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POPULATION DISTRIBUTION AND SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY OF TWO SPECIES OF SHORE CRAB *HEMIGRAPSUS NUDUS* AND *HEMIGRAPSUS OREGONENSIS* AT BAMFIELD, VANCOUVER ISLAND, BRITISH COLUMBIA, CANADA.

BY

HAILIN WAN

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SPRING, 1990

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DEGREE: MASTER OF SCIENCE

YEAR THIS DEGREE GRANTED: SPRING 1990

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled POPULATION DISTRIBUTION AND SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY OF TWO SPECIES OF SHORE CRAB *HEMIGRAPSUS NUDUS* AND *HEMIGRAPSUS OREGONENSIS* AT BAMFIELD, VANCOUVER ISLAND, BRITISH COLUMBIA, CANADA, submitted by Hailin Wan in partial fulfillment of the requirements for the degree of Master of Science.

supervisor

D A Craig

A. N. Spencer

Date: Jan 23, 1990

ABSTRACT

The reproduction and population distribution of the shore crabs *Hemigrapsus nudus* and *Hemigrapsus oregonensis* were studied at Bamfield, Vancouver Island, British Columbia, Canada. Eggs of *H. nudus* developed from October to December. Gravid *H. nudus* females were found from January to May. Larvae hatched in April and May, and megalopae recruited in June and July. Eggs of *H. oregonensis* started to develop in October. Gravid females were found from February to September. The hatching occurred from May to September, with two peaks: one in May and June, and a second in September. This study, compared with the previous studies on more southerly locations, indicates that the seasons of brooding and hatching are related to the latitude of the population localities (the lower the latitude, the earlier the initiation of reproductive events).

H. nudus prefers gravel and exposed shores, while *H. oregonensis* prefers muddy and quiet bays. When the two species are found in the same habitat, *H. nudus* lives higher in the intertidal than *H. oregonensis* . Results of laboratory and field experiments indicate that *H. nudus* could live under drier conditions than *H. oregonensis*, and, behaviorally, *H. oregonensis* is able to make burrows in muddy areas, whereas *H. nudus* tends to hide under gravel. *H. oregonensis* is shown to have greater tolerance of estuarine conditions.

ACKNOWLEDGEMENTS

I would like to express my thanks to every member of my supervisory Committee, Dr. H. F. Clifford, Dr. F. S. Chia and Dr. A. N. Spencer for their suggestions, recommendations, encouragement and criticism. To my supervisor Dr. Chia, I would like to thank him for giving me the opportunity to conduct this research as a graduate student and those helpful discussions and communications. To Dr. Spencer, I am grateful for his sincere support throughout this study. To Dr. Clifford, I would like to thank him for his advice on research and his patience in making this a better thesis in its final stage. I also appreciate the critical reviews and constructive suggestions of Dr. N. E. Stacey and Dr. D. A. Craig as my examination committee members.

I would also like to thank Dr. J. McInerney, director of the Bamfield Marine Station and its staff members for providing research facilities during the one and half year I stayed there. I feel indebted to many Bamfielders for their kindness in helping me when the engine broke-down on my boat or allowing me to dock the boat at their personal docks.

Additional thanks go to G. Jensen, A. Martel, T. Rawlings, L. Green, N. McFadden and J. Przysiezniak for their help and companionship in sampling on those isolated islands in rain or fog and plankton towing on the rough seas at night. I thank specially A. Dittel of Delaware University for the rotifer samples she sent to me a number of times, although they all died by the time of arrival. Finally, the help and criticism of R. Koss, J. Buckland-Nicks and L. McEdward are greatly appreciated.

This research has been supported by National Science and Engineering Research Council of Canada grants to Dr. F. S. Chia, and by Chinese State Commission of Education Scholarship, Western Canadian Universities Marine Biological Society Scholarship and research and teaching assistantships of the Department of Zoology, University of Alberta to myself.

I reserve my deepest and personal appreciation to my wife, Yanling, my parents and my brother for their continuous encouragement, understanding and support through numerous letters and phone calls.

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CHAPTER 1: GENERAL INTRODUCTION

A: LITERATURE REVIEW

Hemigrapsus nudus (Dana) and *H. oregonensis* (Dana) belong to the family Grapsidae. The genus *Hemigrapsus* has 11 species, all distributed along the Pacific rim. *H. nudus* and *H. oregonensis* are the only two species found on the Pacific coast of North America, ranging from Alaska to the Gulf of California (Rathbun, 1918; Hart, 1982). A number of studies on these two species are found in the literature: Hart (1935) on larval development; Jacoby (1981) and Lindberg (1980) on the adult behaviour; and Dehnel (1958, 1960, 1962, 1966) on physiological responses under different physical environmental conditions; Boolootian et al. (1959), Batie (1982a) and Knudsen (1964) on reproductive cycles at California, Oregon coast and Puget Sound in Washington, respectively. Batie (1982b) also examined the population structure of *H. oregonensis* on the Oregon coast. Some of these studies and others (Morris et al., 1980; Hart, 1982; Kozloff, 1983 and Ricketts et al., 1985) pointed out that a trend of characteristic habitat preference by each species existed. Namely, *H. nudus* prefers exposed, gravel areas, while *H. oregonensis* prefers quiet, muddy habitats. However, no sampling has been done to support this conclusion. Low (1970), mainly based on laboratory experiments, concluded that *H. nudus*, because of its gill structure, could not tolerate a muddy substratum, and *H. oregonensis* tended to be eliminated from less muddy areas by predators.

There have been several studies on the population distribution of brachyuran crabs. Willason (1981) examined the crabs *Pachygrapsus crassipes* and *Hemigrapsus oregonensis* and found that the main factors determining their distribution patterns were sediment and salinity. Seiple (1979) extensively studied *Sesarma sinereum* and *S. reticulatum* and also concluded that salinity and sediment were probably the major factors in determining their distributions. Jensen (1989) found that megalopa larvae of the porcelain crabs *Petrolisthes cinctipes* and *P. eriomerus* exhibit differential settlement, and this might be one of the factors affecting their vertical distribution patterns.

B: OBJECTIVES OF THE THESIS

Both crab species are important members in the intertidal community of the Pacific shores of North America. They are consumers of encrusting diatoms, seaweed (Knudsen, 1964), snail egg capsules (Rawlings, pers. comm), barnacles and juvenile mussels (pers. obs). They are also the prey of a number of fish species (Low, 1970). Although their reproductive cycles have been examined by Boolootian et al. (1959) and Knudsen (1964) on the California and Washington coasts, we have no knowledge about their reproductive biology on the coast of Vancouver Island. No research has been done to determine possible factors affecting the distribution patterns of these two shore crab species.

This study was designed to answer the following questions: (1) Is the reproductive cycle of these two species on the west coast of Vancouver Island different from that at more southerly locations? (2) Is there a significant difference in the patterns of distribution between these two species? (3) What are the biological and physical factors that might influence the distribution patterns of *Hemigrapsus*?

C: ORGANIZATION OF THE THESIS

This thesis is organized into two main chapters and two appendices. Chapter 2 examines the distribution pattern of the two crab species vertically and horizontally in the intertidal zone and their reproductive cycle (egg development, ova deposition, hatching, the appearance of free swimming larvae in the water column, and settlement). Chapter 3 examines the possible factors which may affect the distribution patterns through a number of laboratory and field experiments. Appendix I concerns the effect of feeding time after hatching on larval mortality and Appendix II studies differential mortality under laboratory culturing conditions.

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CHAPTER 2: POPULATION DISTRIBUTION AND SOME ASPECTS OF REPRODUCTIVE BIOLOGY

A: INTRODUCTION

Intertidal habitats are generally divided into low, middle and upper zones, and each of these zones is known to support a particular assemblage of intertidal organisms. According to Stephenson and Stephenson (1939, 1949, 1972), intertidal zonation is affected by moisture, light and sediment and it has little to do with the tides. Connell (1961, 1970 and 1972), through a series of field experiments, mainly on barnacles, concluded that the distribution of organisms at the upper limit is controlled by desiccation, temperature and solar radiation, while the lower limits are controlled by competition for space or by predation (reviewed by Carefoot, 1978). Different species have different adaptations, and also different environmental factors could promote different adaptive mechanisms in the assemblage of organisms.

Intertidal environments are influenced by the degree of submergence, temperature, light, moisture, salinity, wave exposure, and sediments. Various combinations of these factors might produce different kinds of habitats which are suitable for different organisms. The presence of one group of organisms might influence the distribution of another group through predation or competition or by modification of abiotic factors. Specialization for one kind of habitat usually means poor adaptation to other types of habitats (Barnes, 1982).

The eggs of crabs are fertilized internally and deposited on the outside of the abdomen. Development of the nauplius larva takes place in broods on the abdomen of the female and the larva is later released into the water column as a free swimming zoea larva. Altogether, five zoeal stages and one megalopal stage are completed in the plankton, the megalopa settles in the intertidal region and metamorphoses into a juvenile crab.

Hart (1935) studied the larval development of *H. nudus* and *H. oregonensis* in the laboratory; she also studied the morphology of zoeae and megalopae. The information from that study makes it possible to identify *Hemigrapsus* larvae in plankton.

Boolootian et al. (1959) studied the reproductive cycles of several crab species including *H. nudus* near Monterey Bay, California and found that the gravid period of *H. nudus* was from October to the following May. The percentage of gravid females in the population increased from October to January, to a maximum of 90-100 % of all the females. From January to May the percentage of gravid females declined continuously. By May, no gravid females could be found. This indicates that the larvae hatched from January to May. Boolootian et al. (1959) mentioned that at the time the larvae were hatching, the water temperature in Monterey Bay was at a maximum period of the year, and no plankton bloom occurred at this time, but shore kelp underwent degeneration. From these observations they concluded that the organic detritus from the degenerating kelp could be the food source for *H. nudus* larvae.

Knudsen (1964) studied the reproductive cycle of *H. nudus* and *H. oregonensis* in the southern Puget Sound area and tried to relate reproductive events to environmental factors. In addition, he studied some aspects of behaviour and mentioned that *H. nudus* preferred the upper intertidal zones, while *H. oregonensis* was most common lower on the shore. As the average particle size of the substrata decreased from boulders to fine sand or mud, *H. nudus* was replaced by *H. oregonensis.* Egg development of *H. nudus* began at the end of September and reached its maximum at the end of November. Egg deposition on female abdomen began in early January, and increased to 70 % of the females at the end of January; by May, 98.6 % of the females were gravid. Only 1 % was gravid by the end of June. For *H. oregonensis*, ova began to develop in October. Egg deposition started in mid February and by May, 90 % of the females were gravid. A second brood (70% of the females) appeared at the end of May and lasted until the beginning of September.

Batie (1982) examined the reproductive cycle of *H. oregonensis* in Yaquina Bay, Oregon. Although the major reproductive season was from February to May, he did find gravid females of *H. oregonensis* present throughout the year. Kuris (1978) reported that in Bodega Bay, California, *H. oregonensis* brood from November to February (first brood, 65 % of the females) and again from June to August (2nd brood, 43 % of the females). On the Oregon Coast, Batie (1982) found that the maximum gravid percentage occurred in March, with 32.8 % of the females being gravid. None of the above mentioned studies dealt with larval abundance in the plankton.

After spending larval stages in plankton, crab competent larvae are ready to settle. Orth et al. (1987) have shown that in Chesapeake Bay, the recruitment of the blue crab *Callinectes sapidus* is much higher in sea-grass meadows than on tidal marsh creeks, and they explained this as being due to either active selection of sea-grass by the megalopa larvae at settlement or to increased predation. According to Jamieson (1986), the annual variation of recruitment of the Dungeness crab *Cancer magister* was due to the interaction between density dependent factors and other environmental factors.

In this Chapter I report results of studies on the population distribution patterns of two crab species at three different study sites, their reproductive cycles from egg development through the egg deposition, larval hatching, and finally the recruitment of juveniles into the intertidal populations.

B: MATERIALS AND METHODS

Studies were conducted from May,1987 through August, 1988 at three sites within the Bamfield Inlet and Grappler Inlet close to Bamfield Marine Station, Vancouver Island, British Columbia ($48^0 05'$ N, $125^0 08'$ W) (Fig. 1A and 1B). Bamfield and Grappler Inlets are two narrow tidal channels 2000-3000 m long. The tides are semi-diurnal with a range of -0.6 to 4 meter (0 meter being the mean low lower tidal level.). The three study sites were Bamfield Station, Wiseman Bay and Grappler Inlet. The Station site was an exposed gravel area; the Grappler site was a sheltered and muddy area, and the Wiseman Bay site was intermediate with respect to sediment nature and the degree of exposure. The slopes of the three sites decreased in the following order: Station, Wiseman Bay and Grappler.

1. POPULATION SAMPLING :

The population structure and population distribution of both crab species were studied from May, 1987 to August, 1988 at the three sites by using a 0.5 by 0.5 meter square quadrat every month. Three horizontal transect lines were laid at each site at approximately 0.6, 2 and 3.4 meter of the tidal levels. At each low tidal period in the middle of the month, three quadrats (0.25 m^2) were sampled at each transect line at each site, with a total of nine quadrats at each site. The positions of quadrats on each transect line were determined in such a way that they divided the horizontal length of the site into four parts with equal distance. The sediment within the quadrat was removed; however, the amount of sediment removed from each quadrat depended on the nature of the substrate. Every attempt was made to collect every *Hemigrapsus* in each quadrat. However, this was not possible at the Grappler site, as some crabs live in burrows and to avoid destruction of the habitat, I did not dig them out. At the Grappler site, extra random collections were done along the transect line to increase the sample size to 100 animals in each collection at this site. The carapace width of the crabs was measured with a vernier caliper accurate to the nearest 0.1 mm. Sex was recorded and the reproductive condition was noted; the deposits were then returned to the original site, and the crabs were released back into the area where they were collected.

2: EGG DEVELOPMENT

Starting in September 1987, 10 female *H. nudus* were collected in Bamfield Inlet and 10 female *H. oregonensis* were collected from Grappler Inlet each month until gravid females of that species appeared in the monthly samplings of natural populations. The female crabs were dissected and five eggs from each individual were measured to the nearest 20 µm for egg diameter, using an eye-piece calibrated ocular micrometer on a dissecting microscope. The mean diameters of the eggs from 10 female crabs were used to represent the egg development of the species for a given month.

3: GRAVID PERCENTAGE AND HATCHING.

During each month, the percentage of gravid females was used as an indicator of the gravid percentage. From the analysis of the gravid-rates throughout the year, hatching could be detected. A sudden and rapid drop in the gravid-rate indicated a peak period of hatching.

4: PLANKTON SAMPLING

From June to October in 1987 and from March to August in 1988, plankton samples (560 µm mesh size, 0.5 m net mouth diameter standard net) were collected at bimonthly intervals at three sites (near the three intertidal sampling sites). Samples were taken during the mid-tide period of the tidal cycle of the day using a 30 horsepower glassfiber boat with the speed kept to a minimum in a direction parallel to the shore and at a distance of about 15 -20 meters from the shore for 5 minutes. The total volume of the water sieved through the plankton net at each tow was recorded with a digital flowmeter counter and then converted into volume. The larval abundance was then standardized based on 50 cubic meters of water. The samples were taken to the laboratory immediately and examined under a dissecting microscope. Zoeae and megalopae of both crab species at each site were identified and their number recorded following the methods of Hart (1935, 1971), Lough (1975) and Rice (1980).

5: MEGALOPAE COLLECTION WITH OYSTER BAGS

This experiment was conducted from mid July to early August 1988 during a two week tidal cycle. Oyster bags approximately 30 cm long, 20 cm diameter with mesh size of about 2cm X 1cm were used (method from Jensen. per. comm.). Each bag was filled with clean oyster shells. Altogether 27 oyster bags were placed at three experimental sites, nine bags per site , three bags per intertidal level (low, mid and high) at each site. Two weeks later, the bags were brought to the laboratory, and examined under a dissecting microscope. *H. nudus* and *H. oregonensis* megalopae and juveniles were identified and recorded.

C: RESULTS

1: RELATIVE POPULATION PERCENTAGE AT THE THREE SITES

The distribution patterns represented by the relative percentage of the two species at each site are summarized in Fig. 2. These data were obtained from the population sampling. The relative percentages of each species were calculated based on the total number of both species collected for all months at each site. A one-way ANOVA analysis indicated that the Station site had the highest percentage of *H. nudus.* The lowest percentage of *H. nudus* was found at the Grappler site. The differences are statistically significant (Table 1).

2: DENSITY DISTRIBUTION.

By plotting tidal height against the mean number of crabs of each species in a quadrat at that height, vertical distribution patterns were obtained. (Fig. 3). At both the Station and the Wiseman Bay site, a one way ANOVA (Table 2) indicated a significant difference in population density at each tidal level between the two species. At low intertidal level of the Station site, *H. oregonensis* had a significantly higher density than did *H. nudus*; the density of *H oregonensis* became significantly lower at the mid and high intertidal levels at the same site. At Wiseman Bay, the density of *H. oregonensis* was significantly higher than that of *H. nudus* at low and mid intertidal areas, but in the high intertidal area the opposite was found. It is clear that the density of *H. oregonensis* decreased from low to high, while the density of *H. nudus* increased from low to high intertidal levels.

Figs. 4 and 5 present the change of density at different times of the year for *H. nudus* and *H. oregonensis* respectively. From Fig. 4 it is shown that the density of *H. nudus* on high intertidal level at both the Station and Wiseman Bay sites dropped significantly during the winter months. The change of density for *H. oregonensis* did not appear to be significant.

3: SIZE FREQUENCY DISTRIBUTION.

All the data collected at the three study sites were pooled and the carapace widths were plotted against the number of crabs collected during each month. By doing this, the size frequency distributions for *H nudus* (Fig. 6A) and *H . oregonensis* (Fig. 6B) were obtained. Fig 6A indicated that June and July were the peak recruitment period for *H. nudus* in both 1987 and 1988. This matched with the larval hatching season. The larvae hatched in April and May, and spent 45-60 days (Appendix II) in the plankton before settling in the intertidal region. For *H. oregonensis* (Fig

6B), the recruitment peak was not obvious, this is perhaps due to the longer hatching period of this species, which scattered the recruitment.

4. SIZE FREQUENCY DISTRIBUTION OF GRAVID *HEMIGRAPSUS* COLLECTED IN 16 MONTHS SAMPLINGS

Fig. 7 shows the size frequency distribution of gravid female *Hemigrapsus* collected in all the 16 months samplings. The minimum gravid female *H. nudus* was 9 mm, the maximum was 22 mm; The minimum gravid female *H. oregonensis* was 6 mm, but since there was only one gravid female found in this category, 7 mm was chosen as the minimum size for gravid *H. oregonensis.* The maximum was 19 mm.

5. EGG DEVELOPMENT, GRAVID PERCENTAGE OF MATURE FEMALES AND HATCHING:

The data pertaining to egg development and larval hatching are shown in Fig. 8. For *H. nudus,* eggs became obvious and could be measured in October, at which time the eggs had an average diameter of 140 µm. The egg diameter increased to 320 µm by November and reached 390 µm in December. At the end of December, gravid females were found in the intertidal populations and therefore egg measurement was stopped at this time. The gravid percentage of the mature females (≥9 mm see Fig. 7) of the *H. nudus* population in January was 6.7 %; thereafter, the gravid percentage increased steadily until April, when the gravid percentage was 43.1 %. It then declined to 18.8 % in May. In June, no gravid females were found in the populations. This indicates that hatching occurred from April to June, and that larvae were released into the plankton during this period.

For *H. oregonensis,* egg development became apparent by October, at which time the average egg diameter was 135 µm; by November it had reached 143 µm. Size increased sharply to 200 µm in December and finally reached 263 µm in January. In February, the gravid percentage of mature females (≥7 mm see

Fig. 7) in the natural population was 1.5 % and this increased to 26.9 % in April. In 1987, the gravid percentage dropped from May (31.4 %) to September (0 %), In 1988, two peaks occurred. The gravid percentage dropped to 19.1 % in May, but rose again to 30.1 % in June, then decreased to 3.7 % in August.

6: PLANKTON TOWS:

Results of the plankton tows at the three sites were pooled to estimate larval abundance at different times of the year. Results are presented in Fig. 9. For *H. nudus,* no data were obtained in 1987. In 1988, larvae began to appear in the plankton at the beginning of April, and were most abundant at the beginning of May. Larvae were found in the plankton until July, 1988. An interesting feature of these data is that the advanced zoeal stages were scarce.

H. oregonensis had a more complex pattern. In June 1987, when the plankton tows started, there were numerous 1st zoeae stage larvae; they decreased steadily until August. But in early September, another peak appeared, and larval abundance then declined to 0 % in October. In 1988, larvae began to appear at the beginning of May, and reached a peak in early July.

7: MEGALOPAE COLLECTED WITH OYSTER BAGS:

The numbers of *Hemigrapsus* megalopae and juveniles collected with oyster bags are listed in Table 3. Because of the small sample size, no statistics could be performed to compare the abundance at different sites. However, there is a possibility of a preference for differential settling of the two species. Namely, more *H. nudus* megalopae and juveniles (combined) were found at the Station site than at the Wiseman Bay and Grappler Site. In contrast, more *H. oregonensis* megalopae and juveniles (combined) were found at the Grappler site than at the Station site. *H. nudus* appeared to be more abundant at higher tidal levels than *H. oregonensis.*

D: DISCUSSION

The three study sites were approximately 300-500 m apart. Even within such a short distance, significant differences in relative percentage of the two species were noted. This difference is more clear in more extreme situations. At Brady's Beach (Fig. 1 B), which was a more exposed site with gravel sediment, only *H. nudus* was found, whereas at the end of the Bamfield Inlet (Fig. 1B), which was a calm muddy bay, only *H. oregonensis* was found.

The vertical distribution pattern of both species was also different; the density of *H. nudus* increased from low to high intertidal levels while that of *H . oregonensis* decreased. The reasons for such a distribution pattern are not clear. It is noted that brown seaweed, *Fucus sp.,* which was believed to be one of the major food sources of both crabs, was abundant at mid and high intertidal levels at the Station and the Wiseman Bay sites, but very scarce at Grappler site where the green seaweed *Ulva sp.* was more abundant.

The density distribution patterns of *H. nudus* at the three tidal levels over a year's period (Fig. 4) indicates that at high intertidal level, *H. nudus* had a significant drop of density in winter, whereas *H. oregonensis* did not (Fig. 5).

When comparing the reproductive cycles of *H. nudus* along the Pacific coast of North America, an interesting trend can be detected. In California (Boolootian et al., 1959), *H. nudus* had a longer (October-May) gravid period than in Puget Sound (January-May) (Knudsen, 1964). On the Oregon coast, Lough (1975) was able to collect *H. nudus* larvae as early as February. The results of my study show that the reproductive cycle of *H. nudus* between the Barkley Sound populations and the Puget Sound populations has a very similar trend.

From the reproductive cycle of *H. nudus* it is estimated that the egg deposition on the abdomen of the gravid females lasts about three months before the eggs are hatched, although the time from deposition to hatching was not measured in this study. This conclusion is supported by the results in the studies by Boolootian et al. (1959) and Knudsen (1964), in which the start of the egg deposition is also three months earlier than the start of hatching. Although there are spectulations that hatching may have occured before the gravid percentage reaches its peak, my plankton sampling results do not support such an idea. Even if there is such an early hatching, it must be trivial. The plankton sampling result also support the conclusion of Knudsen (1964) that there is only one brood per year for *H. nudus.* The duration of egg deposition for *H. oregonensis* is estimated to be about two months. Knudsen (1964) reported two broods per year. My plankton samplings in 1987 (Fig. 9) showed this trend.

There could be a number of factors controlling the reproductive cycles of these two crab species. Boolootian et al. (1959) suspected that the hatching of *H. nudus* was correlated with kelp degeneration. Knudsen (1964) speculated that photoperiod might trigger gametogenesis in *H. nudus* and suggested temperature might be related to the reproductive cycle of *H. oregonensis.*

No megalopae were collected in my daytime surface plankton tows, but they were found from July to September 1988 in night tows using a neuston net. This indicates a possible vertical diurnal migration for larvae of both species.

It was found that the blue crabs, *Callinectus sapidus* (Cronin and Forward, 1982), and fiddler crabs (*Uca sp.*) (Lambert and Epifanio, 1982) have a long larval life; the larvae are released from the adult habitat (estuarine), enter the ocean and return to the estuary again to settle when they become competent. On the other hand, *Rithropanopeus harrisii* retain most of their larvae in the adult habitats (Sandifer, 1975) and the larval life is short. Larval duration of *H. nudus* and *H. oregonensis* is quite long. Hart

(1935) suggested that larvae of both species have a duration of about 1 month; Lough (1975) estimated the larval life of *H. oregonensis* to be 40-70 days; Batie (1983) believed it to be 8-13 weeks. My study (Appendix II) indicated the larval life was ca. 45-60 days. It is not surprising that variations in length of larval life exist since many factors, such as food abundance, temperature and salinity would influence larval durations. What is certain is that the larval life of *H nudus* and *H. oregonensis* is relatively long, and they would be expected to be transported offshore during larval development. If this is the case, a number of physical and biological factors would influence larval survival and distribution.

Epifanio et al. (1988) studied larval distribution of *Uca* along a transect line and found that larvae left the shore in early stages of development and returned to·settle when competent. This is likely the situation for *H. nudus* and *H. oregonensis*. Plankton tows in June 1988 (Fig. 9) collected all five zoeal stages, while the earlier tows collected only 1st and 2nd zoeae stages. The June plankton tows were performed just after a storm, when large waves were sweeping into Barkley Sound and its inlets. The waves might have transported the offshore larvae back to the sound.

Newly metamorphosed *H. nudus* and *H. oregonensis* juveniles have carapace widths of approximately 2 mm, although the smallest width listed in the size frequency distribution was 4 mm (representing crabs with carapace widths from 3.5 mm to 4.5 mm). The reason 4 mm was chosen as the smallest carapace width was because it was difficult to find *Hemigrapsus* crabs smaller than 4 mm with the unaided eye. Also sediment structure of the study site, light conditions and the sampling time of the day provented me from locating them. Very few *Hemigrapsus* juveniles were found during the normal population quadrat sampling without the aid of other methods, such as sieves, oyster bags etc. Laboratory culturing results showed that it took about 15-20 days to grow to a size of 3.5-4.0 mm from a carapace width of 2 mm in both species.

The results of megalopae sampling with oyster bags possibily indicate that the megalopae and juveniles of *H. nudus* are more likely to appear in the high intertidal zone where the sediment particle sizes are large, whereas *H. oregonensis* juvenile were more common lower down the shore in muddy habitats. However, the sample size was very small, and no statistical test could be performed to show whether significant differences existed. In addition, crab juveniles are mobile. Thus, the observed pattern might be the result of immigration and juvenile mortality.

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Table 1: Comparison of two crab species in relative percentages at three study sites.

source of variation	SS	DF	MS	FS	P
between sites	18985.8570	2	9492.929	169.5154	<0.001
within site	2464.0175	44	56.00040		

VARIANCE COMPONENTS

LEVEL	VARIANCE COMPONENTS	PERCENT
BETWEEN SITES	602.62993	91.4975
WITHIN SITE	56.00040	8.5026

By ANOVA. Data were arcsin square root transformed.

Table 2: Comparison of population densities of the two crab species at each tidal level at the Station and Wiseman sites (Based on 12 month collections from May of 1987 to April of 1988). Numbers in this Table indicate the mean number of crabs per quadrat±S. E. One way ANOVA test was applied.

site	tidal level	*H. nudus*	*H. oregonensis*	p
Station	low	0.8±0.3	6.6±0.9	<0.01
	mid	15.8±2.3	1.6±0.4	<0.01
	high	15.6±2.7	0±0	<0.01
Wiseman Bay	low	0.1±0	6.2±1.4	<0.01
	mid	1.2±0.6	7.3±1.1	<0.01
	high	6.8±1.0	3.6±0.8	<0.01

Table 3: Megalopae and juveniles collected with oyster bags at three sites. Data are the number of larvae and juveniles collected two weeks after the oyster bags were placed in the field (from mid July 1988 to early August 1988).

<u>Station site</u>	*H. nudus*		*H. oregonensis*	
	megalopae	juveniles	megalopae	juveniles
low intertidal	0	0	0	0
mid intertidal	0	2	0	1
high intertidal	2	1	0	0
<u>Wiseman site</u>				
	megalopae	juveniles	megalopae	juveniles
low intertidal	0	0	0	0
mid intertidal	0	0	1	0
high intertidal	2	0	0	0
<u>Grappler site</u>				
	megalopae	juveniles	megalopae	juveniles
low intertidal	0	0	0	0
mid intertidal	0	0	3	0
high intertidal	0	1	0	0
total	4	4	4	1

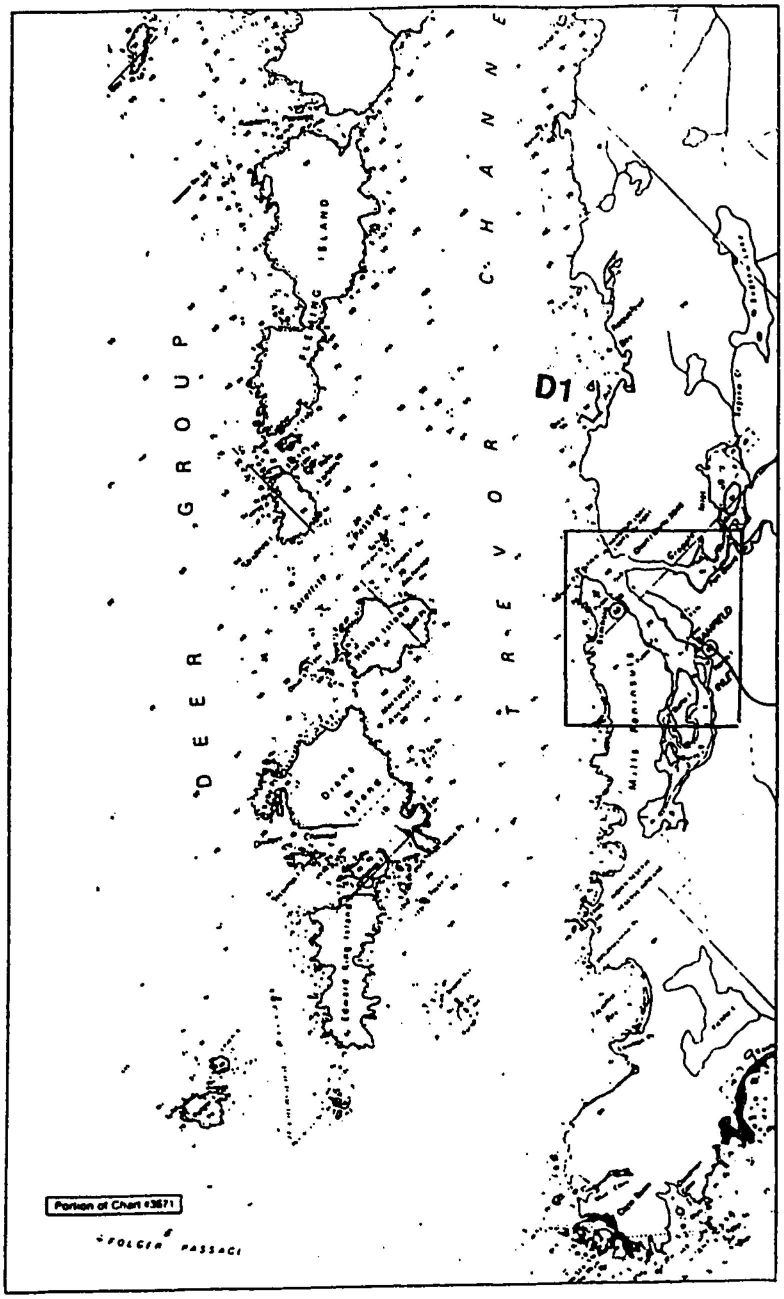


Fig. 1 A. Map of part of Barkley Sound, Vancouver Island, British Columbia. Only one temporary study sites (D1: Dixon Island) beyond the enclosed square (Enlarged in Fig 1 B) is indicated

Fig. 1 B. Photograph showing the study sites (S: Station site. W: Wiseman Bay site. G: Grappler site). It also shows one tempcrary site, Brady's Beach (B). This photograph comes from Bamfield Marine Station photo files.


G

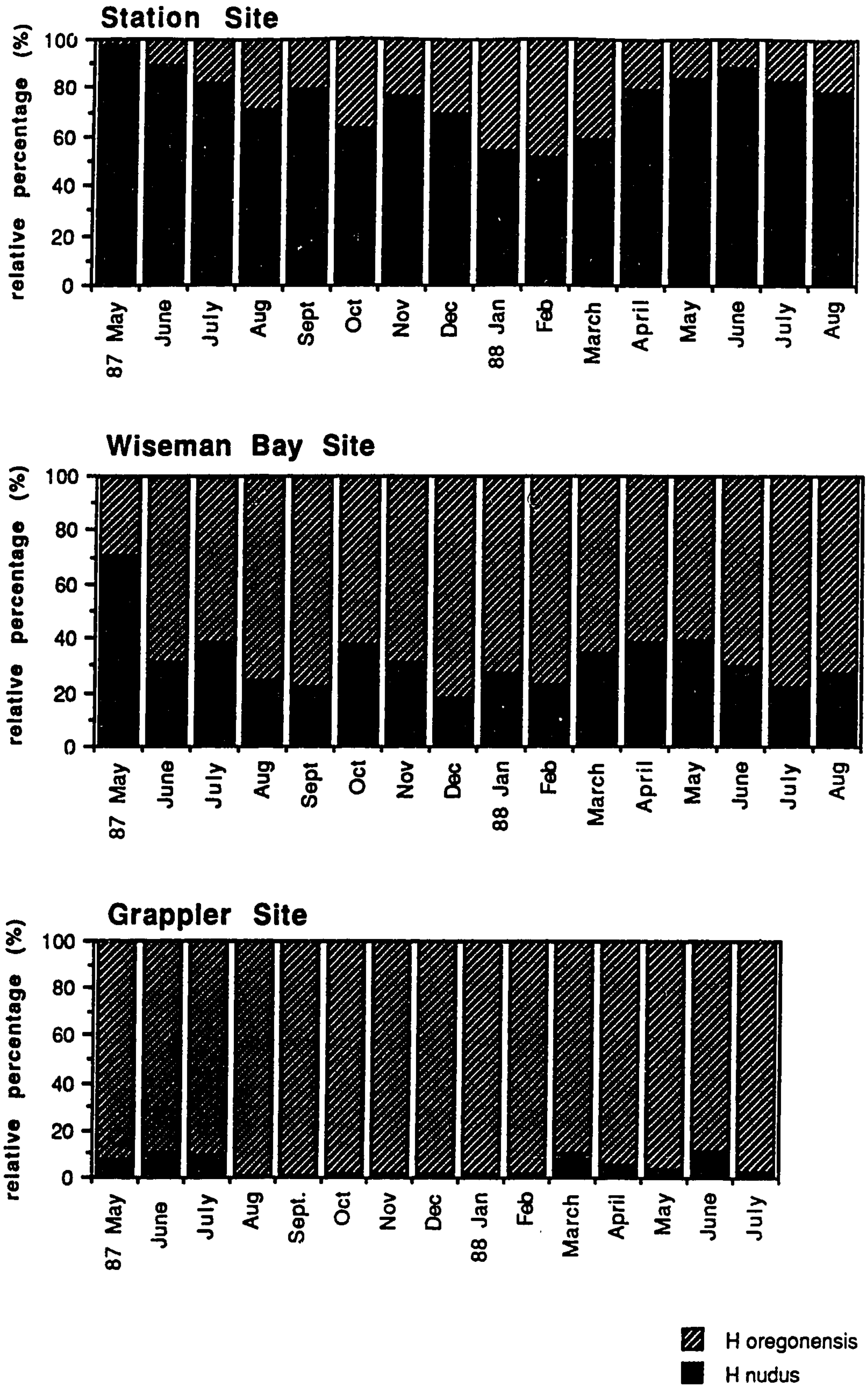


Fig. 2 The relative percentage of *H. nudus* and *H. oregonensis* found in sample quadrats based on monthly collections from May 1987 to August 1988.

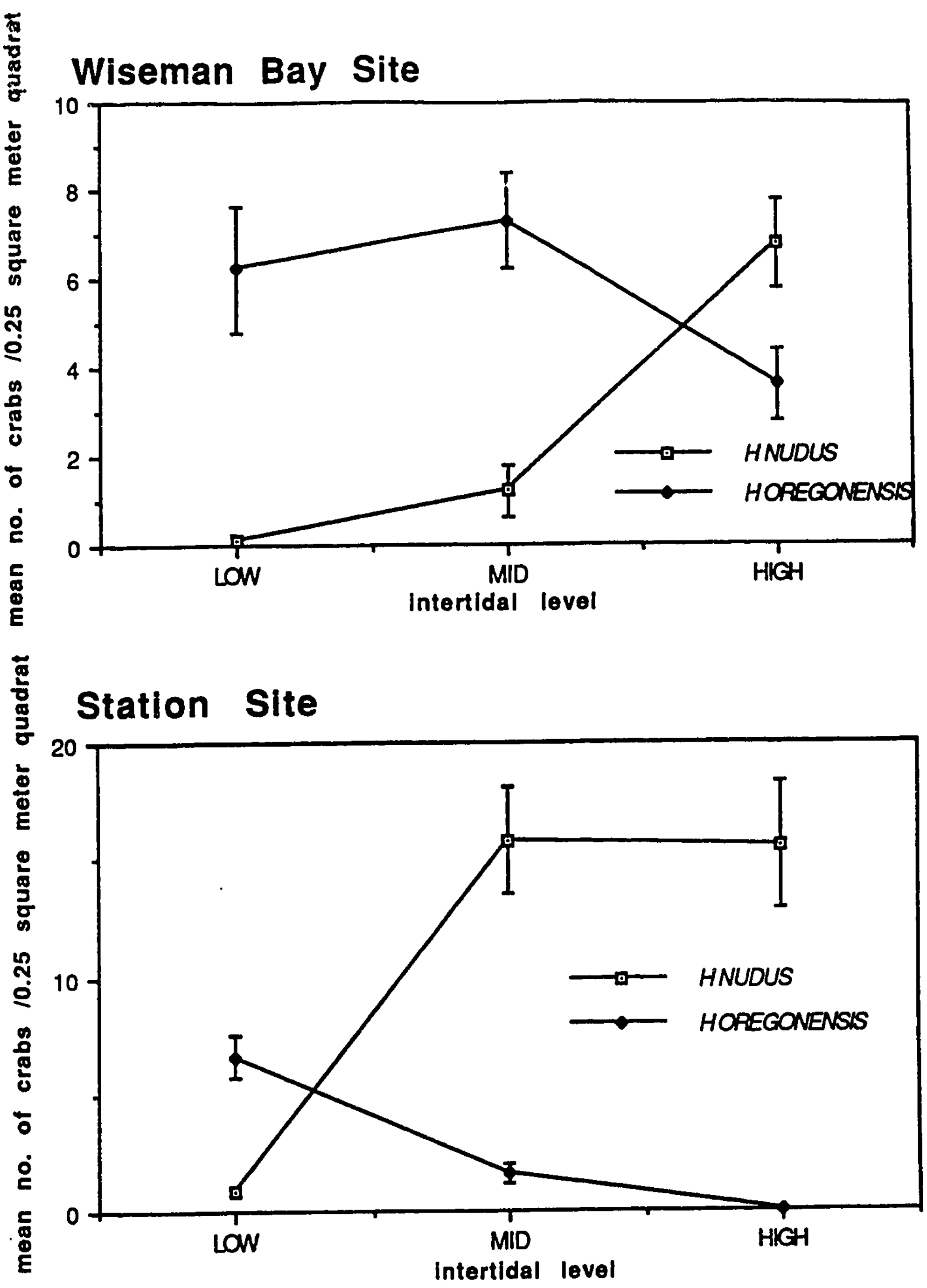
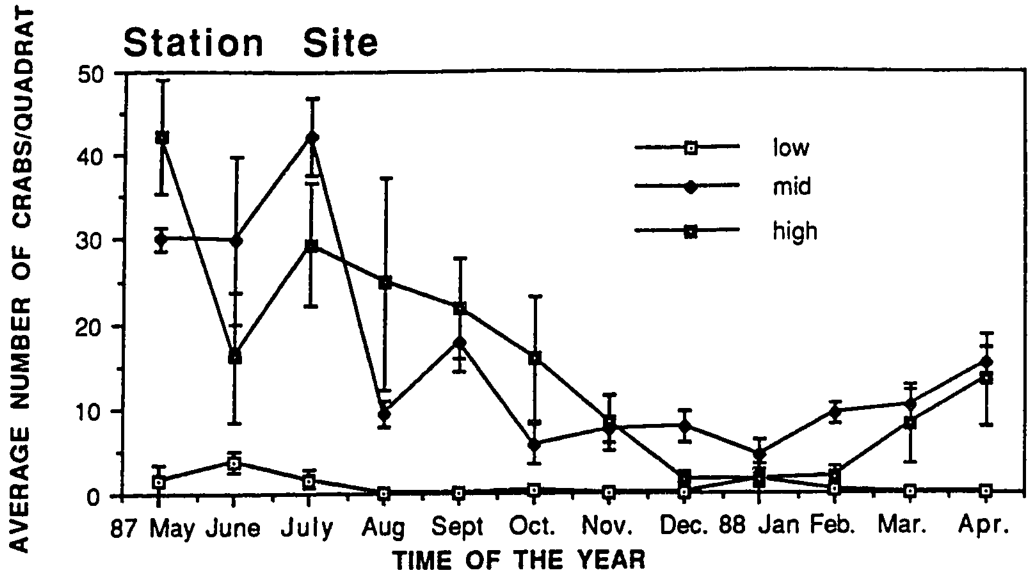


Fig. 3 The population densities of two crab species at low, mid and high intertidal levels (mean number of crabs per quadrat per month±S.E.). Three quadrats at each level. 12 month data were used.



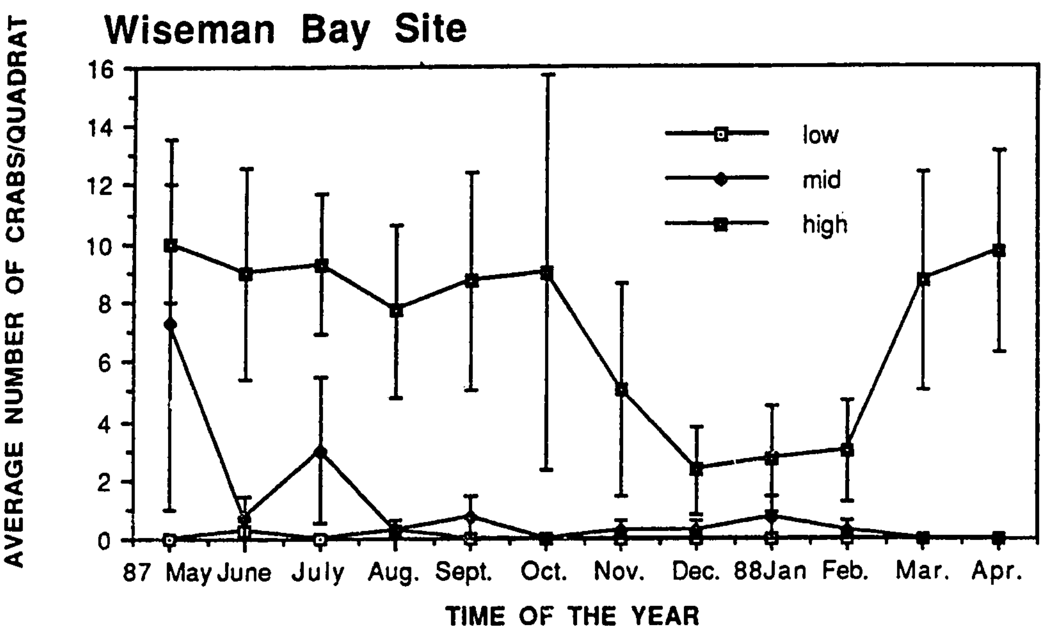
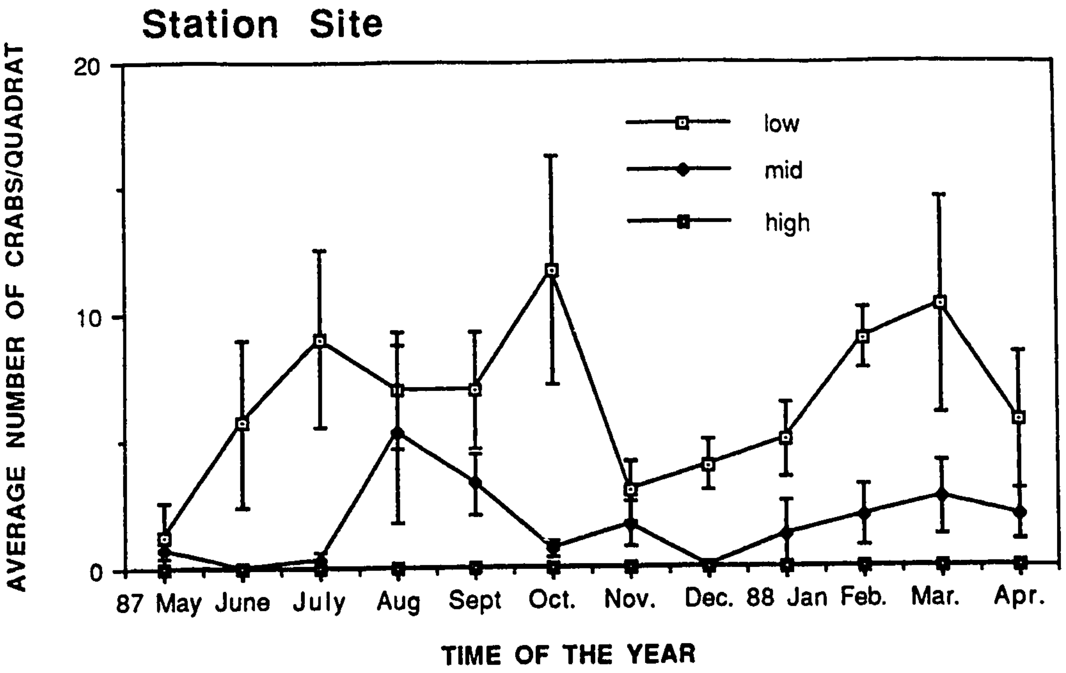
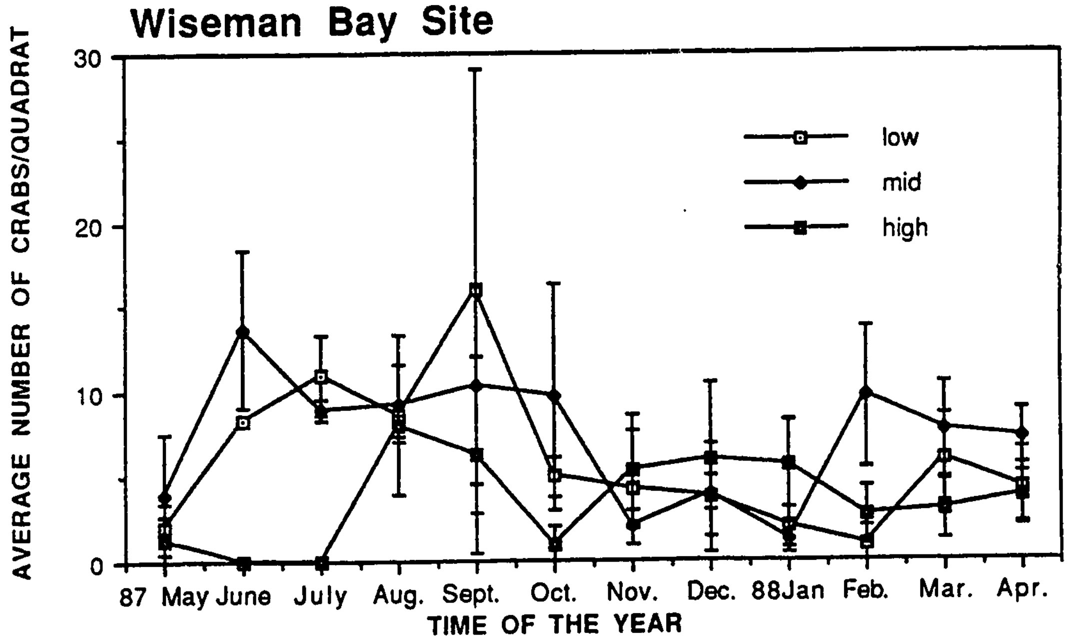


Fig. 4 Densities (mean number of crabs per quadrat±S.E.) of *H. nudus* over a one year period at low, mid and high intertidal levels. Three quadrats were sampled at each level per month.



Station Site



Wiseman Bay Site

Fig. 5 Densities (mean number of crabs per quadrat±S.E.) of *H. oregonensis* over a one year period at low, mid and high intertidal levels. Three quadrats were sampled at each level per month.

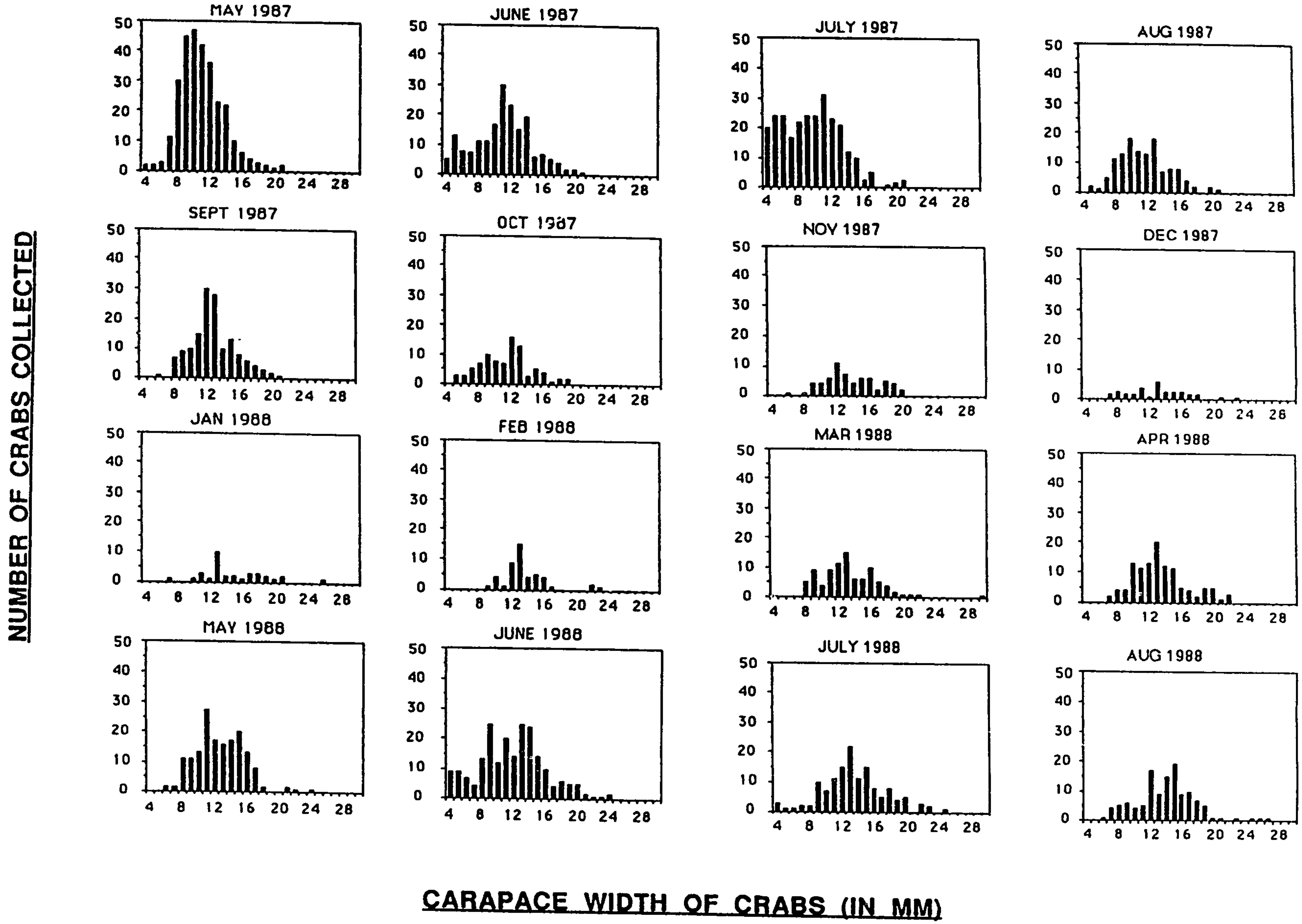


Fig. 6 A Size frequency distribution of *H. nudus* from May 1987 to August 1988 from the pooled data at three study sites (Station, Wiseman Bay and Grappler).

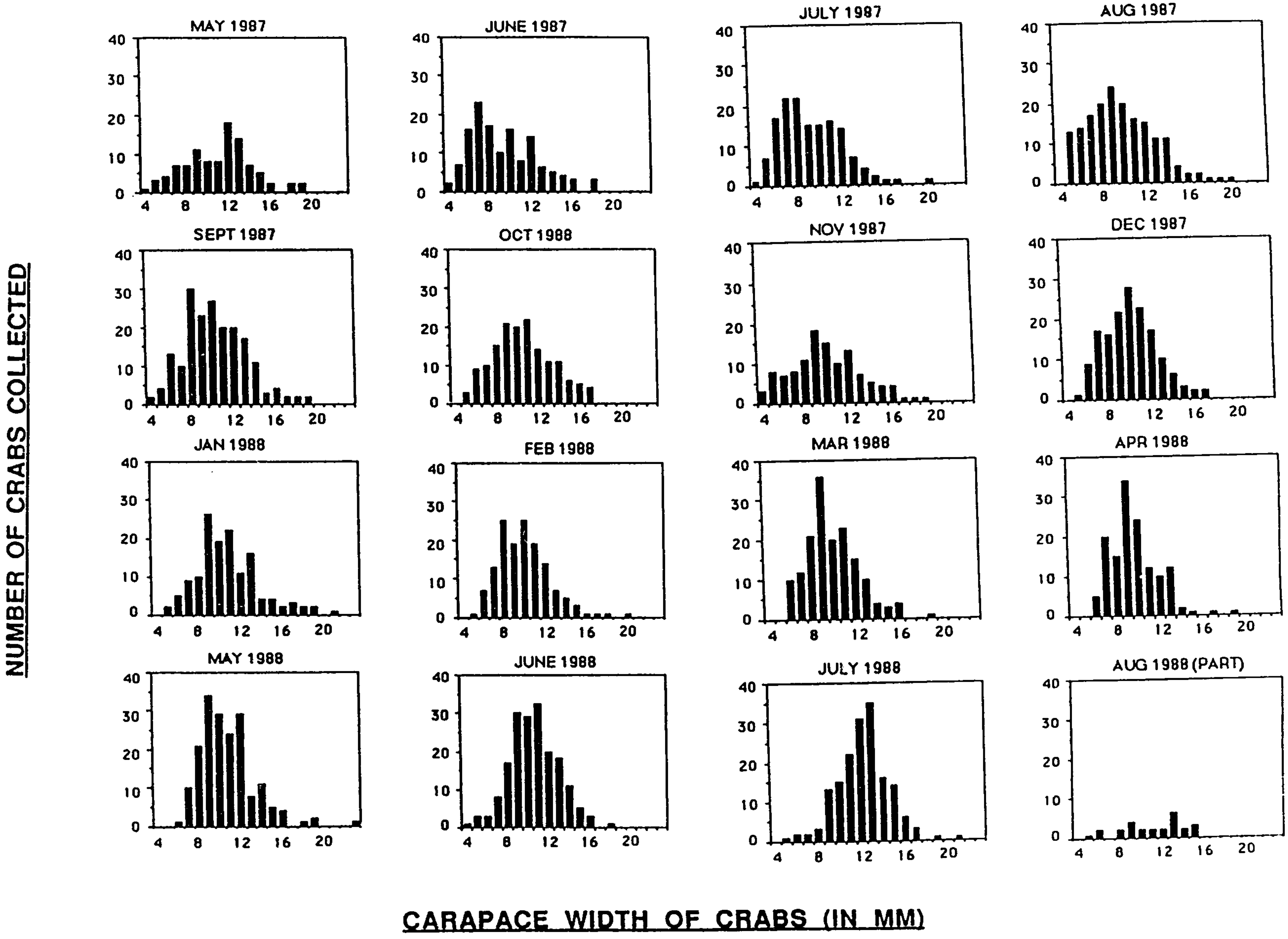


Fig. 6 B Size frequency distribution of *H. oregonensis* from May 1987 to August 1988 from the pooled data at three study sites (Station, Wiseman Bay and Grappler).

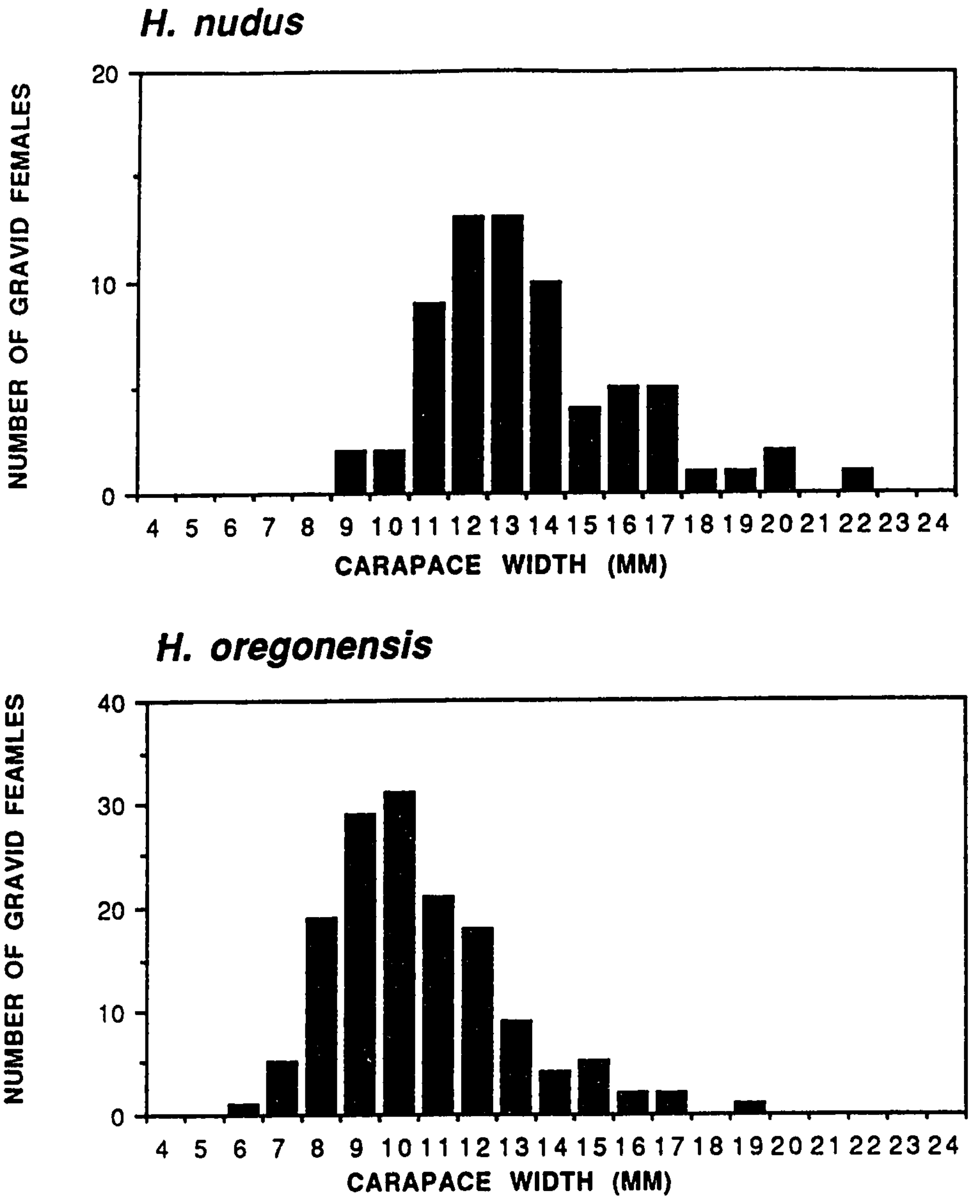


Fig. 7 Size frequency distribution of gravid *Hemigrapsus* collected in all the field samplings.

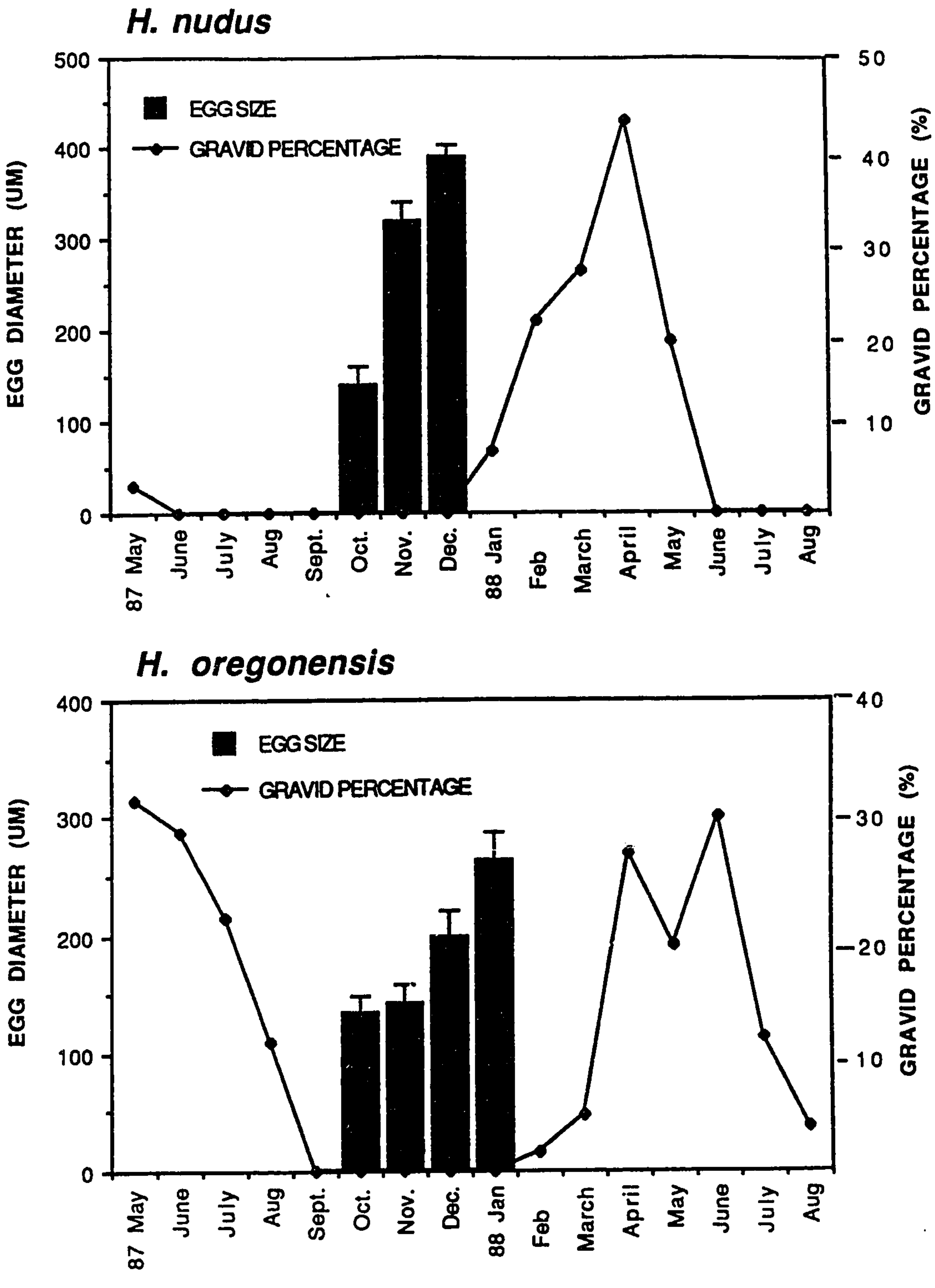


Fig. 8 Reproductive cycle of *Hemigrapsus* during the study period of 16 months. The lines represent the gravid rate of mature female crabs; bars represent the mean egg diameter±S.E.

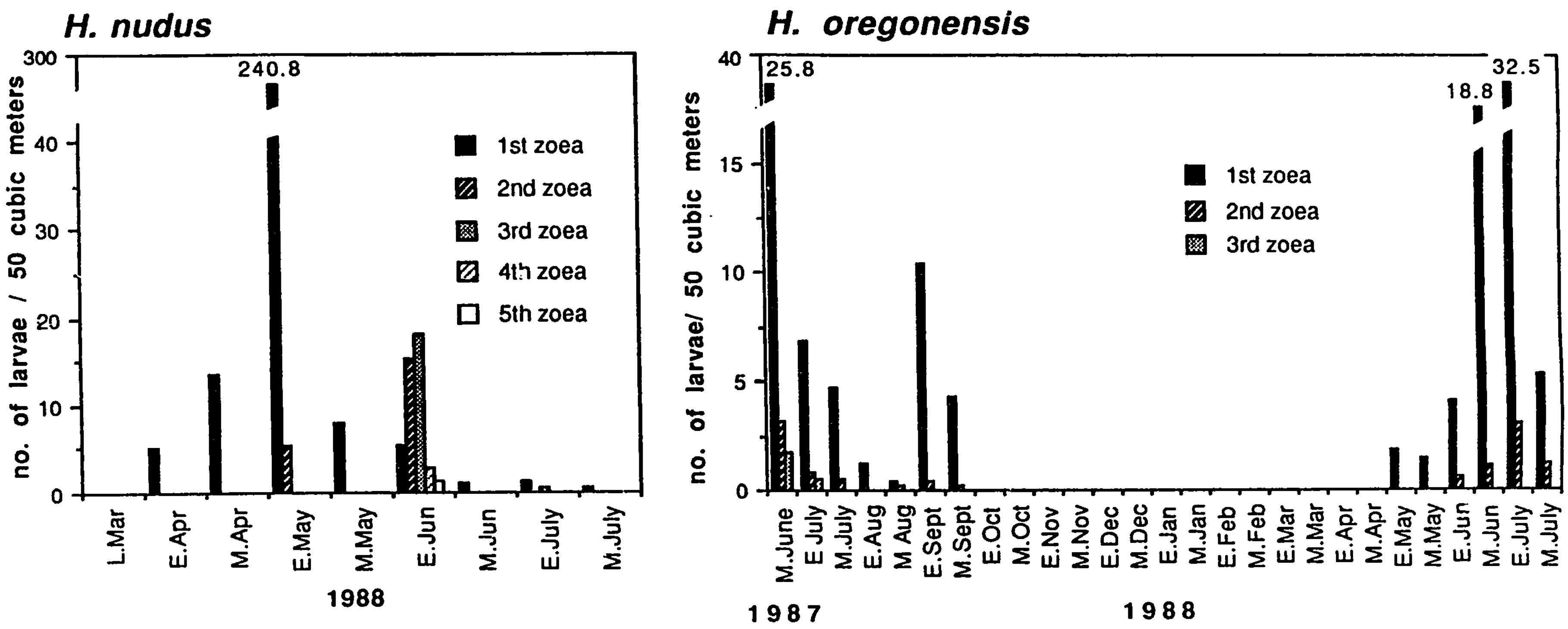


Fig. 9 **Larval density of each larval stage of *Hemigrapsus* in the plankton. (data pooled from three sites)**
E: early M: middle L: late

CHAPTER 3: POSSIBLE FACTORS AFFECTING THE DISTRIBUTION OF THE SHORE CRAB, *Hemigrapsus*

A: INTRODUCTION

It was shown In Chapter 2 that *H. nudus* and *H. oregonensis* have different distributional patterns. *H. nudus* is more abundant on exposed, gravel substrate, while *H. oregonensis* is more abundant on quiet, muddy habitats. When the two species coexist at one site, *H. nudus* lives higher on the shore than *H. oregonensis.* Willason (1981) reported that the burrowing behavior of *H. oregonensis* provided it with partial refuge from predation and *H. oregonensis* is more tolerant of low salinity. Low (1970) demonstrated that *H. nudus* could hide under the gravels and had a better chance of avoiding predation by fish than *H. oregonensis* when the two species were placed together in laboratory aquaria with a number of fish species. He also showed that in muddy areas, the gills of *H. oregonensis* are more resistant to silting, while the gills of *H. nudus* became clogged.

This chapter was designed to examine the effects of some biological and physical factors such as predation, competition, desiccation and salinity that may contribute to the establishment of the observed distribution patterns.

B: MATERIALS AND METHODS

Field experiments were mainly performed at the three long term study sites, as well as some temporary study sites (Fig 1 A and B). Specific locations are indicated in the descriptions of each individual experiment.

1. TETHERING EXPERIMENT

Tethering experiments were designed to determine whether there was differential predation on the two species at the same study site. In this experiment, the methods of Heck and Thoman (1981) were used. *Hemigrapsus* with carapace widths of 5-8 mm were collected and were tethered individually by placing a drop of Crazy Glue (Ross cyanoacrylate cement) on their carapace to stick one end of a 1 m long monofilament fishing line. One experiment (September, 1987) was conducted at Station site, and two experiments (July and September, 1987) were performed at the Wiseman Bay and Grappler sites. For each experiment, 12 -15 pairs of tethered *H. nudus* and *H. oregonensis* were placed in the intertidal areas, with 4 pairs at each level (approximately at 0.6, 2 and 3.4 m). Each pair, one *H. nudus* and *H. oregonensis* ,was tied to a nail by the unglued end of the monofilament fishing line. The nail was hammered tightly into the intertidal sediments. Inspection was made 24 hours later. Individuals that disappeared were assumed to be eaten, possibly by perch or rockfish, which are known predators of *Hemigrapsus* and are common in the study area, especially in summer. However, I also observed cannibalism among *Hemigrapsus,* and this could be another source of mortality.

2. DESICCATION EXPERIMENT

Resistance to desiccation by *Hemigrapsus* was studied in September, 1988 to determine whether *H. nudus* can survive longer than *H. oregonensis* in dry conditions. This may account for their ability to live in higher intertidal zones. Ten *H. nudus* and ten *H. oregonensis* of similar size in matching pair were placed individually in culture dishes. The culture dishes were then placed in an incubator at 10 ^{0}C (83% humidity). Ten degrees centigrade was the air temperature during the day when this experiment was conducted. The crabs were examined every hour and the dead ones were noted until all were dead. Mean number of hours that each species survived was calculated and compared by a paired T-test.

3. SALINITY EXPERIMENT

The salinity tolerance experiment was performed in October, 1988 by placing the crabs in culture dishes with seawater of three salinity levels: 31.5‰, 16‰ and 4‰. At each salinity level two replicates were used and in each replicate, 12 crabs, freshly collected from the field and ranging in size from 8-18 mm, were used. All culture dishes (20 cm in diameter, 10 cm in height, volume of seawater in each culture dish was ca. 3 liter.) were placed in an incubator at 10 ^{0}C. Crabs were checked every 12 hours, at which time the dead ones were removed, the water was changed and the number of surviving ones recorded. The experiment was terminated after 144 hours.

4. CAGE EXPERIMENT

On Sept. 24, 1988, 12 cages (2 mm iron mesh, 20cm X 20cm in area and 10 cm in height) were placed at the Wiseman Bay site; six cages were placed at the 2.6 m level, and 6 were at the 0.6 m level. Among the 6 cages at each level, two contained 20 *H nudus,* two contained 20 *H. oregonensis,* and two contained 10 of each species. The crabs were chosen in such a way that each cage had smaller crabs (8-12 mm), medium size crabs (12-16 mm) and large crabs (16-20 mm) and each cage also contained four fist-size stones. Cages were checked 2 days and 2 weeks later. The purpose of this experiment was to determine the survival of these two species under identical conditions. The cages were presumed to prevent predation, could be used to examine interspecific competitions when two species were put together, and the lower intertidal location could avoid desiccation.

5. REMOVAL EXPERIMENT

The removal experiment was conducted at Dixon Island in September 1987. Quadrats of 0.5 m X 0.5 m were marked with nails at four corners connected by white lines. Four treatments were performed. Before each treatment, the number of crabs in the sediments were counted. Altogether eight quadrats were used. In two quadrats, all crabs were removed, but the gravel was left; in two other quadrats, only the gravel was removed, and for another two quadrats, both the gravel and the crabs were removed. The last two quadrats served as controls. After twenty four hours, all quadrats were examined and changes were recorded. The purpose of this experiment was to find out the effect of gravel on existence of *Hemigrapsus.*

C: RESULTS

1. TETHERING EXPERIMENT

None of the tethering experiments at the three study sites showed a significant difference between the two species (Fig. 10) (t test: $P_a > 0.05$; $P_b > 0.05$. P_a for Wiseman Bay site, P_b for Grappler Site). In all cases, the rate of loss was very high. The high mortality could be due to the negative effects of tethering which prevented crabs from burrowing and hence increased the possibility of predation.

2. DESICCATION EXPERIMENT

The number of hours the two species survived in the dry dishes inside the incubator is given in Fig. 11. A paired T-test indicates that the number of hours *H. nudus* survived was significantly more than that of *H. oregonensis* ($P<0.05$). Death was determined by the change in color from purple or green to grey and lack of movement of crabs when touched with a finger. My previous experience indicated that crabs can survive more than 5 days of starvation in the laboratory.

3. SALINITY EXPERIMENT

Results of the salinity experiment are summarized in Table 4. At 31.5‰ salinity, there was no significant difference in mortality between the two species at the end of 144 hours (ANOVA $P>0.05$); at 16‰, there was no significant difference either (AVOVA $p>0.05$), but at 4‰, the mortality for *H. nudus* was significantly higher than that of *H. oregonensis.* ($p<0.05$). This is consistent with the observations of natural populations. *H. oregonensis* could live in estuarine areas and bays with freshwater runoff, but not *H. nudus.*

4. CAGE EXPERIMENT

Results of this experiment are given in Table 5. It was noted that two days after the beginning of the experiment, there was a high mortality of *H. oregonensis* in the high intertidal region in cages containing only *H. oregonensis* as well as in cages with both species. However, *H. nudus* at both levels, and *H. oregonensis* at the low intertidal zone did not suffer significant mortality.

Two weeks later, most crabs in the high intertidal cages had died, while *H. nudus* in natural habitat around the cages still existed. This is likely due to the fact that the cages could not hold any water, whereas the natural sediment could keep the bottom moist. Results from the cages at the low intertidal, where both species were coexisting, indicated that neither species was dominant at this density. In the cages containing only one species, there was moderate mortality, but there appeared to be no difference in mortality between the two species.

5. REMOVAL EXPERIMENT

Results of the removal experiment are given in Table 6. The results indicate that the distribution of the gravel has an important influence on the distribution of the crabs. When the gravel was removed, the crabs would relocate and find other places to hide; whereas the crabs under gravel tended to remain where they were if no obvious interference occurred. The intertidal distribution of gravel is related to the degree of exposure and the microtopography of the area.

D. DISCUSSION

In Chapter 2 It was suggested that differential settlement of megalope larvae might be an important factor in controlling the distribution patterns of adult populations. This notion was consistent with the findings of Shank (1983, 1985), who showed that the movement of megalopae in the water was controlled by physical oceanographic phenomena, but, in the final phase of settlement, the megalopae may be capable of choosing the most suitable substrate for settlement. One might then ask what is the inducer that attracts the megalopae larvae? Would it be gravel, mud, the presence of some specific organisms, or a chemical cue? When examining large scale recruitment patterns of the fiddler crabs (*Uca minax* and *Uca pugnax*), Epifanio et al. (1988) claimed that salinity was the major factor separating the megalopae of the two species, while the factors determining the microhabitat patterns were unknown. It should be kept in mind, when dealing with the settlement of crab larvae, that crabs are mobile. Moreover, some settled megalopae could resuspend and continue swimming. This means that even if they had settled in one place, they could choose another habitat at the next high tide.

After metamorphosis, juveniles and adults are continuously subject to predation and starvation. Heck and Wilson (1987) , and Heck and Thoman (1981) showed, using tethering experiments, that predation rates on crabs can differ geographically as well as temporally. Results of my tethering experiment did not show any difference in the predation rates between the two species.

As mentioned previously, at more protected sites, such as the end of Bamfield Inlet, only *H. oregonensis* could be found, while in exposed sites such as Brady's Beach, only *H. nudus* occurred. On September 24, 1987, approximately 450 *H. oregonensis* were collected from the end of Bamfield Inlet and transferred to Brady's Beach (Fig. 1 A and B) which was bounded on one side by large rocks, and on the other side by a long stretch of sandy beach. It was intended that the transferred population was to be checked monthly until it was decided to terminate the study. The result of the transferring experiment was surprising since all *Hemigrapsus oregonensis* had disappeared from the the site after 40 days. However, the *H. nudus* population appeared to be unaffected. The substrata at the site were basically fine sand covered with gravel, and the area was more exposed than any of the three permanent study sites. This indicated that the exposed shore (e.g. Brady's Beach) may not be suitable for *H. oregonensis.* This is perhaps due to the combined effects of coarseness of the sediments and strong wave action, which make it impossible for *H. oregonensis* to dig burrows. As Low (1970) described, *H. oregonensis* tended to roam, while *H. nudus* would hide under rocks when the two species were put in the same experimental tanks.

In Barkley Sound, starting in March and continuing until winter, the intertidal areas were covered with seaweeds and encrusting diatoms. These seaweeds and diatoms provide food for *Hemigrapsus.* Since Knudsen (1964) showed that no significant difference existed in the stomach contents of the two species, it is therefore concluded that food distribution is not a factor affecting the distribution of these two crab species.

The desiccation experiment showed a significant difference in the ability to survive when the two species were exposed to the air. Barkley Sound has a semi-diurnal tidal cycle. Organisms living in the high intertidal zones might be exposed to the air for about 10-12 hours. This could bring severe physiological stress to the organisms living at that level, especially at sites that have a steep slope and large particle sizes, which do not retain water. Since *H. nudus* can live longer than *H. oregonensis* under dry conditions, *H. nudus* can live higher in the intertidal area, especially at the Station site, which has a rather steep slope.

In Barkley Sound, heavy rainfalls in fall and winter dilute the intertidal burrows in muddy bays and pools and in gravel habitats. This rainfall could significantly change the salinity for a short period, especially in the muddy bays. At the same time, the sediment structure of small particle size would more easily hold the rainwater and keep a low salinity. This might explain why *H. oregonensis* is more resistant to low salinity than is *H. nudus.*

The drop of *H. nudus* density at high intertidal level during winter season reported in Chapter 2 may also be related with the low salinity at high intertidal level in winter caused by heavy rainfalls.

The results of the cage experiment imply that *H. oregonensis* are less resistant to desiccation in the high intertidal areas. This would restrict the distribution of *H. oregonensis* to lower levels. In low intertidal regions, *H. nudus* and *H. oregonensis* could coexist in the cage, whereas outside the cages only *H. oregonensis* survived. It should also be mentioned here that the cages at low intertidal also received high siltation. This might indicate that siltation is not an important factor in excluding *H. nudus* from inhabiting the low intertidal and muddy areas. The behavior of *H. nudus* , namely to hide under gravel, enables this species to exist higher in the intertidal zone.

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Table 4: Salinity tolerance experiment showing the survival of *Hemigrapsus* under different salinity conditions for a period of 144 hours. Each treatment was carried out in two replicates.

species	time	number of crabs(mean±S.D.)surviving at each salinity		
		31.5%o	16%o	4%o
H. nudus	12 h	12±0	12±0	12±0
	24 h	12±0	11.5±0.7	9.5±0.7
	36 h	12±0	11±0	8.5±0.7
	48 h	12±0	11±0	7.5±0.7
	60 h	12±0	11±0	6.5±0.7
	72 h	12±0	11±0	6±1.4
	84 h	12±0	11±0	5.5±0.7
	96 h	12±0	10.5±0.7	5±1.4
	108 h	12±0	9.5±0.7	5±1.4
	120 h	12±0	9.5±0.7	5±1.4
	132 h	12±0	9±0	4.5±2.1
	144 h	12±0	9±0	3.5±2.1
H. oregonensis	12 h	12±0	12±0	12±0
	24 h	12±0	12±0	11.5±0.7
	36 h	12±0	12±0	9±1.4
	48 h	12±0	12±0	9±1.4
	60 h	12±0	12±0	8.5±0.7
	72 h	12±0	12±0	8.5±0.7
	84 h	12±0	11.5±0.7	8.5±0.7
	96 h	12±0	11.5±0.7	8.5±0.7
	108 h	11.5±0.7	11.5±0.7	8.5±0.7
	120 h	11.5±0.7	11.5±0.7	8.5±0.7
	132 h	11±0	11.5±0.7	8.5±0.7
	144 h	10.5±0.7	11.5±0.7	8.5±0.7

Table 5: Field cage experiment in Wiseman Bay during fall 1988, showing the mortality as affected by tidal height and species interaction. Twenty crabs were placed in each cage at the beginning of the experiment. Central column includes cages with both species: number of *H. nudus* / number of *H. oregonensis*.

0 HOUR:

H. nudus	*H. nudus/H. oregonensis*	*H. oregonensis*
HIGH INTERTIDAL (2.6 m)		
20	10/10	20
20	10/10	20
LOW INTERTIDAL (0.6 m)		
20	10/10	20
20	10/10	20

2 DAYS:

H. nudus	*H. nudus/H. oregonensis*	*H. oregonensis*
HIGH INTERTIDAL (2.6 m)		
19	9/5	13
20	9/6	12
LOW INTERTIDAL (0.6 m)		
19	10/10	17
20	7/8	19

2 WEEKS:

H. nudus	*H. nudus/H. oregonensis*	*H. oregonensis*
HIGH INTERTIDAL (2.6 m)		
0	0/0	0
1	1/0	0
LOW INTERTIDAL (0.6 m)		
18	7/5	9
9	5/5	16

Table 6. Removal experiment at Dixon Island. Values are the number of crabs before and after the removal. The right column gives the percentage of crabs compared with the original 24 hours after removal.

TREATMENT	QUADRAT NO.	ORIGINAL NO. OF CRABS	FINAL NO. OF CRABS	% OF THE ORIGINAL
CRABS REMOVED	1	1 H.N.	1 H. N.	100%
		13 H.O.	4 H. O.	30.8%
	2	0 H.N.	1 H. N.	?
		16 H.O.	33 H.O.	206.4%
GRAVEL REMOVED	3	14 H.N.	6 H.N.	42.9%
		4 H.O.	1 H.O.	25%
	4	12 H.N.	8 H.N.	66.7%
		0 H.O.	0 H.O.	?
BOTH REMOVED	5	0 H.N.	0 H.N.	?
		6 H.O.	0 H.O.	0%
	6	5 H.N.	0 H.N.	0%
		22 H.O.	0 H.O.	0%
CONTROL	7	7 H.N.	6 H.N.	85.7%
		1 H.O.	1 H.O.	100%
	8	4 H.N.	6 H.N.	150%
		0 H.O.	0 H.O	?

When the original number of crabs was "0", the percentage is left as "?"

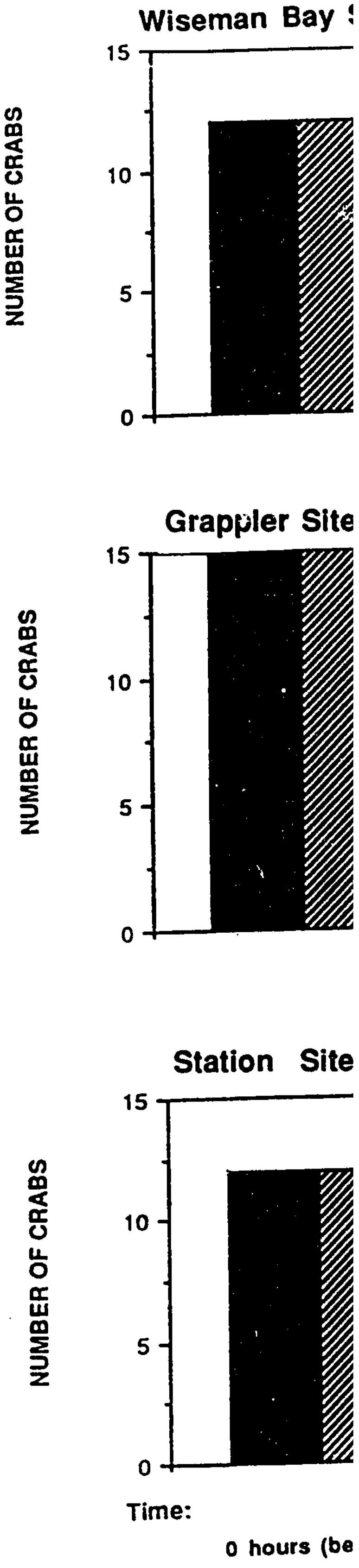

Fig. 10 Field tetl
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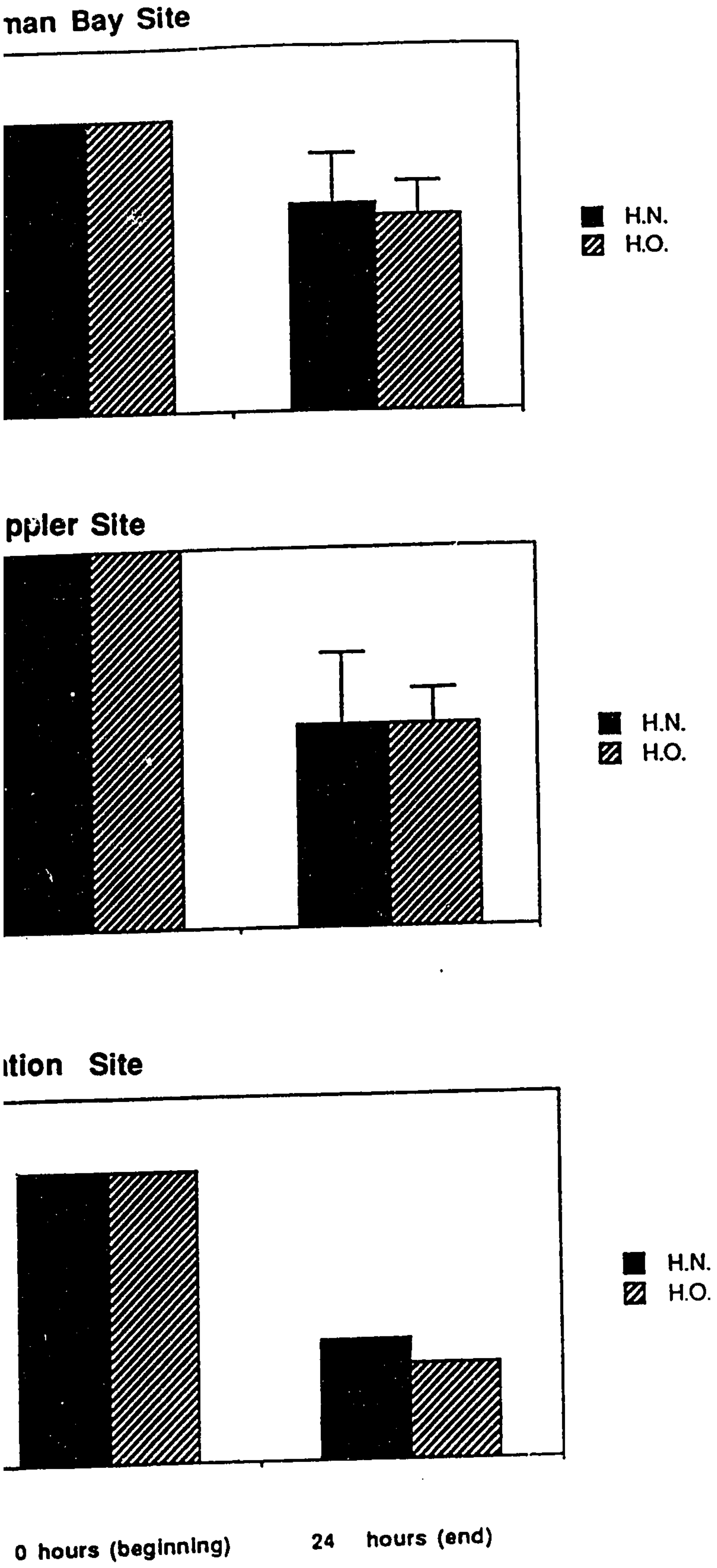

Field tethering experiments showing the number of crabs surviving after 24 hours (mean±S.D.). Experiments were conducted in July and September 1987.

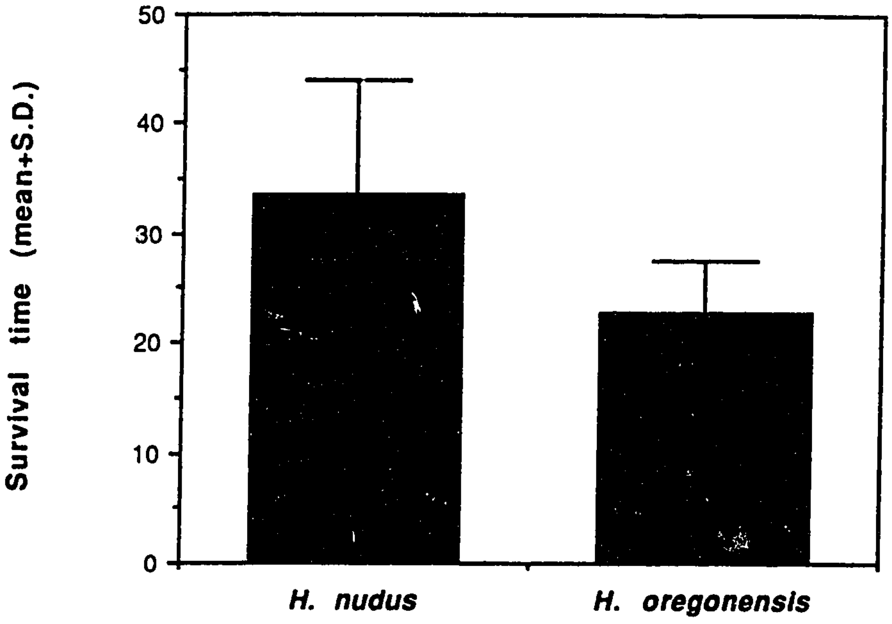

Fig. 11 Desiccation experiment showing the number of hours crabs survived (Mean+S.D.) in an incubator at 10°C and 83% humidity.

CHAPTER 4: GENERAL CONCLUSION

As Willason (1981) noted, sympatry of two or more species of brachyuran crabs is often found in members of the families Grapsidae and Ocypodidae. This study was an attempt to examine the reproduction and population distribution of the two sympatric species, *H. nudus* and *H. oregonensis* in Barkley Sound.

It was found (Chapter 2) that the reproductive season of *H. nudus* was different from those recorded for California (Boolootian et al. 1959) and the Oregon coast (Lough, 1975), but similar to that found by Knudsen (1964) at Tacoma, Washington. Although factors contributing to the initiation of gametogenesis , mating, egg deposition and hatching are still unknown, studies at different latitudes are important for us to understand these questions better. Boolootian et al. (1959) studied *H. nudus* at 37 °N and found brooding females from October to the following May, and hatching mainly from January to May. In Lough's study (1975) at 44°N, although no detailed information on reproductive biology is available, the larvae of *H. nudus* were collected as early as February. My study (at 48°N) is similar to that of Knudsen (1964) (at 47°N) with regard to the brooding period, from January to May, while hatching occurred from April to May.

For *H. oregonensis,* Kuris (1978) reported two brooding periods at Bodega Bay (39°N), the first from November to next May, and the second from June to August. Batie (1982) found brooding females year round with a peak in March, and the hatching season extended from March to May. In my study as well as that of Knudsen (1964), broodings were found from February to August, and hatching was approximately from May to September.

It is interesting that at higher latitudes, both the time of first brooding and hatching are delayed. These sites are certainly different with regard to water temperature and day length and thus one conclusion that can be drawn is that the breeding season of these crabs is related to temperature or photoperiodicity. Controlled laboratory studies on the influence of these two factors on reproduction should be rewarding.

This study showed for the first time that *H. nudus* and *H. oregonensis* have significantly different distribution patterns both vertically and horizontally. *H. nudus* prefers exposed, gravel shores, while *H. oregonensis* prefers sheltered, muddy bays. *H. nudus* lives higher on the shore than *H. oregonensis* when they occupy the same habitat.

Chapter 3 concerns the possible factors which may contribute to the observed distribution pattern of *Hemigrapsus.* My study showed that *H. nudus* has greater tolerance to desiccation than *H. oregonensis.* Since the average sediment particle sizes are larger higher on the shore, this may provide better protection for *H. nudus,* whereas *H. oregonensis* tends to dig burrows in muddy areas. These factors seem to decide the distribution of the two species, whereas gill structure and differential predation do not appear to be important factors. If the gill structure of *H. nudus* is susceptible to clogging, then differential mortality would be expected to occur in the cage experiment at lower intertidal zone at Wiseman Bay. This was not the case. The result of the salinity experiment is in concordance with the large scale distribution pattern of *Hemigrapsus*: namely *H. oregonensis* can survive in estuaries, but *H. nudus* can not.

This study has suggested many new questions. Although I have shown that larval abundance in the plankton differs seasonally, the distribution patterns of larvae in the water columns are still unknown. It is possible that the distribution patterns of *Hemigrapsus* larvae are similar to those of the fiddler crabs. Epifanio et al. (1988) showed that fiddler crabs *Uca minax* and *U. pugnax* larvae have long distance dispersal offshore and return for settlement when ready. How selective are the megalopae of *H. nudus* and *H. oregonensis* when they settle? Do they select for sediment type or use other cues such as chemicals, or are patterns established after settlement through migration and differential juvenile mortality? A better knowledge of these processes at the different stages of their life cycles will enable us to appreciate the life history adaptations of these ecologically important creatures in the intertidal communities.

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Appendix: I

LABORATORY OBSERVATIONS ON LARVAL MORTALITY AND DURATION BETWEEN HATCHING AND THE FIRST MOULT OF THE SHORE CRAB *HEMIGRAPSUS NUDUS* AS INFLUENCED BY DIFFERENTIAL STARVATION

ABSTRACT

Differential starvation experiments on 1st stage zoea larvae of *H. nudus* show that, over an 8 day feeding period, initial feeding during the first two days does not supply the larvae with enough food to moult to the 2nd stage zoeae. However initial feeding does sustain larvae longer than the non-feeding control larvae. Three days initial feeding reaches the point of saturation (larvae can live to moult if they stop feeding in the rest of the experimental period) as well as reduces mortality. When the larvae were starved for the first 3 days but were fed for the rest of the experimental period, the latency to moult increased, but some of them can still moult to the 2nd zoeal stage; however, when the larvae were starved for the first 4 days and were fed for the remaining 4 days, none of them could moult to the 2nd zoeal stages and this is defined as the point of no return.

A: INTRODUCTION

Among the possible causes of larval mortality, starvation or availability of food have been considered as critical factors (Anger et al. 1981). It is reasonable to assume that the duration between moults of crustacean larvae is related to food supply and that it has a direct effect on larval mortality, e.g. the longer the duration; the higher the mortality. Up to now, studies of the effects of food availability on the duration between moults and moulting success of crab larvae are limited. Paul and Paul (1979) found that the lack of food supply for king crab zoeae immediately after hatching has a serious damaging effect on their feeding success at later stages. Anger et al. (1981) have shown that starvation of newly hatched zoeae causes high mortality and that there is a point of no return (time after which feeding could no longer sustain the larvae to the next stage) for the first zoeae. However, in their study the duration of the 1st zoeae under natural feeding conditions is quite short (3-4 days), and this dictated their experimental design, in which feeding was terminated on the 4th day. This could result in the lower estimate of the point of no return, because if those larvae that were starved for the first 3 days are fed continuously from the 4th day, they may be able to reach the 2nd zoeal stage. In contrast, the natural duration of the 1st zoeal stage of *H. nudus* is about 7-9 days depending on the food conditions (Hart, 1935; and personal observations), which allows a more detailed design for starvation and mortality experiments. The purpose of this study is to examine the effects of differential feeding on the mortality and duration of moulting of the 1st zoeal stage of the shore crab *H. nudus.*

B: MATERIALS AND METHODS

Gravid female *H. nudus* were collected intertidally from Barkley Sound, Vancouver Island (48°50' N, 125°08' W) in April, 1988. They were transferred to the lab at Bamfield Marine Station and kept in plastic containers with mesh screens on two sides and kept in running sea water at a temperature of 10-12°C. When the larvae hatched, they rose to the water surface and were collected immediately and transferred to culture beakers so that the larvae used for the experiments were the same age. Altogether, 510 newly released 1st zoeae larvae were used in 17 different treatments based on the duration of feeding (Fig 12 C), with 3 replicates for each treatment and 10 larvae for each experimental beaker. The experiment was run for 8 days. The observation lasted until the last zoeae moulted or died. Treatment 1 is the control, in which the larvae were not fed for the entire 8 days. In treatment 2, larvae were fed for the 1st day but starved for 7 days, in treatment 3, the larvae were fed for the first 2 days, and starved for the remaining 6 days and so on. Larvae of the 9th to 11th treatments were fed for all the 8 days; in the 12th treatment, larvae were starved for the 1st day and fed for the remaining seven days; in the 13th treatment, the larvae were starved for the first 2 days, in the 17th treatment the larvae were starved for the 1st 6 days.

Each experimental beaker contained 50 ml of 1 µm filtered sea water with a concentration of 5 mg/L penicillin G added. The water was changed daily, and in all feeding experiments, 50 newly hatched *Artemia franciscana* nauplii (Great Salt Lake Strain) were placed in the beaker for food immediately after the water had been changed; in all the non-feeding cases only the water was changed. The experiments were conducted in an incubator set at 14°C, and the beakers were examined daily at the time of changing water. The nauplii, the dead zoeae, the moult and moulted larvae were removed from the beakers and recorded at the time of examination, so that only the live first stage zoeae were left in the beakers.

The total number of zoeae that died in the 1st zoeal stage out of the original 30 in each treatment is defined as the mortality under each treatment. For those treatments in which all the zoeae died in the 1st stage, the duration means the average time (days) of survival in this stage; for the treatments where larvae moulted to the 2nd stage, the duration is defined as average time (days) spent in 1st zoea stage. In each experimental beaker duration is calculated as $(d1*n1+d2*n2+d3*n3....)/n1+n2+n3......$ etc. $d1$ = 1st day, $d2$ = 2nd day........... ; $n1$ = the number of zoeae that died in the 1st day in the 100% mortality situation; but in other situations means the number of zoeae which moulted to the 2nd zoeal stage.

C: RESULTS

The mortality and duration of the 1st zoeae are shown in Fig 12 A and B. It is clear from Fig 12 A that there is no difference in mortality rate between starved zoeae and zoeae fed for the first 2 days; but when zoeae were fed for the first 3 days after hatching, the mortality dropped considerably, and was not significantly different from the mortality of those zoeae that were fed through the whole period (P>0.05 one way ANOVA). This indicates that 3 days is the point of saturation, whereby enough food has been acquired for the 1st zoeae to survive to the time of moulting. When the zoeae were starved for the 1st day and fed through the remaining 7 days, the mortality increased. The starvation of the zoeae for 1, 2 or 3 days at the beginning of the experiment led to an even higher mortality, but still some zoeae were able to survive to moult. Feeding from the 5th days onward could not provide the zoeae enough food to regain the potential to moult. Thus, starvation of 4 days was the point of no return.

As mentioned previously, two different calculations are employed to express the duration. For those treatments in which some zoeae moulted successfully to the 2nd zoea stage, the duration means the average time needed to complete the 1st zoea stage from all the moulted ones. For those that did not have any zoeae moult, the duration means the mean survival time in the 1st zoeae stage. Fig 12 B shows that the starved group can live for an average of 6.3 days. One day of feeding prolongs the survival time to 8.4 days, and two days of feeding can sustain the zoeae for 9.1 days. It is clear that although the initial feeding of 1 or 2 days does not help the zoeae to moult to the 2nd zoeal stage, it does help them to survive longer. Three days feeding enables some zoeae to moult in a duration of 8 days. Feeding for the first 4-6 days gives a duration of 8.1; 7.9 and 7.7 days respectively. Treatments 8 to 11 shorten the duration in the 1st zoea stage further. Starvation for the first 1, 2 or 3 days retards moulting, and the duration required to moult becomes progressively longer (Fig 12 B), with the increase in the length of the starvation, but some larvae still could moult to the 2nd zoeal stage. Starvation longer than 3 days could not provide the energy for larvae to moult, but compared with the control group (treatment 1), feeding from the 5th day still extends their life significantly, but feeding from the 6th day and later does not result in increased survival when compared with the starved group.

The mortality and duration are plotted against the number of days that the larvae were starved in the 8 days experiment based on whether the larvae were starved at the beginning or the end of the experiment (Fig 13 A and B). It is clear that in terms of mortality, feeding from the beginning of the experiment results in an improved survival rate than feeding at the end (Fig. 13). When duration to the first moult is considered, it is also true that the larvae that were starved at the end have a shorter duration, and more of them moult to the 2nd zoeae (larvae starved from day 4 can still moult, while the larvae starved until day 4 can not.). On the other hand, although larvae cannot survive to moult when fed at the beginning, they live longer than the larvae fed at the end of the experimental period.

D: DISCUSSION

Anger et al. (1981) examined the effects of differential feeding in terms of the duration of the 1st zoeae of five crabs, *Menippe mercenaria, Panopeus herbstii, Neopanope sayi, Sesarma cinereum* and *Libinia emarginata.* They found that starvation periods commencing immediately after larval hatching have a much more severe effect than those at later times. Based on this, they proposed that there is a sensitive early stage in which larvae need food to survive. The present study confirms their finding. My results show that larvae which were starved for the first three days have higher mortality than those starved for three days at the end of the 8 day experiment. And similarly, those starved at the beginning of larval life take a longer time to moult than those starved in the latter part of the larval life.

In another paper, Anger (1984) suggested that the crab larvae moult cycle is divided into four stages. Postmoult stages A and B are independent on food supplies, in that the larvae can survive without food. C, Intermoult stage, is a critical one, in which the larvae can not survive without food, and the so-called point of no return occurs in this stage. Stage D is again independent of food. Although the A and B stages do not rely on food, their nutritional supplies can directly affect stage C. In other words, stage C can be lengthened based on the food supplies of A and B (similar to our treatment 12, 13 and 14). This means that although the A and B stages are not dependent on food supplies, larvae in stages A and B could accumulate food reserves for the C stage. When the initial starvation passed the point of no return, they would never be able to moult. In Anger's study (1984), the A and B stages occur during the first day after hatching. In my study, the critical point lies in the 3rd day after hatching, and the A and B stage occur in the first two days.

The larvae of *H. nudus* hatch from early April to late May in Barkley Sound, which coincides with the spring phytoplankton bloom. This is essential as the larvae must have an adequate food supply in the first three days after hatching to survive and moult to the next zoeal stage. This result agrees with that of Paul and Paul (1980) on king crab larvae and Kon (1979) on *Chionoecetes opilio.*

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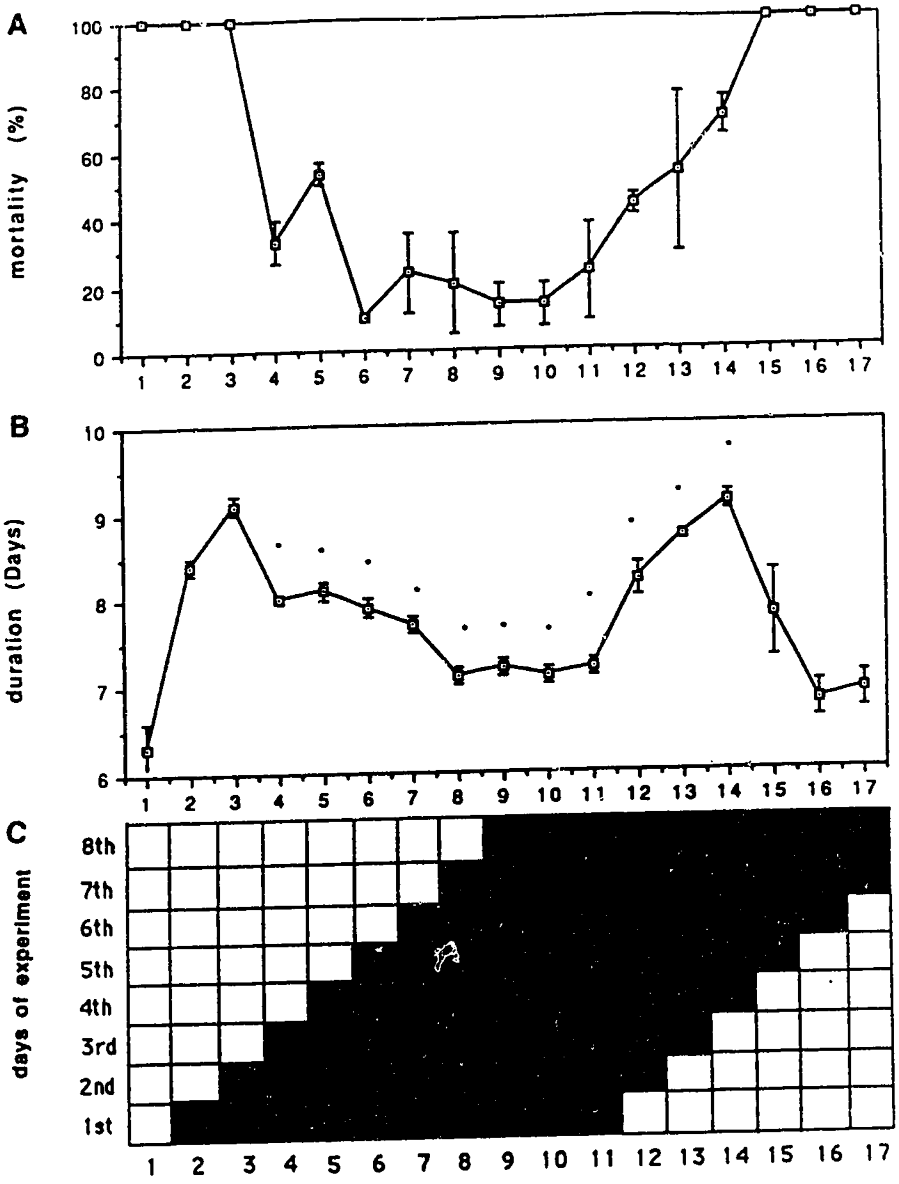

Fig. 12 The effects of differential feeding on mortality and duration of 1st zoeae of shore crab *Hemigrapsus nudus*.

A: mortality (%. Mean±S.E.)

B: development or survival duration (days. mean±S.E.)

C: experimental design

The number on X axis in all three figures indicates different treatments. In points with * indicate the days required to moult; points without * indicate the average days the zoeae survived. In C, the black and white mosaic pattern indicates days of feeding (black) and starvation (white).

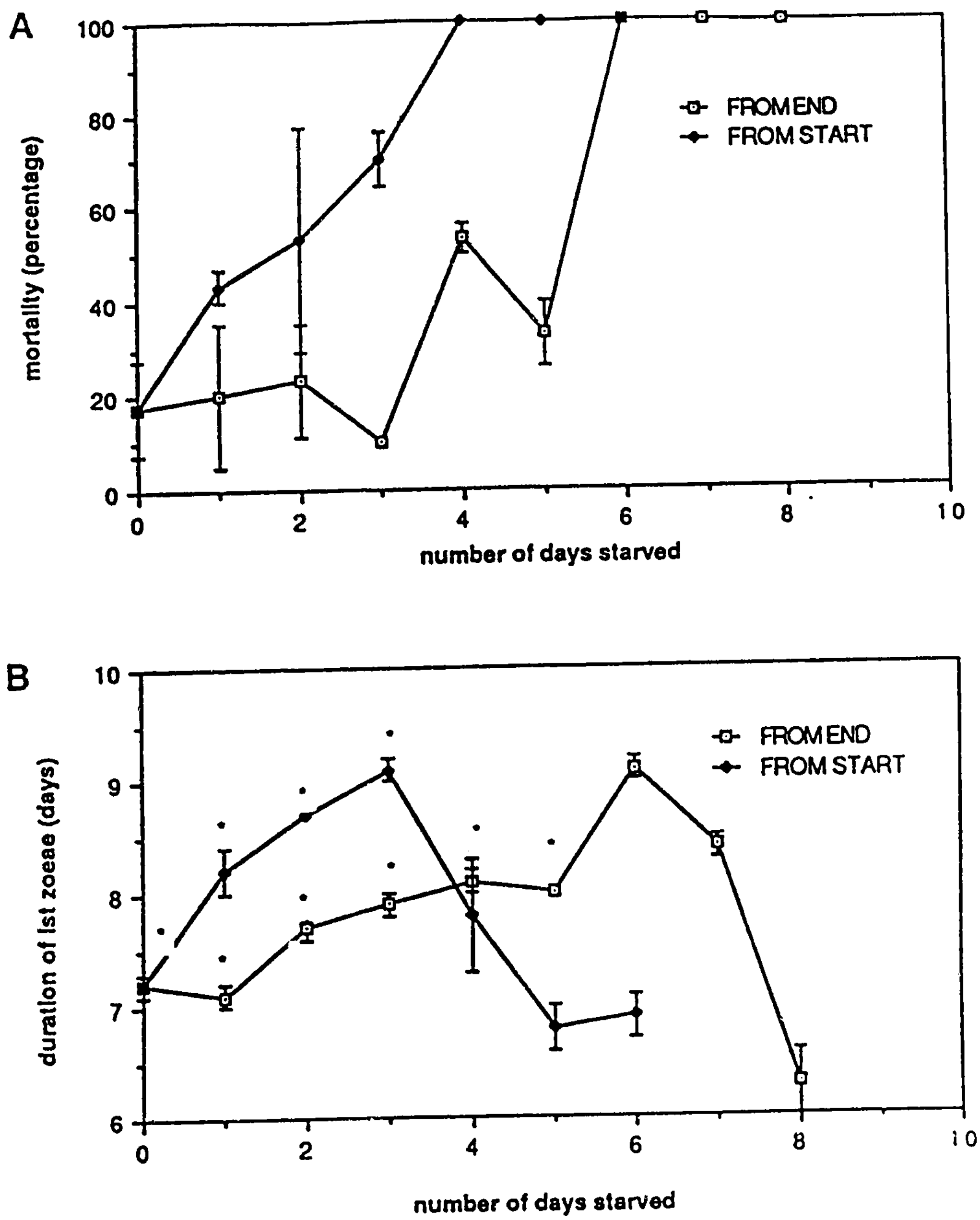

Fig. 13 Mortality and duration of first zoeal stage in relation to the number of days starved.

A: Relationship between mortality and the length (days) of larval starvation, either from the beginning or the end of the experiment. Mortality is represented as % (mean±S.E.)

B: Relationship between the duration of 1st zoeae and the length (days) of larval starvation, either from the beginning or the end of the experiment. Refer to Fig. 9 C. Points with * means the days it took to moult to the 2nd zoeal stages, points without * means the number of days the zoeae survivedg in the 1st zoeal stage.

Appendix: II

DIFFERENTIAL MORTALITY RATE OF DIFFERENT STAGES OF LARVAE OF TWO SPECIES OF SHORE CRABS CULTURED UNDER IDENTICAL CONDITIONS

ABSTRACT

This study reports that under identical rearing conditions the 1st zoeae of both species of the shore crabs *Hemigrapsus nudus* and *Hemigrapsus oregonensis* have a significantly higher mortality than the 2nd to 4th zoeal stages Further, the mortality rate from 5th zoeae to megalopa is again much higher than that in the earlier zoeal stages. The causes for this phenomenon are unknown.

A: INTRODUCTION

There have been a number of studies on larval development of brachyuran species under different temperatures, salinities and food levels (Hartnool and Mohamedeen,1987; Costlow & Bookhout,1959; Costlow et al,1960; Anger,1984; Sulkin,1975), and these studies have provided information on larval heat tolerance, and effects of salinity and differences in food quantity and qualitye. But no studies have been reported on the differential mortality rate of zoeal stages of a single species under identical rearing conditions. Based on field sampling results (McConaugha et. al., 1983) as well as our own experience, it seems much easier to obtain the 1st zoeal stage larvae than other stages. Vertical migration alone does not explain this phenomenon adequately, nor does predation. This study is designed to examine , under identical rearing conditions, the mortality rate of different zoeal stages.

B: MATERIALS AND METHODS

Gravid females of *H. nudus* and *H. oregonensis* were collected from intertidal areas in Barkley Sound, Vancouver Island (48°50' N, 125°08' W) in April, 1988. They were transported to the laboratory at Bamfield Marine Station and kept in plastic beakers with a mesh screen on two sides in a shallow tank with running sea water at 10-12°C (salinity 31‰). When the larvae hatched, the zoeae rose to the surface and were immediately collected and transferred to culture beakers so that the larvae used in the experiment were the same age. Altogether 1000 newly hatched zoeae of *H. nudus* and 800 zoeae of *H. oregonensis* were used for the experiments.

The 1000 *H. nudus* larvae were divided evenly into 10 plastic culture beakers, each containing 600ml of 1µm filtered sea water, while the *H. oregonensis* larvae were divided evenly into 8 beakers. The experiments were conducted in an incubator set at 14 °C under constant dim light. The sea water was treated with a concentration of 5mg/L penicillin-G and was changed daily. Newly hatched *Artemia franciscana* nauplii (Great Salt Lake strain) were used to feed the zoeae at a concentration of ca. 2 nauplii/ ml/day. The removal of uningested nauplii and the supply of new ones were done daily when the water was changed. The number of dead zoeae and the moults were recorded each day. The amount of filtered sea water and number of *Artemia salina* nauplii used in each beaker during the later part of the experiment was adjusted depending on the number of zoeae left, so that the cultures were kept under the same concentration. At the end of the experiment, the numbers of zoeae that successfully moulted to 2nd, 3rd, 4th and 5th zoeal stage in each jar were recorded and the survival rate (r) from one zoeal stage to the next was calculated by using the formula $N(t)= N(0) \times e^{-rt}$. N(t) is the number of larvae at the end of each stage. N(0) is the number of larvae at the beginning of each stage. r is the mortality rate; t is the duration (days) in a stage from N(0) to N(t) and it is calculated as the days on which 50% of the larvae have moulted to the next stage.

One way ANOVA were run to compare mortality rates of the different zoeal stages. Since it was suspected at the beginning of the experiment that the mortality rate from 1st zoeae to 2nd zoeae was higher than the mortality rate from the 2nd to 3rd, 3rd to 4th and 4th to 5th zoeal stage, the planned comparisons were run to check whether this is the case.

C: RESULTS

The numbers of zoeae surviving at each stage are given in Table 7 A for *H. nudus* and Table 7 B for *H. oregonensis*. Mortality rates of each stage for each beaker were calculated based on the formula given in MATERIALS AND METHODS and were summarized in Table 8 A for *H. nudus* and Table 8 B for *H. oregonensis*. (In some beakers no 5th zoeae successfully moulted to the megalopa stage, making calculation of mortality impossible; thus they are not included.). From the data in Table 8 A and B, one-way ANOVA were run to compare the mortality rate at the 1st, 2nd, 3rd and 4th zoeal stages for both species, with 10 replication for *H. nudus* and 8 replication for *H. oregonensis*. The ANOVA tables are as follows:

H. nudus:

source:	**df:**	**sum of squares:**	**Mean square:**	**F-test:**	**P value:**
between stages	3	0.014	0.00461	7.83	0.0006
residual	27	0.016	0.00059		

H. oregonensis:

source:	**df:**	**sum of squares:**	**Mean square:**	**F-test:**	**P value:**
between stages	3	0.00227	0.00076	4.119	0.0191
residual	21	0.00386	0.000184		

From the ANOVA tables it is obvious that there is a significant difference in mortality rate at different zoeal stages for both species. These results show clearly that *H. nudus* has a significantly higher mortality rate at the 1st zoeal stage than at the other stages ($P<0,01$), but no significant difference among the other stages ($P>0.05$); for *H. oregonensis,* there is also a significantly higher mortality rate at the 1st zoeal stage than at the other stages ($P<0.01$), but not among the rest ($P>0.05$).

D: DISCUSSION

The results of the present study indicate that, under similar laboratory culturing conditions, the 1st zoeae of the shore crabs *H. nudus* and *H. oregonensis* are more vulnerable than the other zoeal stages and subject to higher mortality. The reason for this higher mortality could be due to some unknown environmental factors, but food shortage or water quality could hardly be the case. It is noted that later zoeal stages have a greater food consumption rate than the 1st zoeae under the same level of food supplies (unpublished observation). It is possible that the first stage zoeae, due to their small size, may have greater difficulty capturing nauplii. However, *Artemia franciscana* nauplii have been used widely as food for laboratory culturing of crab larvae (Mootz & Epifanio, 1974; Sulkin, 1975; Bigford, 1978; Brick,1974), and the laboratory observation did show that the 1st zoeae of both species can capture and successfully ingest newly hatched *Artemia franciscana* nauplii. The temperature of 14°C, representing the surface water temperature during summer months, should be more suitable for the 1st zoeae than later stages, because later stages tend to move down the water column. Hartnoll and Mohamedeen (1987) studied six crab larvae in the laboratory and hinted that there was an increasing mortality rate at later stages, and they suspected it was due to more difficulty in later moults, or that the experimental conditions had cumulative ill effects. They also found that different batches of the same species could show marked difference under the same experimental condition . Indeed, variation in crab larval mortality rates in laboratory culturing systems could be very large; also with the small sample size of most experiments by previous researchers (mostly around 100 or less), the comparison of the mortality rates of crab larvae at different stages becomes difficult. My research has a larger sample size and reduces the possibility of experimental errors.

The reason why mortality from the 5th zoeae to the megalopa was so high in my study needs further research. Perhaps it is because the transformation from a zoea to megalopa is much more drastic than that between zoeal stages. It is also possible that *Artemia* from Great Salt Lake did not supply the necessary nutrition for this drastic metamorphosis. Johns et al. (1981) suggested that species undergoing a drastic metamorphosis during development do not survive well when fed *Artemia* from the Great Salt Lake. In addition, the transformation from the 5th zoeal stages to the megalopa may require proper substrates which were not provided in my experimental set up. It is my experience that I can locate the megalopa in the intertidal sediment, but few exist in the plankton samples.

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Table 7: Larval mortality experiment showing number of *Hemigrapsus* surviving at the beginning of each stage. Duration represents the number of days when 50% of the larvae had moulted to the next stages among all moulted to that stage. A: *H. nudus* B: *H. oregonensis*

A:

replicates	1st zoea	2nd zoea	3rd zoea	4th zoea	5th zoea	megalopa
1	100	26	24	21	19	1
2	100	47	37	34	26	0
3	100	66	48	46	37	0
4	100	66	57	52	44	0
5	100	25	20	20	17	1
6	100	87	75	73	50	1
7	100	67	60	58	47	0
8	100	83	74	70	64	1
9	100	67	59	54	37	0
10	100	65	58	51	35	0
mean±S.E.	100±0	59.9±6.7	51.2±6.0	47.9±5.8	37.6±4.6	0.4±0.16
duration (days)	10	8	8	9	9	

B:

replicates	1st zoea	2nd zoea	3rd zoea	4th zoea	5th zoea	megalopa
1	100	62	41	35	35	0
2	100	77	65	50	45	3
3	100	80	75	62	46	3
4	100	68	47	38	33	4
5	100	60	48	41	30	3
6	100	80	60	54	39	2
7	100	64	40	36	29	0
8	100	60	42	30	23	2
mean±S.E.	100±0	68.9±3.1	52.3±4.6	43.3±3.9	35.0±2.8	2.1±0.5
duration (days)	9	8.5	9.5	8	10	

Table 8: The daily mortality rate of crab larvae calculated from Table 8 (see text for formula). R1 to R4 are the mortality rates from 1st to 2nd, 2nd to 3rd, 3rd to 4th and 4th to 5th zoea respectively.

A: *H. nudus* B: *H. oregonensis*

A:

replicates	R1	R2	R3	R4
1	0.13471	0.01000	0.01670	0.01111
2	0.07550	0.02990	0.01056	0.02981
3	0.04155	0.03981	0.00531	0.02420
4	0.04115	0.01833	0.01148	0.01857
5	0.13863	0.02790	0.00000	0.01806
6	0.01393	0.01855	0.00338	0.04206
7	0.04005	0.01379	0.00424	0.02337
8	0.01863	0.01435	0.00695	0.01000
9	0.04005	0.01589	0.01108	0.04201
10	0.04308	0.01424	0.01609	0.04182
mean	0.05873	0.02028	0.00858	0.02610
S.D.	0.04422	0.00930	0.00552	0.01241

B:

replicates	R1	R2	R3	R4
1	0.05312	0.04865	0.01665	0.00000
2	0.02934	0.01993	0.02762	0.01316
3	0.02480	0.00759	0.02003	0.03730
4	0.04285	0.04345	0.02236	0.01764
5	0.05675	0.02626	0.01659	0.03905
6	0.02480	0.03384	0.01079	0.04068
7	0.04958	0.05529	0.01109	0.02723
8	0.05675	0.04196	0.03542	0.03321
mean	0.04225	0.03462	0.02007	0.02603
S.D.	0.01393	0.01591	0.00836	0.01453