expressed in logarithmic scale) on the mean percentage mortality (expressed as probit) of *Hyale* sp. after 24 hours exposure. The regression equations of the best-fit lines in adults and juveniles are $Y = 2.00 \log_{10}X + 3.09$ ($r^2 = 0.943$) and $Y = 3.89 \log_{10}X + 1.42$ ($r^2 = 0.915$), respectively. In the equations, X represents the concentration of cadmium solution and Y represents the probit value.

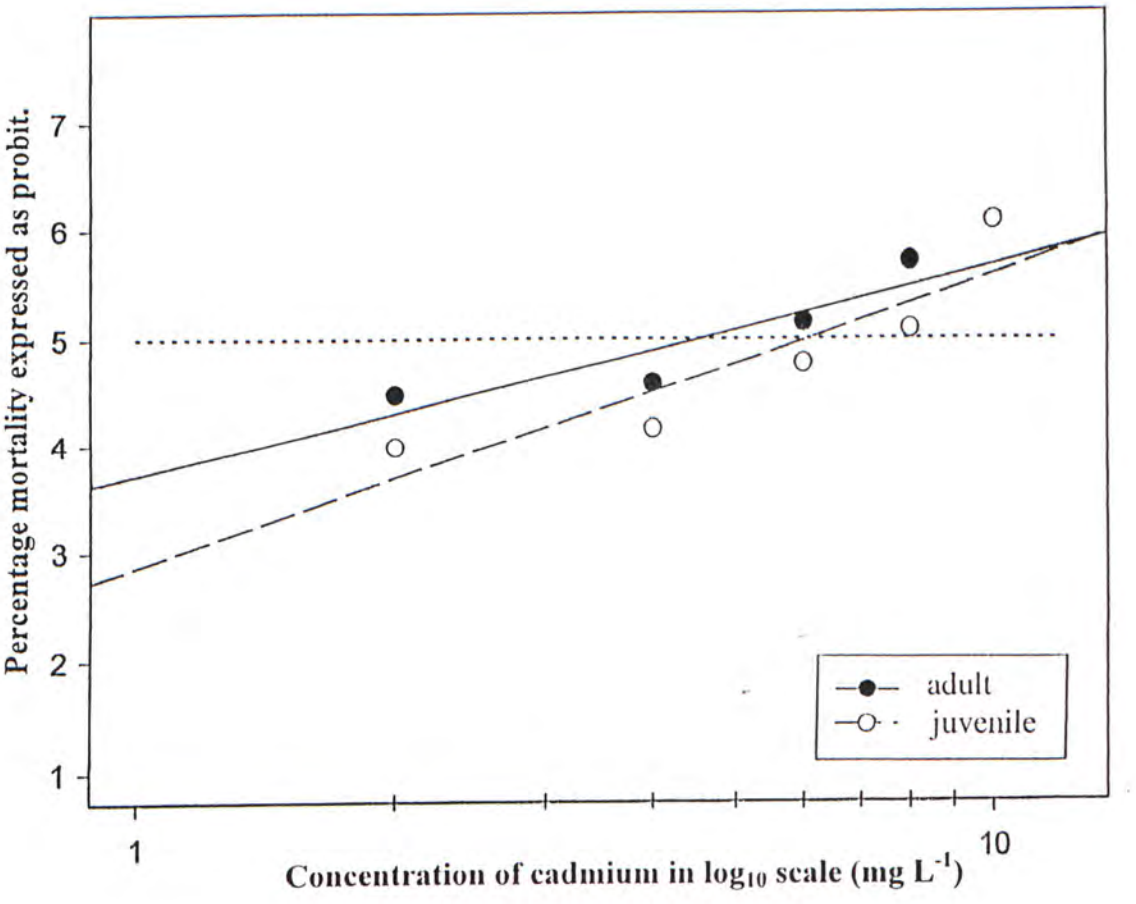


Figure 6.4 Effects of cadmium concentrations (expressed in logarithmic scale) on the mean percentage mortality (expressed as probit) of *Hyale* sp. after 48 hours exposure. The regression equations of the best-fit lines for adults and juveniles are $Y = 1.95 \log_{10}X + 3.72$ ($r^2 = 0.900$) and $Y = 2.72 \log_{10}X + 2.87$ ($r^2 = 0.806$), respectively.

that the toxicity of cadmium to juveniles and adults was not significantly different as the exposure time was lengthened to 48 hours.

The 72h LC_{50} values of adults and juveniles were 3.00 (1.45 to 4.25) mg L^{-1} and 2.12 (1.64 to 2.57) mg L^{-1}, respectively (Figure 6.5). The two values were not significantly different from each other (P = 0.117; Student's *t*-test). When percentage mortality expressed as probit was regressed against the concentration of cadmium in $\log_{10}$ scale, the slopes of the regression lines in adults and juveniles were not significantly different from each other (P = 0.206; Student's *t*-test). The equations of the best-fit regression lines in adults and juveniles are $Y = 1.91 \log_{10}X + 4.13$ (r^2 = 0.956) and $Y = 2.07 \log_{10}X + 4.29$ ($r^2 = 0.998$), respectively. The analysis indicated that no significant difference was found in the toxicity of cadmium between juveniles and adults after 72 hours exposure.

The 96h LC_{50} values of adults and juveniles were 1.38 (0.98 to 1.76) mg L^{-1} and 0.96 (0.74 to 1.18) mg L^{-1}, respectively (Figure 6.6). The two values were not significantly different from each other (P = 0.136; Student's *t*-test). When percentage mortality expressed as probit was regressed against the concentration of cadmium in $\log_{10}$ scale, the slopes of the regression lines in adults and juveniles were not significantly different from each other (P = 0.429; Student's *t*-test). The equations of the best-fit regression lines in adults and juveniles are $Y = 2.12 \log_{10}X + 4.71$ (r^2 = 0.928) and $Y = 2.08 \log_{10}X + 5.02$ ($r^2 = 0.974$), respectively. The results indicated that no significant difference was found in the toxicity of cadmium between juveniles and adults after 96 hours exposure. The LC_{50} values of the adult and the juvenile under different periods of exposure time were summarized in Table 6.1.

The statistical analysis showed that there was no significant difference among the slopes of all regression lines in different exposure periods in adults ($P > 0.05$; Student's *t*-test). The similar result was observed in the juvenile group, except for the duration of 24h. It was found that the slopes of the regression lines was significantly steeper in the duration of 24h than those of 72h (P = 0.0008; Student's *t*-test) and 96h (P = 0.0008;

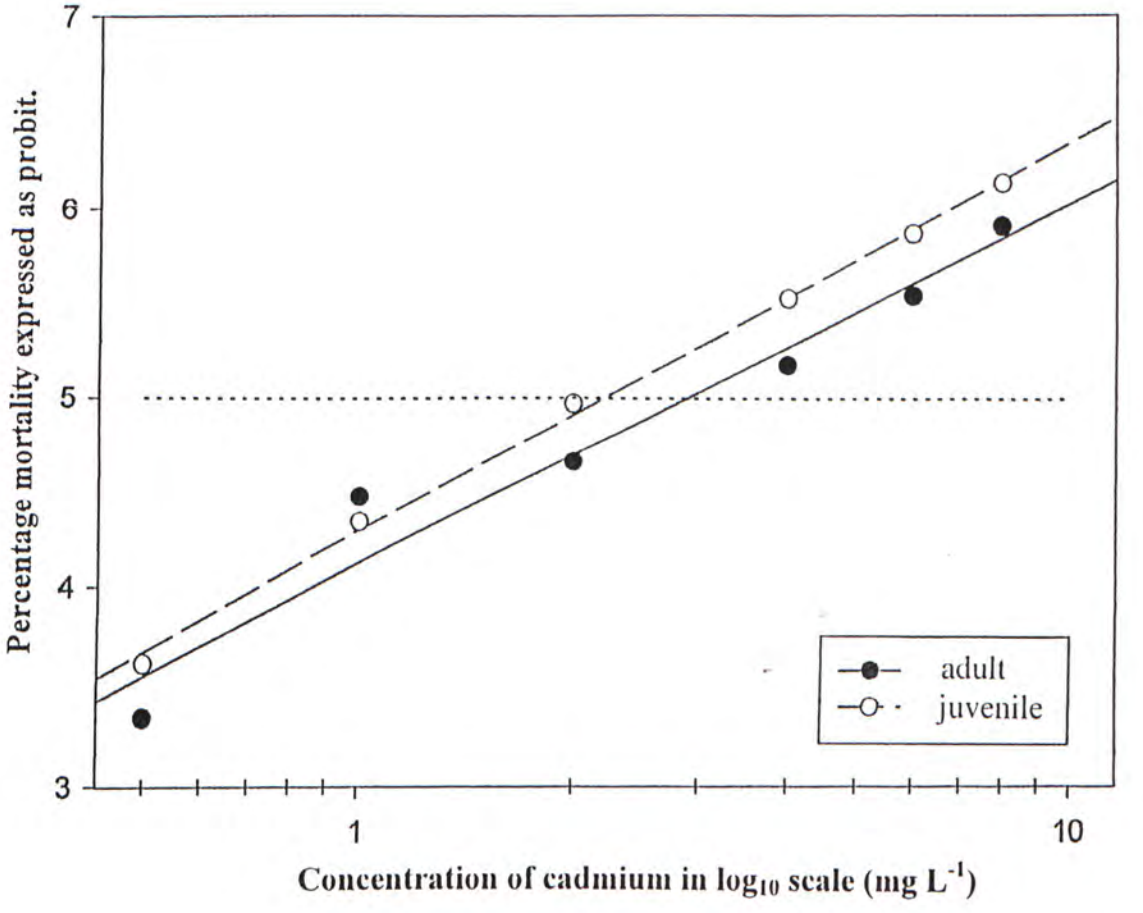


Figure 6.5 Effects of cadmium concentrations (expressed in logarithmic scale) on the mean percentage mortality (expressed as probit) of *Hyale* sp. after 72 hours exposure. The regression equations of the best-fit lines in adults and juveniles are $Y = 1.91 \log_{10}X + 4.13$ ($r^2 = 0.956$) and $Y = 2.07 \log_{10}X + 4.29$ ($r^2 = 0.998$), respectively.

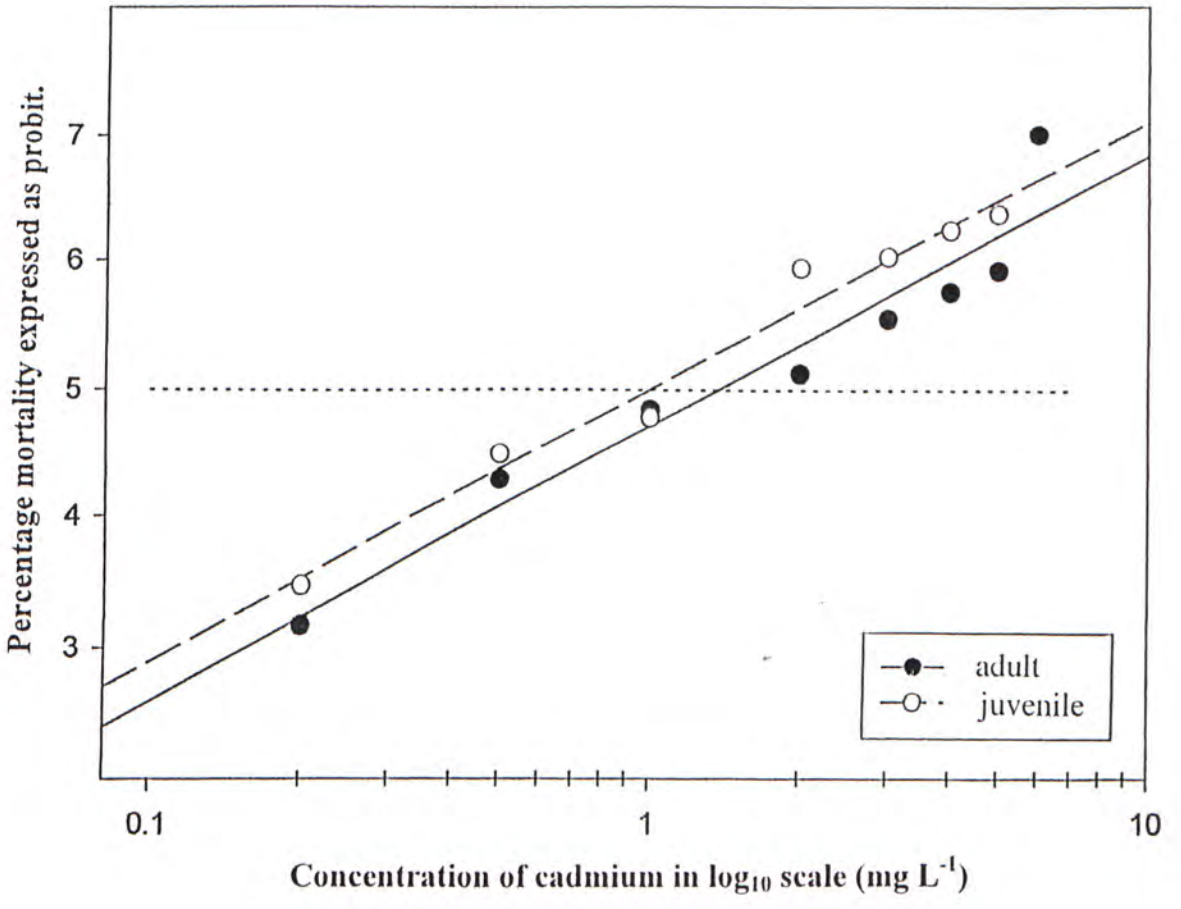


Figure 6.6 Effects of cadmium concentrations (expressed in logarithmic scale) on the mean percentage mortality (expressed as probit) of *Hyale* sp. after 96 hours exposure. The regression equations of the best-fit lines in adults and juveniles are $Y = 2.12 \log_{10}X + 4.71$ ($r^2 = 0.928$) and $Y = 2.08 \log_{10}X + 5.02$ ($r^2 = 0.974$), respectively.

Table 6.1 The values of the median lethal concentration (LC_{50}) of the adult and the juvenile *Hyale* sp. to cadmium in different exposure duration of 24h, 48h, 72h and 96h.

LC_{50} ($mg\ L^{-1}$)	Adults	Juveniles
24h	8.91 (5.65 to 12.44)	8.80 (6.90 to 10.53)
48h	4.47 (2.08 to 7.85)	5.83 (3.43 to 8.24)
72h	3.00 (1.45 to 4.25)	2.12 (1.64 to 2.57)
96h	1.38 (0.98 to 1.76)	0.96 (0.74 to 1.18)

( ) The 95% confidence limit is shown in the parentheses

Student's *t*-test). It indicated that juveniles were more sensitive to higher dose of cadmium than adults were.

6.4 Discussion

The median lethal concentration LC_{50} is a common measure of ecotoxicological tests to amphipods (ASTM 1992; Bhat & Vamsee 1993; Zanders & Rojas 1992). The 24h, 48h, 72h and 96h LC_{50} values of the adult *Hyale* sp. to cadmium are 8.91, 4.47, 3.00 and 1.38 mg L^{-1}, respectively (Section 6.3 & Table 6.1). The most common value employed for the toxicity index is 96h LC_{50}. (ASTM 1992; DeWitt *et al.* 1989; Hong & Reish 1987; Swartz *et al.* 1985b; Zander & Rojas 1992). According to Zanders & Rojas (1992), the amphipod *Elasmopus rapax* is the most sensitive marine organisms to cadmium toxicity. Its 96h LC_{50} value is 0.18 mg L^{-1} and lower than that of the *Hyale* sp. The 96h LC_{50} value to cadmium of *Leptocheirus plumulosus* and *Eohaustorius estuarius* are 1.06 (ASTM 1992) and 9.33 mg L^{-1} (DeWitt *et al.* 1989), respectively. The values of median lethal concentration of different aquatic invertebrates and different amphipods to cadmium are summarized in Tables 6.2 and 6.3, respectively. In general, the LC_{50} values of *Hyale* sp. are relatively larger than those of the invertebrates shown in Table 6.2. However, the value of the 96h median lethal concentration of *Hyale* sp. obtained from the present study falls within the range of the values of marine amphipods. The sensitivity of *Hyale* sp. to cadmium is similar to those of amphipod species commonly used in environmental bioassays (Table 6.3).

It is observed that the values obtained from freshwater species were generally lower than those of marine species (Table 6.3). The 96h LC_{50} values to cadmium of the freshwater amphipods *Hyalella azteca*, *Chaetocorophium lucasi* and *Gammarus pulex* are 0.014 mg L^{-1} (ASTM 1993), 0.061 mg L^{-1} (Collyard 1994) and 0.020 mg L^{-1} (Wright & Frain 1981a), respectively. However the value of the most sensitive marine species *E. rapax* is 0.18 mg L^{-1} (Zanders & Rojas 1992). The 96h median lethal concentration is about 13 times greater in the marine species *E. rapax* than in freshwater species *H. azteca*. The lowering in cadmium sensitivity of marine species is

Table 6.2 The comparison of LC_{50} values of marine and freshwater invertebrates to cadmium.

Organisms	Exposure durattion	LC_{50}	Reference
Vibrio fischeri (Microtox Test)	5min EC_{50}	1070	De Zwart & Sloof 1983
Vibrio fischeri	15min EC_{50}	218	De Zwart & Sloof 1983
Vibrio fischeri	30min EC_{50}	14	Sloof *et al*. 1983
Spirostomum ambiguum (Spirotox Test)	24h	0.20	Madoni *et al*. 1994
Spirostomum ambiguum	48h	0.17	Madoni *et al*. 1994
Brachionus plicatilis (Rotifer)	24h	39	Snell & Moffat 1991
Hydra vulgaris (Hydra bioassay)	24h	0.89	Beach & Pascoe 1998
Hydra vulgaris	48h	0.45	Beach & Pascoe 1998
Hydra vulgaris	72h	0.23	Beach & Pascoe 1998
Hydra vulgaris	96h	0.12	Beach & Pascoe 1998
# *Daphnia magna* (Cladocera)	48h	0.23	Barata *et al*. 1998
# *Mysidopsis bahia* (Mysidacea)	96h (juvenile)	0.02	Cripe 1994
#*Neomysis interger* (Mysidacea)	96h (juvenile)	0.004	Wildgust & Jones 1998
# *Penaeus duorarum (pink shrimp)*	96h (post larvae)	0.51	Cripe 1994
# *Hyale* sp. (amphipod)	24h	8.91	this study
# *Hyale* sp.	48h	4.47	this study
# *Hyale* sp.	72h	3.00	this study
# *Hyale* sp.	96h	1.38	this study

The LC_{50} values expressed as mg L^{-1}

crustaceans

Table 6.3 The 96h LC_{50} values of marine and freshwater amphipods to cadmium.

Amphipods	96h LC_{50}	Reference
Hyalella azteca *	0.014	ASTM 1993
Chaetocorophium c.f. lucasi *	0.061	Collyard 1994
Gammarus pseudolimnaeus *	0.056	Collyard 1994
Gammarus pulex *	0.02	Wright & Frain 1981a
Elasmopus rapax	0.18	Zanders & Rojas
Ampelisca abdita	0.33	ASTM 1992
Rhepoxynlus abronius	0.92	Swartz *et al*. 1985b
Leptocheirus plumulosus	1.06	ASTM 1992
Grandidierella japnnica	1.17	Hong & Reish 1987
Hyale sp. (adults)	1.38	this study
Eohaustorius estuarius	9.33	DeWitt *et al*. 1989

* freshwater species

mainly due to the salinity effect (Wright & Frain 1981a). Increasing in salinity tends to diminish toxic effects of cadmium by hydrophilic complexation of free cadmium ion with inorganic chloride ligand (Rainbow 1995b). The high concentration of calcium ion in seawater also exerts an antagonistic effect on cadmium toxicity (Wright & Frain 1981a; Bhat & Vamsee 1993). The free cadmium ion (0.097 nm) has a similar ionic radius to free calcium ion (0.099 nm) (Liptrot 1984). Other than ionic radius, ionic charge (+2) and coordination number (= 6) of the two ions are also similar. Thus calcium ion competes with cadmium ion for absorption into the body (Rainbow & Kwan 1995).

The sensitivity to cadmium is inversely correlated to the age of the animal (Bhat & Vamsee 1993). For example, the values of 96h LC_{50} of the adult and juvenile *Allorchestes compressa* to copper are 0.55 and 0.11 μg L^{-1}, respectively (Ahsanullah 1982). The juvenile *A. compressa* is 4.5 times more sensitive to copper than the adult. The 96h LC_{50} values of the adult and juvenile *Marinogammarus obtusatus* to cadmium are 13.3 and 3.5 μg L^{-1}, respectively (Wright & Frain 1981b). However, the sensitivities of adult and juvenile *Hyale* sp. to cadmium are generally similar (Section 6.3 and Table 6.1). The statistical analysis showed that there was no significant difference in the values of 24h, 48h, 72h and 96h LC_{50} between the adult and the juvenile stages of *Hyale* sp.

Other than LC_{50} value, the slope of the regression line also indicates the time-dependent toxic effect on amphipods to the cadmium sensitivity (Bhat & Vamsee 1993). The 24h LC_{50} values of the adult and juvenile *Hyale* sp. to cadmium are 8.91 and 8.80 mg L^{-1}, respectively. The two values are not significantly different from each other. However, the slope of the regression line is significantly greater in juveniles (3.89 ± 0.56) than in adults (2.00 ± 0.29) at 24h exposure (Section 6.3). The time-dependent toxic effect of the juvenile is significantly greater and nearly two times than that of the adult. It is shown that the juvenile is more susceptible and sensitive to higher concentrations of cadmium in the 24h exposure period than that of the adult. In the study of heavy metals toxicity to the amphipod *Parhalella natalensis*, the relative

toxicity of the metals, cadmium, copper and zinc was also based on the different slopes of the regression lines instead of the insignificantly different LC_{50} values (Bhat & Vamsee 1993).

Chapter 7 Conclusions

The conclusions of this study are listed as follows:

1. The amphipod species being studied in this project is referred as *Hyale* sp. The mean life span of males and females are 178 and 175 days, respectively. The maximum life span recorded is 262 days in males and 258 days in females. The developmental stages of the species are divided into succession of instars due to its discontinuous growth in nature. The mean number of instars throughout the life span of males and females are 14 (range, 12 to 19) and 17 (range, 11 to 21), respectively. No evidence of any terminal ecdysis or final instar is found in *Hyale* sp. Thus the species undergoes indeterminate growth process. The mean body length of the amphipod is significantly longer in males than in females. The body length increment stabilizes in the range from 9 to 10 mm in males and 8 to 10 mm in females after instar 14. The maximum body length may reach 11.38 mm in a female. The other body parameters also effectively indicate growth of the species. The relationship between dry body weight and body length is positively allometric in ovigerous females, but isometric in males and non-ovigerous females.

2. The following characters: body length, length of antennae 1 & 2, number of articles on antennae 1 & 2, propodus length of gnathopods 1 & 2, and merus length of pereopod 7 show distinct sexual dimorphism. Among all morphological structures measured, only eye length is not significantly differentiated between the two sexes.

3. Two growth phases are differentiated in the allometric growth of the sexually dimorphic characters. The allometric growth patterns of eye length, length of antennae 1 & 2, and propodus length of gnathopods 1 & 2 to body length effectively identify the sexually immature and mature phases in the development of the amphipod. The transition from sexually immature phase to sexually mature phase generally occurs as the body length reaches the range of 3 to 5 mm (equivalent to instar 4 to 6 in males, and instar 4 to 8 in females)

4. Salinity exerts significant effects on the growth of the juvenile *Hyale* sp. Juveniles exhibits the highest growth rate in 20‰ salinity. The relative growth rate of juveniles reared in different salinities is 20 > 30 > 40 > 10‰.

5. Sexual maturation starts to occur at the range from instar 5 to 7 (age, 38 to 53 days) in males and instar 5 to 8 (age, 34 to 44 days) in females. The mean brood size is 13 (range, 3 to 33). The total number of offspring generated throughout the life span of females varies from 16 to 290 juveniles in a total of 2 to 17 broods (mean, 6 broods). The fecundity is dependent upon the life span of females. The mean incubation period and recuperative period of *Hyale* sp. are 7 days (range, 4 –11 days) and 3 days (range, 0 – 18 days), respectively. A positively linear correlation is observed between the brood size and the body length of females.

6. Adults and juveniles of *Hyale* sp. can tolerate the temperature range between 6°C and 30°C. Within the range, more than 90% of the amphipod can survive for 4 days. The amphipod is unable to survive when the temperature reaches below 0°C and above 34°C. The values of low range 96h median lethal temperatures (96h $LTemp_{50}$) in adults and juveniles are 3.2°C (2.6 to 3.6°C) and 4.2°C (3.9 to 4.3°C), respectively. The values of high range 96h median lethal temperatures in adults and juveniles are 31.7°C (31.1 to 32.3°C) and 31.5°C (29.7 to 32.5°C), respectively.

7. *Hyale* sp. is tolerant to salinities between 10 and 30‰. Within the range, more than 90% of the amphipod can survive for 4 days exposure. No amphipod can survive in salinities below 2‰ and above 62‰. The values of low range 96h median lethal salinity (96h LS_{50}) in adults and juveniles are 2.4‰ (1.7 to 2.9‰) and 1.6‰ (0.9 to 2.2), respectively. The values of high range 96h median lethal salinity in adults and juveniles are 55.5‰ (50.9 to 58.1‰) and 56.9‰ (42.1 to 62.4‰), respectively. There is no significant difference between adults and juveniles in tolerance to all range of salinity and high range of temperature. Yet the adult has a significantly higher tolerance to low range of temperature than the juvenile.

8. The 24h, 48h, 72h and 96h LC_{50} values of the adult *Hyale* sp. to cadmium are 8.91, 4.47, 3.00 and 1.38 mg L^{-1}, respectively. The 24h, 48h, 72h and 96h LC_{50} values of juveniles to cadmium are 8.80, 5.83, 2.12 and 0.96 mg L^{-1}, respectively. The LC_{50} values of adults and juveniles to cadmium are not significantly different from each other, except for 24h acute toxicity test. In 24h test, the time-dependent toxicity to cadmium is significantly greater in juveniles than in adults. This indicates that the juvenile *Hyale* sp. is more sensitive to higher dose of cadmium than the adult in 24h exposure. The sensitivity of *Hyale* sp. to cadmium is similar to those of amphipods commonly employed for environmental bioassays.

9. *Hyale* sp. is a suitable amphipod to be used in environmental bioassays. The information on its general biology, tolerance to environmental stress and sensitivity to cadmium is established in this study. Based on the information, this amphipod species is potentially developed into a heavy metal biomonitor and biological monitor in local environmental bioassays.

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