DOCTOR OF PHILOSOPHY

Behavioural responses of the shore crab Carcinus maenus to salinity variation.

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BEHAVIOURAL RESPONSES OF THE SHORE CRAB *CARCINUS MAENAS* TO SALINITY VARIATION

A thesis

Submitted to the University of Wales

by

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SUMMARY

Behavioural responses of the colour forms of *Carcinus maenas* to salinity variation were investigated, and related to their physiology and distribution in an estuary.

Red males, characterised by a thicker carapace were unable to survive in as low salinities as green males; this was reflected in their poorer osmoregulatory capabilities. Haemolymph osmolality and ion concentrations of red crabs decreased at a faster rate and reached lower levels than in green crabs. Haemolymph osmolality and choice behaviour did not vary with size.

In the tidally mixed estuary male and female crabs occurred in roughly similar proportions. Most were green and generally smaller than their open shore counterparts. Migration out of the estuary in winter was reversed in late spring.

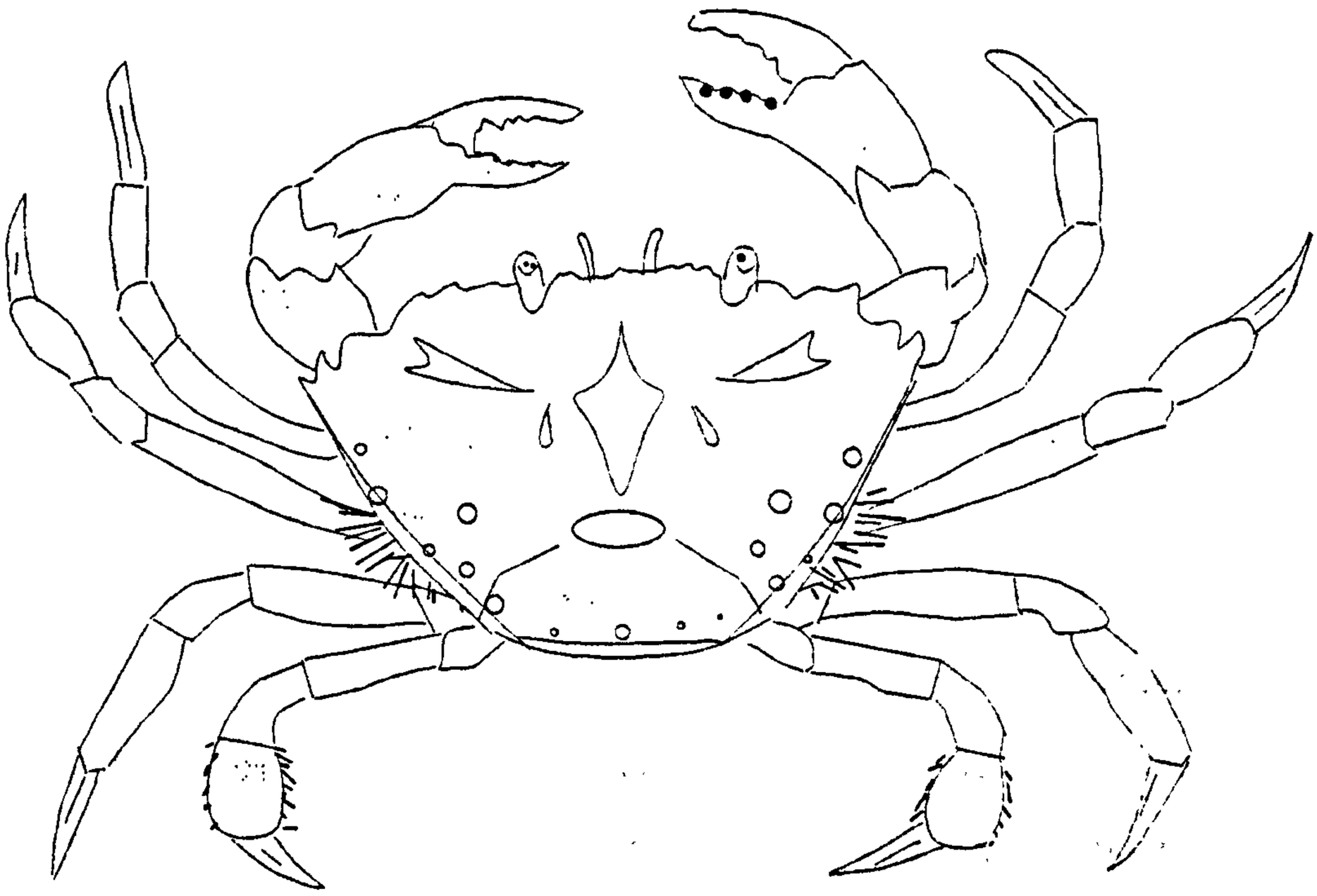
Differences in salinity tolerance of red and green crabs were reflected in salinity preference behaviour. Green crabs persisted longer in the lowest range of salinities tested, especially if a shelter was available. Prior acclimation affected the timing of choice behaviour; the lower the salinity of acclimation the faster the time of exit from the lowest range of salinities tested, and vice versa.

Estuarine green crabs exhibited endogenous locomotor activity of circatidal periodicity and were less responsive to episodes of low salinity than open shore red and green crabs. Constant low salinity initiated a rhythm of circatidal periodicity in arhythmic red and green crabs; red crabs reacted faster and were more active upon salinity change than green crabs. The amount of locomotor activity induced after prior acclimation was similar within each acclimation salinity tested.

Carcinus detected salinity variation by responding to the concentrations of Na and Cl in seawater, and was able to differentiate between salinities separated by as little as 0.5ppt.

General physiological changes appear to occur before behavioural responses are mediated; they probably act as cues for the behavioural responses, which appear not to be triggered by specific receptors. Behavioural and physiological responses combine to enhance the survivability of crabs in changing salinities.

CONTENTS



I should have been a pair of ragged claws

Scuttling across the floors of silent seas

George Eliot (1819-1880)

GENERAL INTRODUCTION

The common shore crab *Carcinus maenas* is a typical euryhaline crab and is classified as a hyperosmotic regulator (Rankin and Davenport, 1981). It is ubiquitous both on the open shore and in estuaries where it can survive indefinitely in salinities as low as 4ppt. (Poulsen, 1922, 1949, Broekhuysen, 1936). Its osmoregulatory physiology has been extensively studied but, as in other animals (Davenport, 1985), knowledge of its osmotically-related behaviour is rather fragmentary (for refs. see Ameyaw-Akumfi and Naylor, 1987).

The aim of the present study was to investigate various aspects of the behaviour of *Carcinus maenas* in relation to variations in salinity, against the general background of its ecology and physiology. Chapter 1 investigates the differing osmoregulatory capabilities of red and green colour forms of *Carcinus*. The distribution of crabs within a tidally mixed estuary was studied over the course of a year (Chapter 2) and related to their reactions to salinity variation in laboratory conditions (Chapter 3). In addition a study was made as to how their endogenous rhythmic locomotor activity patterns were modified by or affected their salinity-related behaviour (Chapter 4). Finally the methods of detection of the medium and the thresholds of salinity discrimination were investigated (Chapter 5) to determine if *Carcinus* was able to detect and orientate

along salinity gradients.

Ameyaw-Akumfi and Naylor (1987) state that behavioural and physiological reactions in response to reduced salinity have, so far, often been considered separately. This present study set out to determine for *Carcinus maenas* if and how behavioural and physiological responses to salinity are linked. Do the crabs exhibit behavioural reactions before physiological changes occur, thus reducing the requirement to expend energy altering their internal condition? Alternatively do physiological changes act as cues for the behavioural responses? Also, if the latter is true, what effect does differing physiological condition have on the behaviour of individuals? Combined field and laboratory studies were planned to seek to explain the observed behavioural and physiological responses of *Carcinus* in relation to its distribution in the wild.

GENERAL MATERIAL AND METHODS

Adult male *Carcinus maenas* of 40-70mm carapace width were collected both subtidally and intertidally using baited pots from Ynys Faelog (Grid Ref. SH 722560 O.S. Map 115) in the Menai Straits. Soft shelled crabs and those close to moult were discarded. Except where specified, females were not used due to difficulty in collecting large numbers of a similar size and colour. Crabs were held in the aquarium in filtered running seawater of 34ppt. at 12°C ± 2°C on a normal light/dark regime for at least five days. They were fed fish and squid, though some cannibalism did occur. Crabs were isolated from food supplies for two days prior to experimentation.

Salinities below 34ppt. were prepared by diluting seawater with tap water and checked using a Salinity Temperature Bridge Type M.0.5 salinometer. Salinities above ambient seawater were prepared by adding seasalts (Sigma Chemical Co.) to seawater, determinations being carried out using a field salinometer refractometer.

In each experiment freshly prepared salinities were used and aerated throughout. Experiments were run in a constant temperature of 12°C ± 2°C, in either a constant light or constant dark regime. In some experiments crabs were initially acclimated to either, low salinity 17ppt. (50% seawater), normal salinity 34ppt. (seawater) or high salinity 50ppt. (150%

seawater). Such crabs were acclimated for a period of 2 - 4 days in these salinities before introduction to the experiment. Design of individual experiments is discussed separately in each chapter.

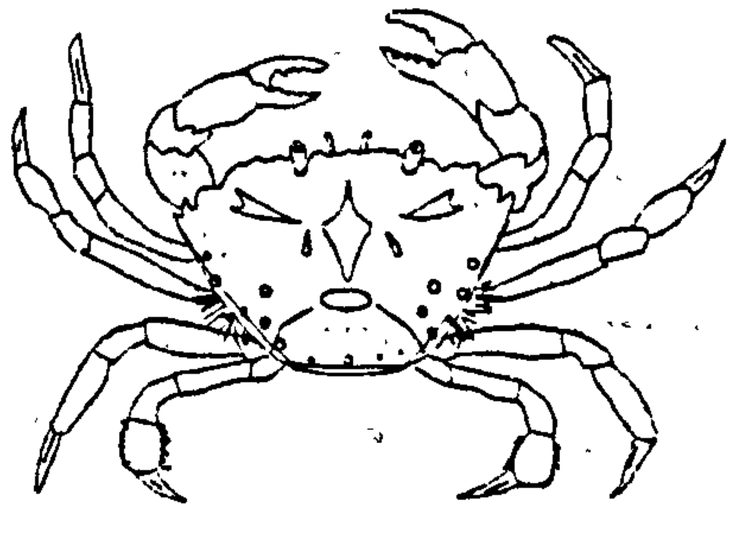
Statistical analysis of results was performed on the UCNW, Bangor mainframe computer using minitab (Minitab Inc. Ltd 1985) and SPSS-X (SPSS Inc.) packages and, unless otherwise stated, were analysed at the 95% confidence limit. All graphics were produced using Harvard graphics, on a Viglen 1 personal computer.

CHAPTER 1:

Physiological differences between red and green colour forms of *Carcinus maenas*

SUMMARY

Carcinus exhibits a variety of carapace colours from green, through orange to red. Red males were characterised by a thicker carapace and greater load of epibionts, and were unable to survive in as low salinities as green males. This was reflected in the poorer osmoregulatory capabilities of red crabs. In low salinities the haemolymph osmolality and ion concentrations of red crabs decreased at a faster rate and reached lower levels than those of green crabs. There was no relationship between the size of crabs and haemolymph osmolality, or size and choice behaviour of individuals.



INTRODUCTION

Interspecific variation of physiological responses to salinity between closely related species has been well documented. Differences are reported between *Hemigrapsus oregonensis* and *H. nudus* (Dehnel, 1962), *Crangon crangon* and *C. allmani* (Spaargaren, 1971), *Porcellana platycheles* and *P. longicornis* (Davenport, 1972), *Carcinus maenas* and *C. mediterraneus* (Lucu *et al*, 1973), *Callinectes sapidus* and *C. similus* (Engel, 1977), *Palaemonetes* species (Knowlton and Kirby, 1984) and *Pachygrapsus marmoratus* and *P. transversus* (Warburg *et al*, 1987).

Non-genetic intraspecific variation can occur as a result of adaptation to different salinities. This leads to a shifting of either the upper or lower limits of tolerance (Kinne, 1964 c d), and usually occurs in geographically isolated populations. Animals that inhabit areas with a wider range of salinities were found to be more efficient osmoregulators. Examples include *Mesidotea entomon* from the Baltic and freshwater environments (Lockwood and Croghan, 1957, Croghan and Lockwood, 1968), and *Praunus flexuosus* and *Crangon crangon* from the Baltic which were found to have significantly lower blood concentrations than animals from Loch Etive, Scotland (McLusky and Heard, 1971, McLusky, 1979, McLusky *et al* 1982). Similar findings are reported for *Carcinus* from the Baltic and North Sea

(Theede 1969, Winkler *et al*, 1988), in which the differences were reduced but not abolished after 3 weeks in laboratory conditions (Theede, 1969).

Differences in osmoregulatory ability also occur seasonally in some species and are usually the result of changes in temperature. Blood concentrations were found to be higher and survival was enhanced at lower temperatures in *Hemigrapsus* species (Dehnel, 1962), *Crangon crangon* (Weber and Spaargaren, 1970), *Callinectes sapidus* (Lynch *et al*, 1973), *Carcinus maenas* (Taylor *et al*, 1977) and *Helice crassa* (Jones, 1981).

Intraspecific variation also occurs between sexes. Male *Callinectes sapidus* were found to have a lower blood osmotic pressure in dilute seawater compared to females (Tan and Van Engel, 1966, Tagatz, 1971, Lynch *et al*, 1973), and the converse was found for *Carcinus maenas*, (Gilbert, 1959). Osmoregulatory abilities also vary with size of the animal, larger animals tending to be less efficient osmoregulators than smaller individuals, in *Carcinus maenas* (Gilbert, 1959), *Orconectes limosus* (Andrews, 1967) and *Pagurus bernhardus* (Davenport, 1972b). However larval stages tend not to be as tolerant of low salinities as the adults (Kinne, 1964a). Eggs and larvae of *Carcinus* can only tolerate salinities in the 28ppt. to 40ppt. range, whereas adults can survive indefinitely in salinities as low as 4ppt. (Remane and Schlieper, 1971). Similarly

Jones (1981) found the larvae of *Helice crassa* to be stenohaline, whereas juveniles and adults tolerated salinities in the 3.5ppt. to 52.5ppt. range. Variation of osmoregulatory ability at different stages of the moult cycle has been reported in *Gammarus duebeni* (Lockwood and Andrews, 1969) and in *Carcinus* (Robertson, 1960, Adelung, 1971)

Geographical isolation may eventually lead to genetic resistance adaptation to salinity (Kinne, 1963a), where genetically different animals of the same species exhibit a wider salinity tolerance range. This has more recently been confirmed in copepod species of the genus *Tisbe* (Battaglia and Bryan, 1964) and in the prawn *Palaemonetes varians* in the estuaries of the Rhine, Meuse and Scheldt (Hummel *et al*, 1989).

Recent work suggests that physiological variances may occur in *Carcinus* in individuals of different colours (Reid *et al*, 1989). *Carcinus maenas* exhibits a wide variety of carapace colours from pale green through orange to a deep red/brown, most apparent on the ventral side and limbs (Plate. 1). The red coloration is thought to be due to a prolonged intermoult, such individuals often being characterised by a greater number of parasites, epibionts, and a heavier carapace (Kaiser *et al*, 1990).

Crothers (1968) found a variation in the distribution of different colours of crabs on the shore.

The red crabs tended to be dominant in the subtidal zone, and were less abundant in the high intertidal zone or in the salt marshes, where green crabs tended to predominate. The difference in relative abundance of the colour forms in the intertidal zone may be due to the difference in ability to cope with changing environmental conditions (Reid, *et al* 1989). The red crabs have been shown not to be as efficient osmoregulators as green forms and could therefore not survive in low salinity for extended periods (Reid *et al*, 1989). Also their metabolic rate was higher and they could not survive in hypoxic conditions as long as the green crabs (Reid and Aldrich, 1989). However red crabs were found to be more aggressive and the chelae could exert greater forces compared with the green crabs (Kaiser *et al* 1990). This has a selective advantage, since the red crabs have a greater mating success when in competition with green crabs for a female (Reid, Abello and Naylor, in prep.).

The mechanism behind the colour differences is unknown, though a percentage of the red colour forms are probably in terminal anecdysis. It is unclear if all green crabs become red at some stage, but all red crabs that moult become green (Reid, pers comm.).

The aim of this chapter was to investigate various aspects of the osmoregulatory physiology of the red and green colour forms of *Carcinus maenas*, with a view to

discussing these in the context of additional studies on the detailed distribution pattern of the colour forms (Chapter 2).

PLATE 1

Green, orange and red colour forms of *Carcinus maenas*.

GREEN

ORANGE

RED

MATERIAL AND METHODS

MORTALITY RATES

Salinities of 0,5,11,22, and 34ppt. were used to examine lethal time for 50% mortality between equal numbers of red and green males, green females and small green males (<35mm). Crabs were kept in plastic tanks 130cms x 70 cms x 30cms deep, in which the water was changed in each tank daily, when any mortalities were recorded and removed. Crabs were not fed during any of the experiments. Experiments maintained in freshwater and 5ppt. salinity were carried through to 100% mortality. Mortality rates of green males freshly collected from the Foryd estuary (Chapter 2 Fig. 2.1.) were examined in the freshwater treatment conditions. In addition mortality rates of crabs were monitored in a six hour freshwater/ six hour seawater cycling system which changed salinities on a square wave basis, water flow being controlled by solenoid valves connected to timer switches. This cycling system produced a pattern of salinity variation similar to that experienced by crabs at the head of the Foryd estuary (Fig. 2.2.A).

HAEMOLYMPH OSMOLALITY

Salinities of 5,11,17,22,28,34 and 40ppt were prepared, and 8 to 15 red and green crabs were kept in these salinities for at least two days to ensure haemolymph osmolality reached a stable level (Theede,

1969, Siebers *et al*, 1972). Crabs were kept in 5ppt. for only 18 hours owing to the high mortality rate in this salinity (Fig. 1.2.B). Haemolymph samples were taken with a hypodermic needle by puncturing the arthrodial membranes of the walking legs and withdrawing approximately 0.5ml of blood. Samples were centrifuged at 12000 revs./min. for 5 minutes. Osmolality was measured from depression of freezing point on an osmometer (Knauer, Berlin) using 150µl aliquot samples.

HAEMOLYMPH ION LEVELS

Haemolymph samples were collected from between 12 and 15 individual crabs of each colour at each salinity using the method described above. 50µl serum samples were used for sodium analysis. These were diluted 1:2000 in deionised water and concentrations of sodium present were determined by means of a flame atomic absorption spectrophotometer (EEL 240). Magnesium, Calcium and Potassium concentrations in the serum samples were determined from 100µl samples diluted 1:100 in 10% nitric acid solution and were read on an atomic absorption spectrophotometer (10-Varian). The concentration of each of these ions was calculated from calibration curves of commercial cation standards (Spectrosol, BDH Ltd.).

FALL IN HAEMOLYMPH OSMOLALITY WITH TIME

Crabs were acclimated to low, normal and high salinities for 2 days. They were then introduced to a salinity of 5ppt and, at 1 hour intervals thereafter for a period of 12 hours, 5 crabs of each colour were removed and haemolymph samples were taken. These crabs were not replaced. A final sample was taken after 18 hours to establish that osmolality had reached a stable level. Samples were centrifuged and refrigerated prior to osmolality being determined using an osmometer (Knauer, Berlin).

HAEMOLYMPH OSMOLALITY AS A FUNCTION OF SIZE AND TIME OF EXIT

The two choice chamber described in chapter 3 (Fig. 3.1.B) was used. One chamber was filled with a salinity of 5ppt. and the other with seawater (34ppt.). Crabs were acclimated to low, normal and high salinities for two days prior to the experiment. They were introduced into the lower salinity in batches of 50, equal numbers of each colour over a wide size range being used. The experiment was monitored for 7 hours. When a crab exited from the low salinity the time was noted, the crab measured for carapace width and a haemolymph sample collected. Haemolymph samples were stored under refrigeration and centrifuged prior to their osmolality being determined using an osmometer

(Knauer, Berlin). This was repeated four times giving information for 100 crabs of each colour at each acclimation salinity. Crabs that did not exit within the time period were also measured for carapace width and a haemolymph sample taken. This experiment was designed to assess a number of variables, to determine if haemolymph osmolality and the time of exit (choice behaviour) were related to the size of the crab, and to investigate whether *Carcinus* exited the low salinity when the haemolymph osmolality reached a certain critical level.

HEART-RATE

Heart-rate was monitored by use of a continually emitting ultrasound transmitter and a receiver, which detected and amplified any distortion of this sound wave. A small probe was secured to the dorsal surface of a crab directly above the heart. The crab was placed in a tank that permitted minimal movement and allowed to settle for a number of hours before the experiment commenced. Heart-rate was recorded for 3 minutes at hourly intervals. Beats per minute were calculated as a mean value for each 3 minute recording period. Heart-rate was monitored initially during a 2 hour control period in seawater (34ppt.), after which a known amount of freshwater was then added to reduce the salinity to 5ppt. and heart-rate was monitored for a further 12 hours in constant darkness. Seven repetitions were

carried out for both the red and green crabs and mean values were calculated from the results obtained.

DETERMINATION OF MOULT STAGE

The moult stage of green, orange and red crabs was determined by examination of shell rigidity and setal changes on the exopodites (O'Halloran and O'Dor, 1988). Crabs were divided into moult stages A to E as defined by Drach (1939,1944). Sampling was carried out at regular intervals during the months May to August. Premoult and early postmoult crabs do not feed (Adelung, 1971), therefore to avoid a biased sample by trapping alone hand collection was also employed.

CARAPACE THICKNESS

Carapace thickness as a function of carapace width was examined to determine if red crabs possess a thicker carapace. Crabs of > 45mm were used. The carapaces were cleaned and dried, and then cut in half antero-posteriorly along the midline. Ten measurements were made at regular intervals along the midline using a dissecting microscope and eyepiece graticule at a magnification of x25. An average thickness was then calculated from these ten measurements, and plotted against the carapace width.

RESULTS

MORTALITY RATES

Fig. 1.1.A shows the time for 50% mortality in groups of large red and green males, green females and small green males. Red crabs exhibited the fastest mortality rate over the range of salinities tested. Both red crabs and small males show a steady decrease in time for 50% mortality with decreasing salinity over the range tested. Females and large green males survived better than red males and small green males in reduced salinities, and indeed showed maximum survival at 22ppt. rather than 34ppt. Assuming that an LT.50 greater than 500 hours (20days) is considered as 'survivability' as defined by McLusky (1967) on *Corophium volutator* and applied to survival times of *Porcellana* (Davenport, 1972a), then all *Carcinus* studied here can be considered as being able to survive in salinities of 11ppt. and above. Only large green males showed such survivability in salinities below 11ppt., having an LT.50 of 26.5 days in a salinity of 5ppt.

Fig. 1.1.B illustrates mortality rates of crabs kept in a constant flow through system in which salinity was varied on a tidal basis from freshwater to full seawater as could occur in an estuary, or a rockpool exposed to freshwater runoff at low water. In this experiment red crabs showed an initially high mortality rate, with the LT.50 reached within 10.6 days,

suggesting that red crabs are unable to survive in an estuarine environment. Mortalities in all the other groups tested occurred at a steady rate from the outset of the experiment with no initial large scale mortality. Green males survived for the longest period, and LT.50's for green males, females and small males were well in excess of the 20 day period suggesting that all these groups would be able to survive in estuarine conditions in the wild. Table 1.1. shows pairwise comparisons of the mortality rates of each of the groups obtained from a survival test (SPSSX). Pairwise comparisons show that red crabs have a higher mortality rate compared with the other groups tested. Small green males show a similar pattern of mortality to that of green females and large green males, but the mortality rates of females were significantly higher than for large green males.

Mortality rates in constant low salinity were analysed in more detail (Fig.1.2.A and B). Mortality rates in freshwater were rapid, and all crabs were dead within 73 hours. Mortality rate was fastest for the red males, whilst green males showed the greatest survival time, the LT.50 being almost three times greater than that for red males. Green male crabs collected from the Foryd estuary had a very similar mortality rate to those collected from the open shore. A survival test (SPSSX, see Appendix) was carried out to deduce which groups differed from each other and Table 1.2. shows the

results obtained. Crabs collected from the Foryd estuary were no better able to survive in freshwater than similar sized green males collected from the open shore. Red male crabs had a faster mortality rate compared to all other groups, whereas small green males and females followed a similar pattern of mortality to each other. Except in very general terms biologically meaningful conclusions cannot be drawn from this experiment since freshwater is rapidly lethal to all groups tested.

Fig. 1.2.B shows mortality rates in 5ppt., used since Broekhuysen (1936) states that this is the lowest salinity that *Carcinus* is able to survive. The mortality rate of red male crabs is initially very high , the LT.50 being reached in 2.3 days. The majority of red crabs were dead within 10 days and 100% mortality was reached before that of all the other groups tested. Females and small males appear to show very similar mortality patterns. Initial mortalities were fairly high, but they slowed down after about 15 days thereafter continuing at about 0.6% per day. Green males were the only group tested that can be regarded as being able to survive in a salinity of 5ppt. the LT.50 of 26.5 days being greater than the critical level of 20days (McLusky, 1967). Mortality was again about 0.6% per day, and was initially not as rapid compared with the other groups tested. Table 1.3. shows the pairwise comparisons of mortality rates obtained from a survival

test on SPSSX (see Appendix). Comparisons between the groups of crabs in 5ppt. salinity show a similar pattern to that found when crabs were maintained in freshwater (Table 1.2.). Red crabs showed a faster mortality rate compared to all the other groups tested. Mortality rates of small green males followed a similar pattern to those of the females and large green males. However mortality rates of females and large green males were significantly different, the large males apparently being better adapted to survive in 5ppt. salinity. Thus the survival patterns in the freshwater and 5ppt. salinity systems were similar to those found in the cycling freshwater-seawater system.

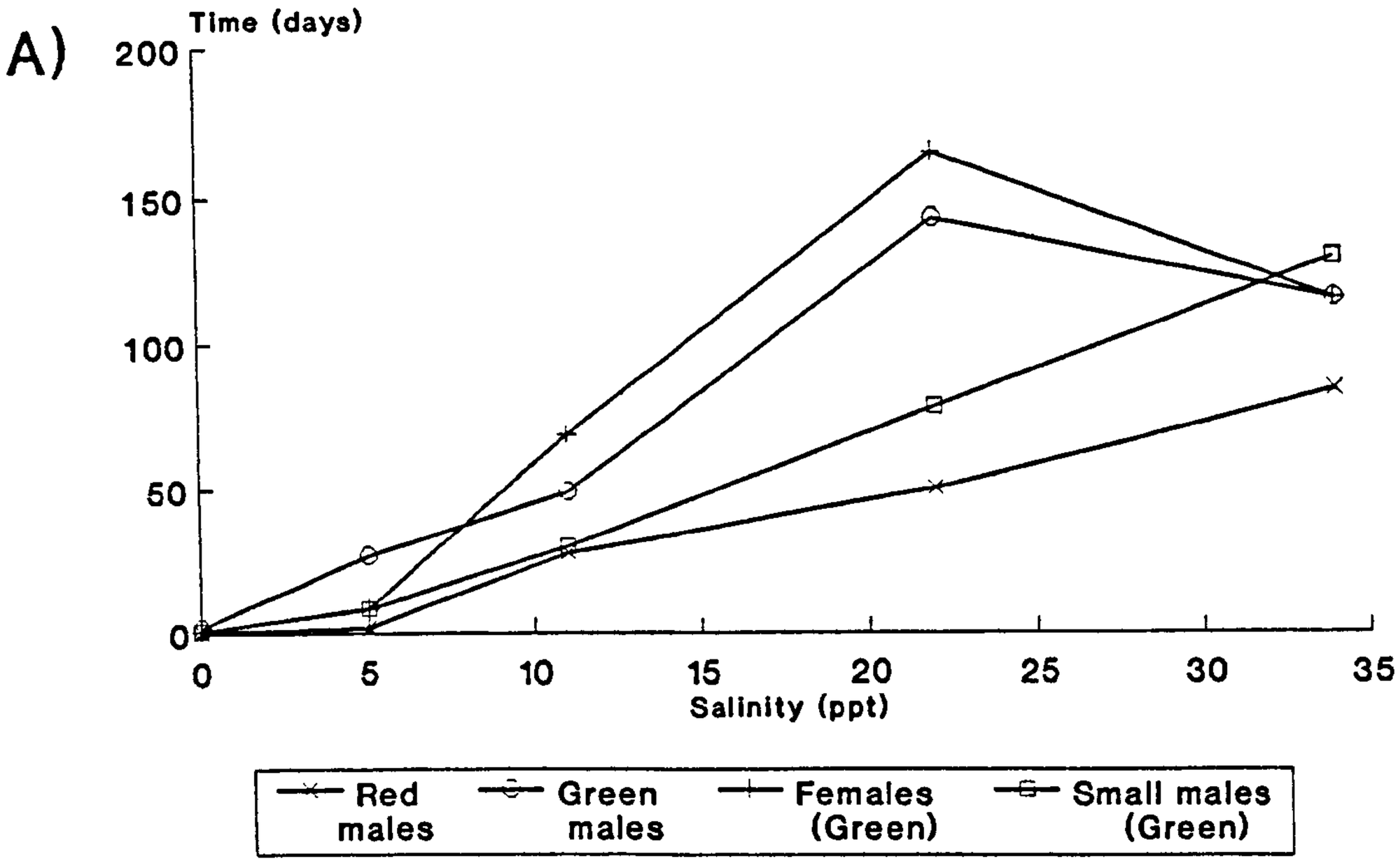
Fig. 1.1.

A) Time taken for 50% mortality in groups of large red and green males (>50mm carapace width), females and small green males (<35mm carapace width), in salinities of 0, 5, 11, 22 and 34ppt.

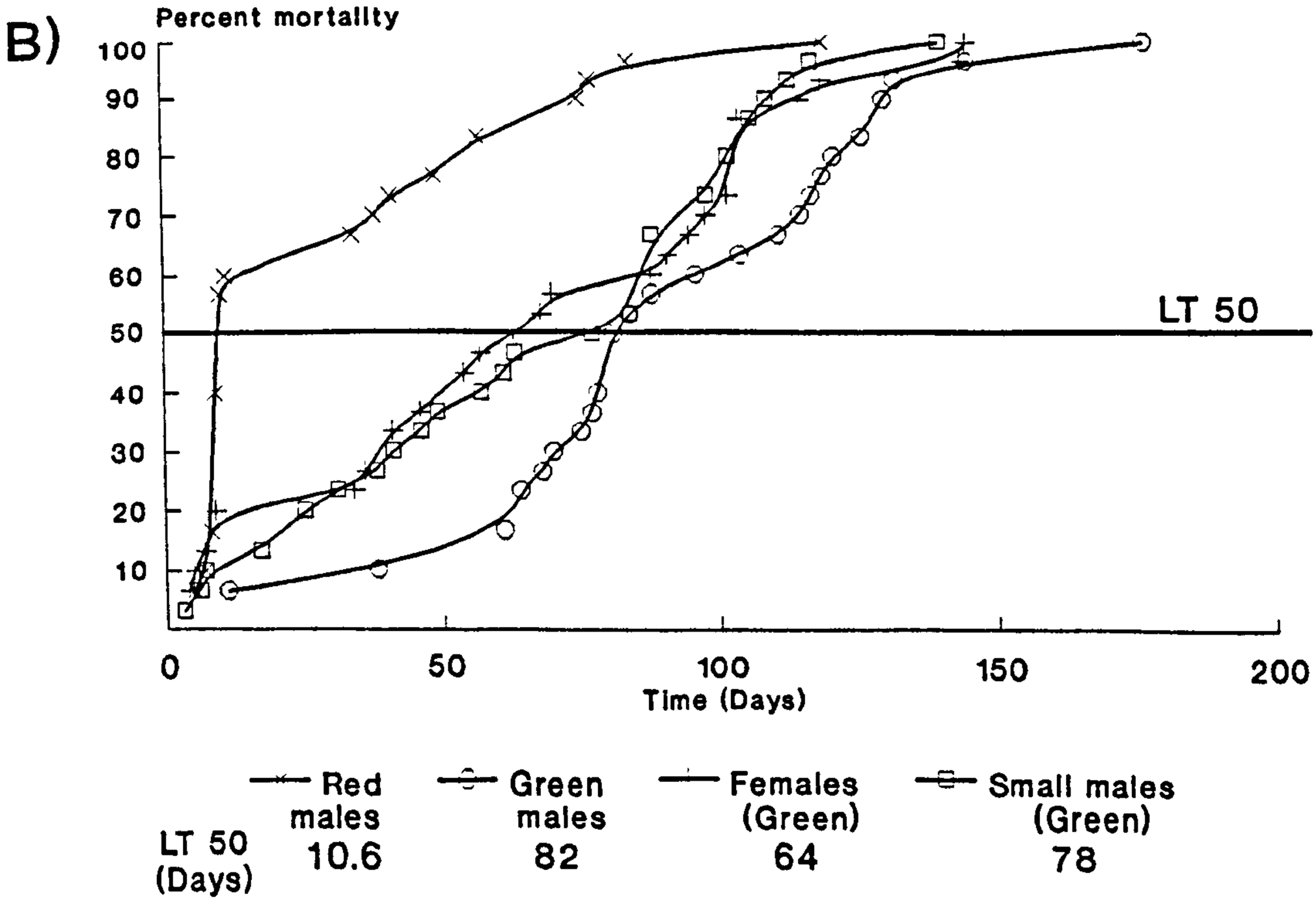
B) Mortality rates (expressed as a percentage) in groups of 30 individual large red and large green males, green females and small green males, kept in a salinity cycling system of alternating 6 hour periods of freshwater (0ppt.) and seawater (34ppt.). The times taken for 50% mortality (LT.50) for each of the groups of crabs are given on the graph.

Fig. 1.1.

Time to 50% mortality
Salinities 0ppt.-34ppt.



Mortality rates in FW/SW cycling system



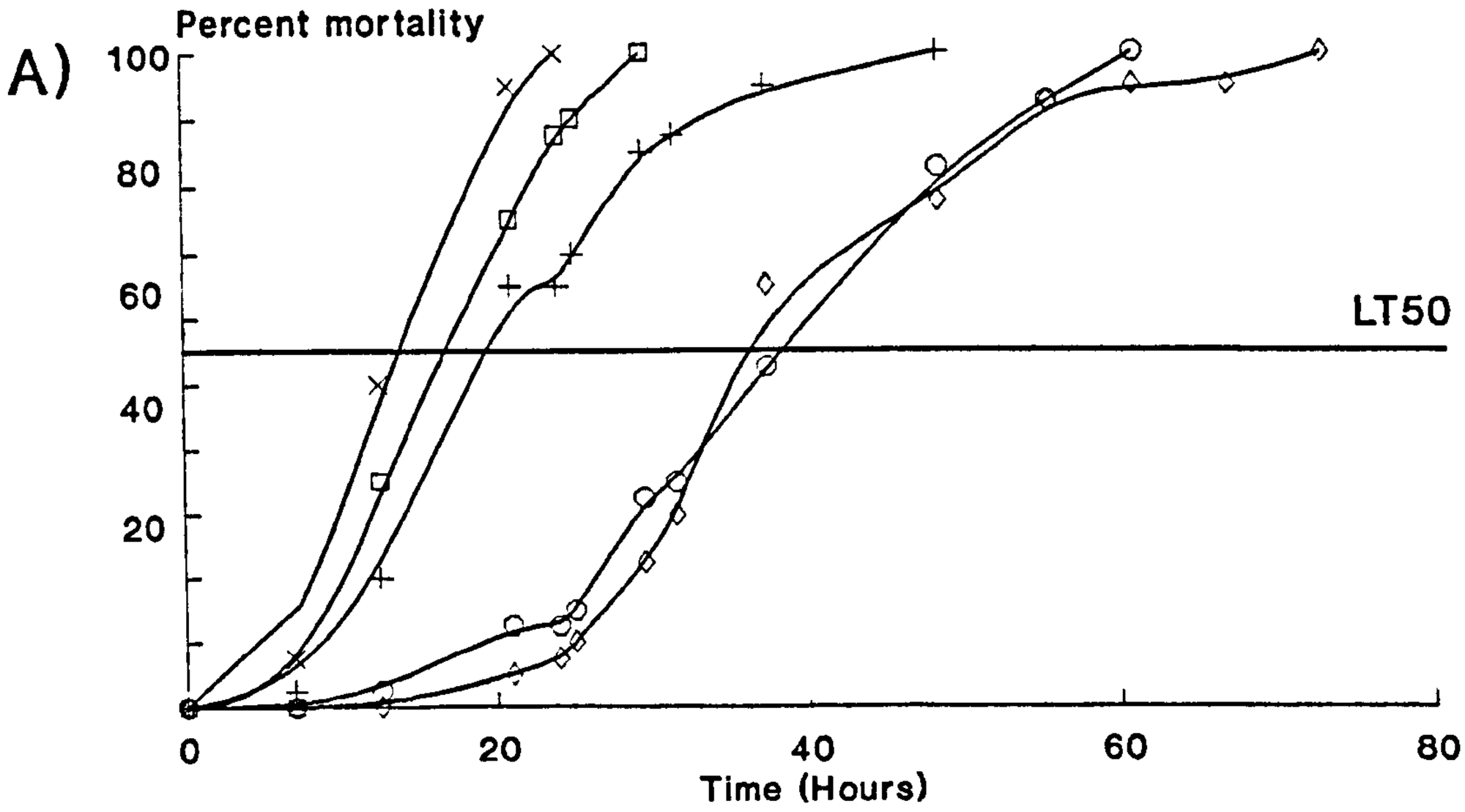
	Red males	Green males	Females (Green)	Small males (Green)
LT 50 (Days)	10.6	82	64	78

Fig. 1.2.

A) Mortality rates (expressed as a percentage) in groups of 40 individual large red and green males (>50mm carapace width), green females, small green males (<35mm carapace width) and large green males freshly collected from the head of the Foryd estuary, held in freshwater. Times taken for 50% mortality (LT.50) to occur in each group are also given.

B) Mortality rates (expressed as a percentage) in groups of 40 individual large red and green males, green females and small green males, held in a constant salinity of 5ppt. Times taken for 50% mortality are shown on the graph.

Fig. 1.2. Mortality rates in freshwater



	Green males	Red males	Est. Green males	Small males (Green)	Females (Green)
LT 50 (Hrs.)	37.9	13	37.8	21.2	21.5

Mortality rates in salinity of 5ppt.

	Red males	Green males	Females (Green)	Small males (Green)
LT 50 (Days)	2.3	26.5	8.3	12.1

TABLE 1.1. Pairwise comparisons between groups; FW/SW cycling system (D-statistic with probability, *=P<0.05, **=P<0.01)

	GREEN MALES	GREEN FEMALES	SMALL GREEN MALES
RED MALES	28.881 (**)	7.916 (**)	13.701 (**)
GREEN MALES	-	4.728 (*)	3.557 (*)
GREEN FEMALES	-	-	0.014 (NS)

TABLE 1.2. Pairwise comparisons between groups in freshwater(D-statistic with probability *=P<0.05, **=P<0.01)

GREEN MALES	GREEN FEMALES	SMALL GREEN MALES	ESTUARINE GREEN MALES
RED MALES	50.888 (**)	13.919 (**)	5.185 (*)	57.720 (**)
GREEN MALES	-	29.456 (**)	43.647 (**)	0.016 (NS)
GREEN FEMALES	-	-	3.472 (NS)	35.681 (**)
SMALL MALES (GREEN)	-	-	-	52.261 (**)

TABLE 1.3. Pairwise comparison between groups; salinity of 5ppt.(D-statistic with probability, *=P<0.05, **=P<0.01)

	GREEN MALES	GREEN FEMALES	SMALL GREEN MALES
RED MALES	39.161 (**)	21.265 (**)	35.611 (**)
GREEN MALES	-	6.174 (*)	3.089 (NS)
GREEN FEMALES	-	-	1.381 (NS)

HAEMOLYMPH OSMOLALITY

Fig. 1.3. shows the haemolymph osmolality (with 95% C.L. of the mean) for red and green crabs over a salinity range 5ppt. - 40ppt. At salinities from 40ppt. down to 28ppt. the haemolymph is iso-osmotic to the surrounding medium. In salinities in the range 5ppt. to 22ppt. haemolymph osmotic pressure is maintained hyperosmotic to the surrounding medium. However, whereas red and green forms initially maintained equal levels of hyperosmotic haemolymph pressure at 22ppt., below that value green crabs exhibited higher haemolymph osmolalities than red crabs, down to the lowest experimental salinity tested of 5ppt. This difference between the red and green crabs is confirmed by a GLM test (Minitab) in which a significant interaction is obtained (Colour*salinity, 6DF, $F=16.51$, $P<0.001$). A Scheffe pairwise comparison test performed on the data also shows that the haemolymph osmotic pressure in red crabs is significantly lower in salinities of 5,11 and 17ppt. There is no difference in haemolymph osmolality between the two colour form in salinities of 22ppt. and above.

Fig. 1.3.

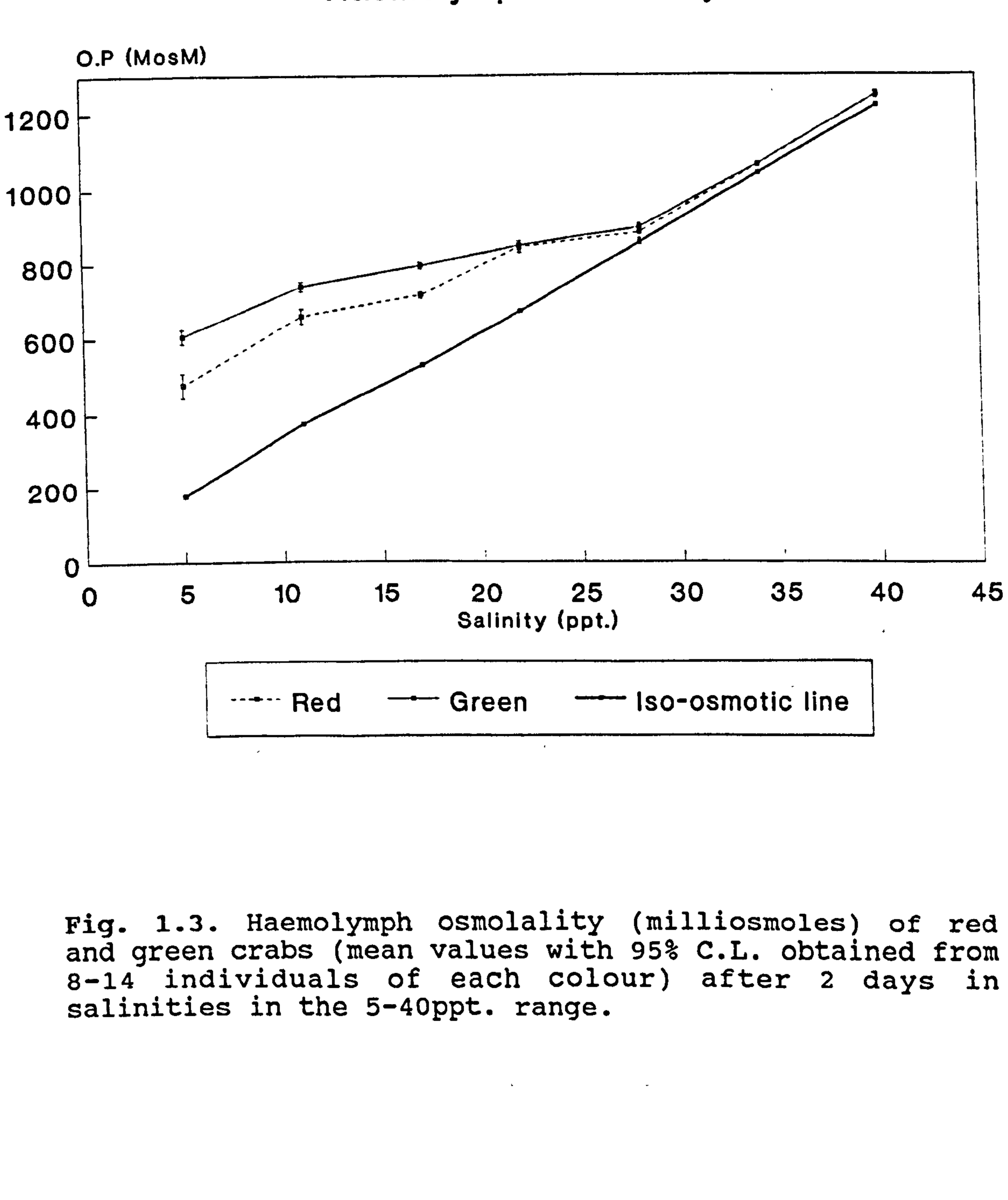


Fig. 1.3. Haemolymph osmolality (milliosmoles) of red and green crabs (mean values with 95% C.L. obtained from 8-14 individuals of each colour) after 2 days in salinities in the 5-40ppt. range.

HAEMOLYMPH ION CONCENTRATIONS

Haemolymph cation concentrations were examined to determine whether they followed a similar pattern to the haemolymph osmolality. Fig. 1.4.A. shows the haemolymph sodium concentrations (with 95% CL. of the mean) over the salinity range 5ppt.- 40ppt. Sodium levels are hypertonic to the medium over the range 5ppt.- 28ppt., and isotonic at 34ppt. and above. Red crabs have lower sodium concentrations than green crabs over the range 5ppt.- 22ppt., but above this range haemolymph sodium concentrations appear to be similar. A GLM test (Minitab) confirmed a significant interaction between colour and salinity (6DF, $F=3.30$, $P<0.01$), but a Scheffe pairwise comparison test established that the differences in sodium concentrations between the two colour forms at each salinity were not significant. However the error bars do not overlap in the lower range of salinities, indicating that there could be a difference haemolymph in sodium concentrations at these salinities. Paired T-tests were therefore applied to the data obtained for each salinity separately. Table 1.4. shows the results obtained. At salinities of 5, 11, 17 and 22ppt. red crabs had a significantly lower haemolymph sodium concentration compared to the green crabs. At salinities of 28ppt. and above haemolymph sodium concentrations of the two forms were similar.

Fig. 1.4.B shows haemolymph magnesium

concentrations over a similar salinity range. Magnesium is regulated hypotonically to the medium at all salinities except for the lowest (5ppt.). Magnesium concentrations are lower in the red crabs at salinities of 5,11, and 17ppt., but red crabs have higher magnesium concentrations in the salinity range 22ppt. - 40ppt. A GLM test (Colour*salinity, 6DF, $F=7.78$, $P<0.001$), followed by a Scheffe pairwise comparison test showed that the only significant difference between the red and green crabs occurred at a salinity of 40ppt., red crabs having higher magnesium concentrations compared to green crabs at that salinity.

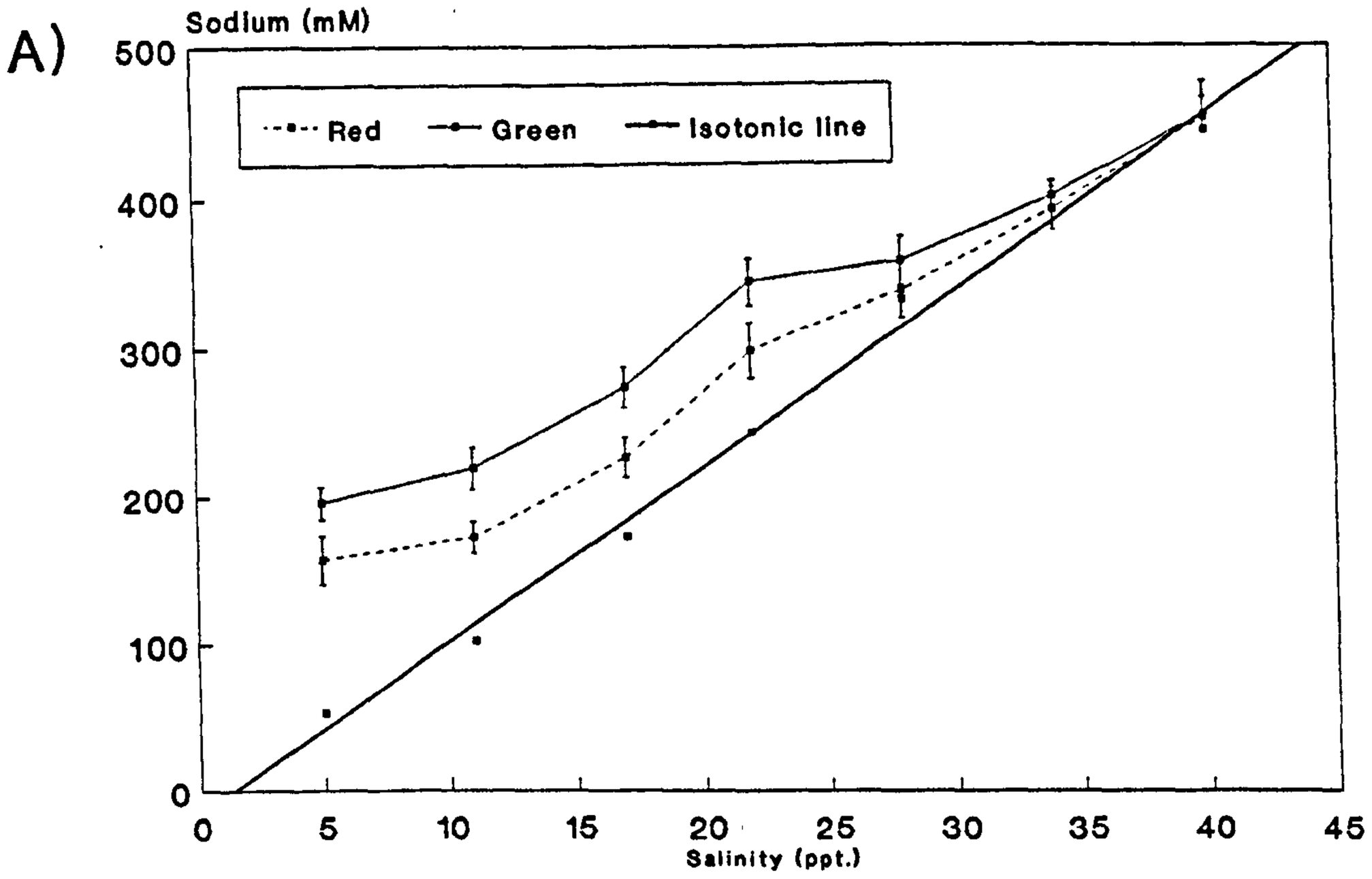
Fig. 1.5.A shows haemolymph calcium concentrations of red and green crabs over the salinity range 5ppt.-40ppt. Haemolymph calcium is regulated hypertonically to the medium over the entire range of salinities tested. Calcium levels are higher in green crabs over the salinity range 5ppt.-22ppt. and higher in red crabs over the salinity range 28ppt.- 40ppt. A significant interaction was obtained from application of a GLM test (Colour*salinity, 6DF, $F=21.83$, $P<0.001$). A Scheffe pairwise comparison test shows that significant differences occur at salinities of 5,11 and 17ppt., green crabs having a higher haemolymph calcium concentration. Significant differences were also found at salinities of 28ppt. and 40ppt., red crabs having higher haemolymph calcium concentrations compared with

green crabs.

Fig. 1.5.B shows haemolymph potassium concentrations over the same salinity range as the other cations. Haemolymph potassium levels are regulated hypertonically to the external medium over the entire salinity tested. Compared with the other cations investigated no trend is apparent in the data: green crabs have higher potassium concentrations in salinities of 5,11 and 28ppt., whereas red crab haemolymph potassium concentrations are higher over the remaining salinities. A GLM test confirms a significant interaction (Colour*salinity, 6DF, $F=8.70$, $P<0.001$), and a Scheffe pairwise comparison test shows that significant differences occur at salinities of 5ppt., when green crabs have higher levels of potassium and at 34ppt., when red crabs have higher haemolymph potassium concentrations.

Overall, the haemolymph ion studies reveal a consistent pattern whereby, green crabs have a higher haemolymph osmolality and ion levels at salinities of 5,11 and 17ppt., although the differences are not statistically significantly at the 5% level in all cases. At salinities of 22ppt. and above haemolymph osmolality and sodium levels are similar in both colours of crabs. At these higher salinities concentrations of magnesium, calcium and potassium are variable, but usually greater in red crabs than in green specimens.

Fig.1.4. Haemolymph sodium concentrations



Haemolymph magnesium concentrations

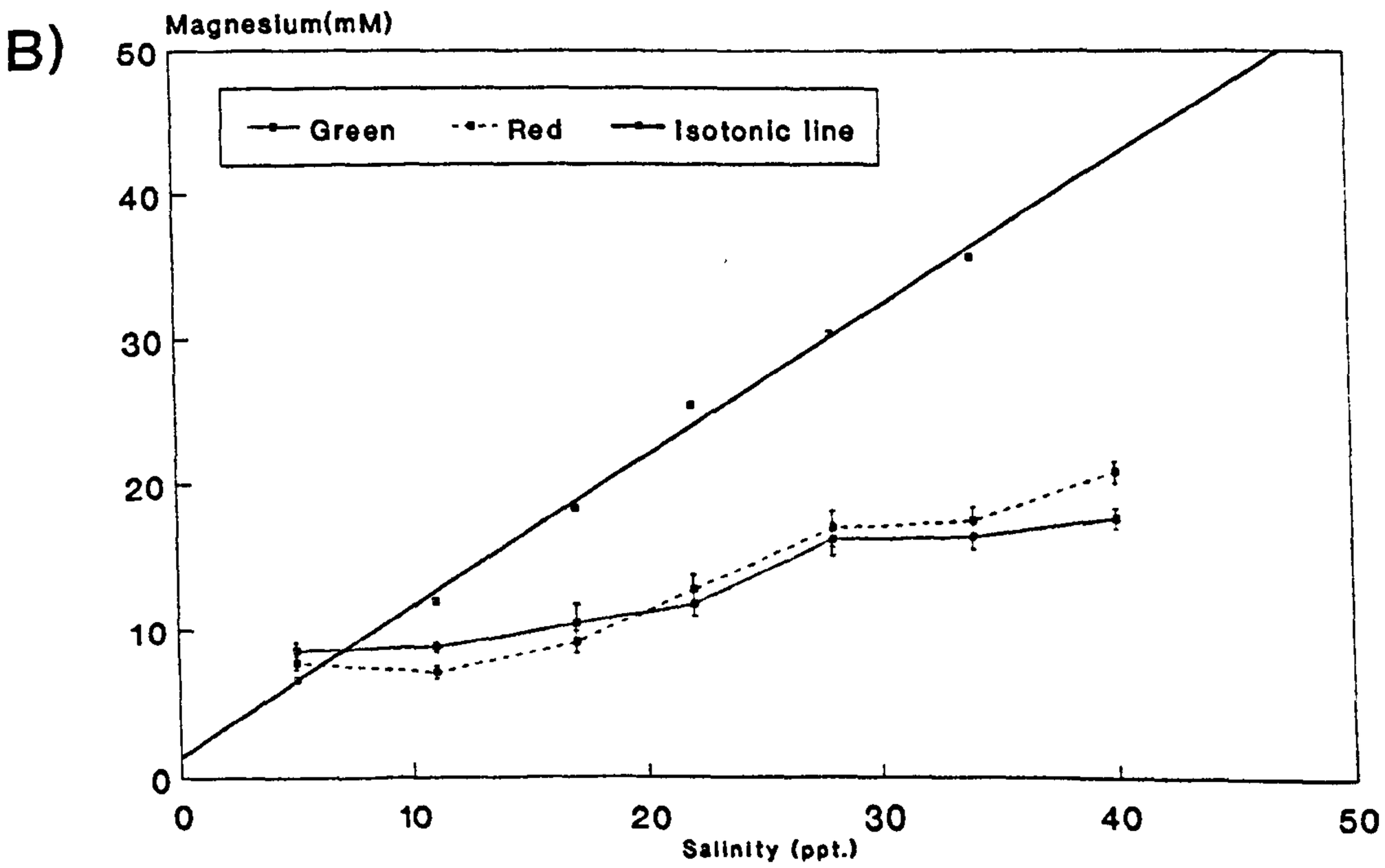
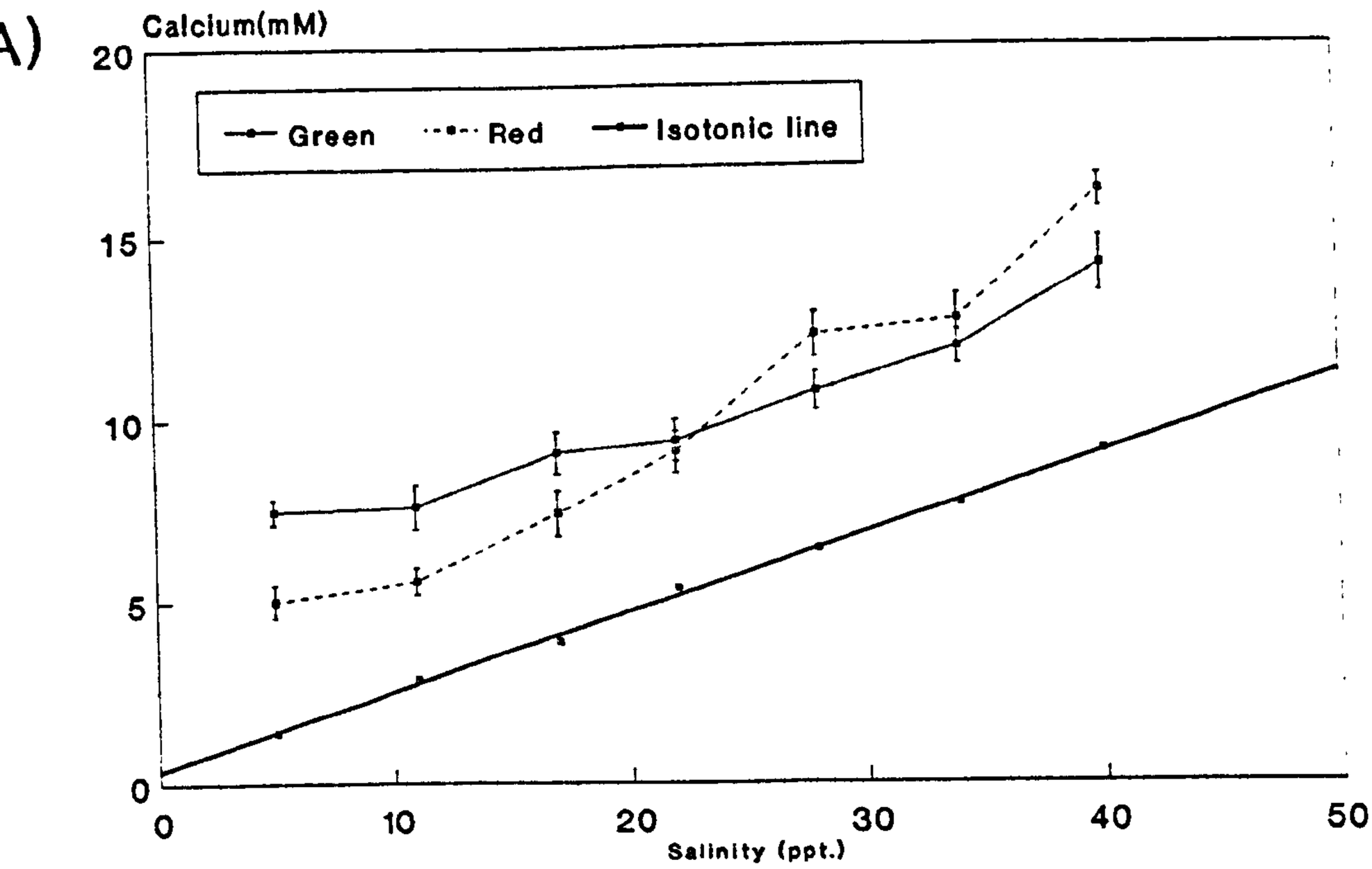


Fig. 1.4. Haemolymph A) Sodium and B) Magnesium concentrations (millimoles) of red and green crabs (mean values with 95% C.L. obtained from 12-15 individuals of each colour) after two days in salinities in the 5-40ppt. range.

Fig. 1.5. Haemolymph calcium concentrations



Haemolymph potassium concentrations

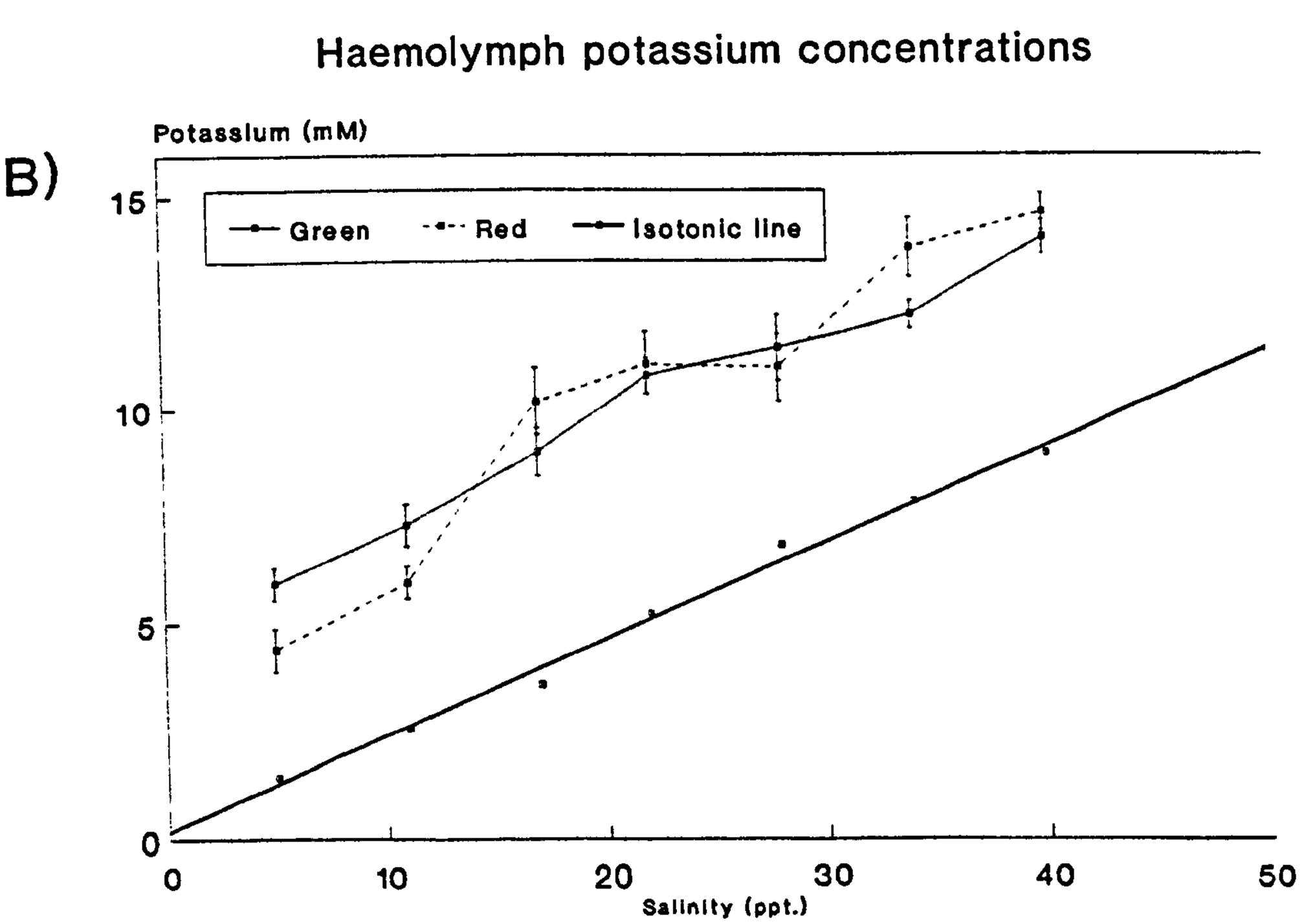


Fig. 1.5. Haemolymph A) Calcium and B) Potassium concentrations (millimoles) of red and green crabs (mean values with 95% C.L. obtained from 12-15 individuals of each colour) after 2 days in salinities in the 5-40ppt. range.

TABLE 1.4. Paired T-tests, comparisons of haemolymph sodium levels at each salinity.

SALINITY(ppt.)	5	11	17	22	28	34	40
T-VALUE	3.88	5.15	4.87	3.68	1.57	1.02	0.21
PROBABILITY	(**)	(**)	(**)	(**)	NS	NS	NS

(**= P<0.01)

DECREASE IN HAEMOLYMPH OSMOLALITY WITH TIME

Fig. 1.6. A,B,C illustrates the fall in haemolymph osmotic pressure after acclimation to low, normal and high salinities. Regression equations and R^2 values of the Log transformed data are included on the graphs and exponential curves were fitted to the data following Margaria (1931) and Shaw (1961). Differences in the rate of change of the haemolymph osmolality (slope) and the insertion (elevation) (Zar, 1974), were compared between the red and green crabs at each acclimation salinity.

Fig. 1.6.A shows the fall in haemolymph osmolality after acclimation to 17ppt. salinity. In both red and green crabs haemolymph osmolality fell by about 200 mosM over the 18 hour period. The rate of decrease appeared to be similar for both crab colours, as confirmed by testing for differences in slope (Z=0.91, 121 DF, P>0.05, NS). Red crabs have a haemolymph osmolality about 50 mosM lower than green crabs, this difference remaining constant over the 18 hour period. On this basis a difference in the intersect would be expected, but statistical analysis showed that this was not significant (T=0.17, 121 DF, P>0.05, NS). It is concluded that after acclimation to low salinity , and upon transfer to 5ppt. there is no difference in the rate of change in haemolymph osmolality with time between the red and green crabs.

Fig. 1.6.B. shows the fall in haemolymph osmolality

after acclimation in normal salinity (34ppt.). Both red and green crabs have a similar haemolymph osmolality in normal salinities. This starts to decrease relatively rapidly after transfer to 5ppt. salinity, falling by about 150 mosM within the first hour. The decrease in osmolality slows down after 10 hours, but it is still falling after a period of 18 hours. A difference between the red and green crab starts to become apparent after 3-4 hours; from then onwards the red crab haemolymph osmolality decreases at a faster rate compared to that of the green crabs. This is confirmed by statistical analysis of differences between the two slopes ($Z=3.75$, 126 DF, $P<0.05$, S).

Fig. 1.6.C. shows the fall in haemolymph osmolality after acclimation to high salinity (50ppt.). The decrease is rapid, falling by about 200 mosM within the first hour. After 10 hours the rate of decrease has slowed down, however, it is still falling at 18 hours. Differentiation between the red and green crabs starts to occur after about 4 hours, thereafter haemolymph osmolality falling at a faster rate in the red compared to green crabs and reaching a maximum difference of 70 mosM after 18 hours. Statistical analysis of the slopes of the two regression lines confirms a difference ($Z=1.71$, 126 DF, $p<0.05$, S).

R^2 values for crabs acclimated to normal and high salinities are high, indicating a good fit of points

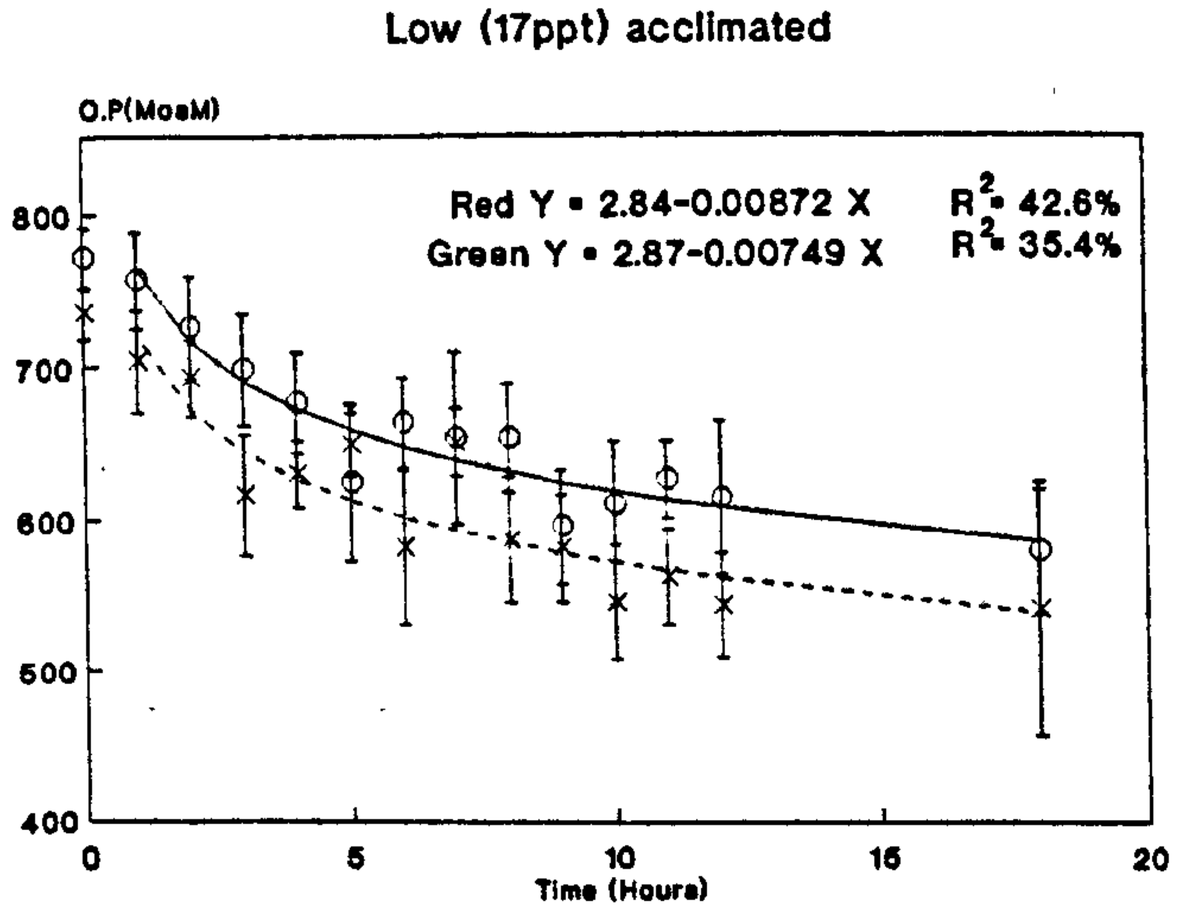
around the regression lines. Those of crabs acclimated to low salinity are not as high, indicating a larger variation in haemolymph osmolality with time. This variability perhaps accounts for the lack of statistically significant differences between the patterns of response of red and green crabs in Fig. 1.6.A.

Fig. 1.6.

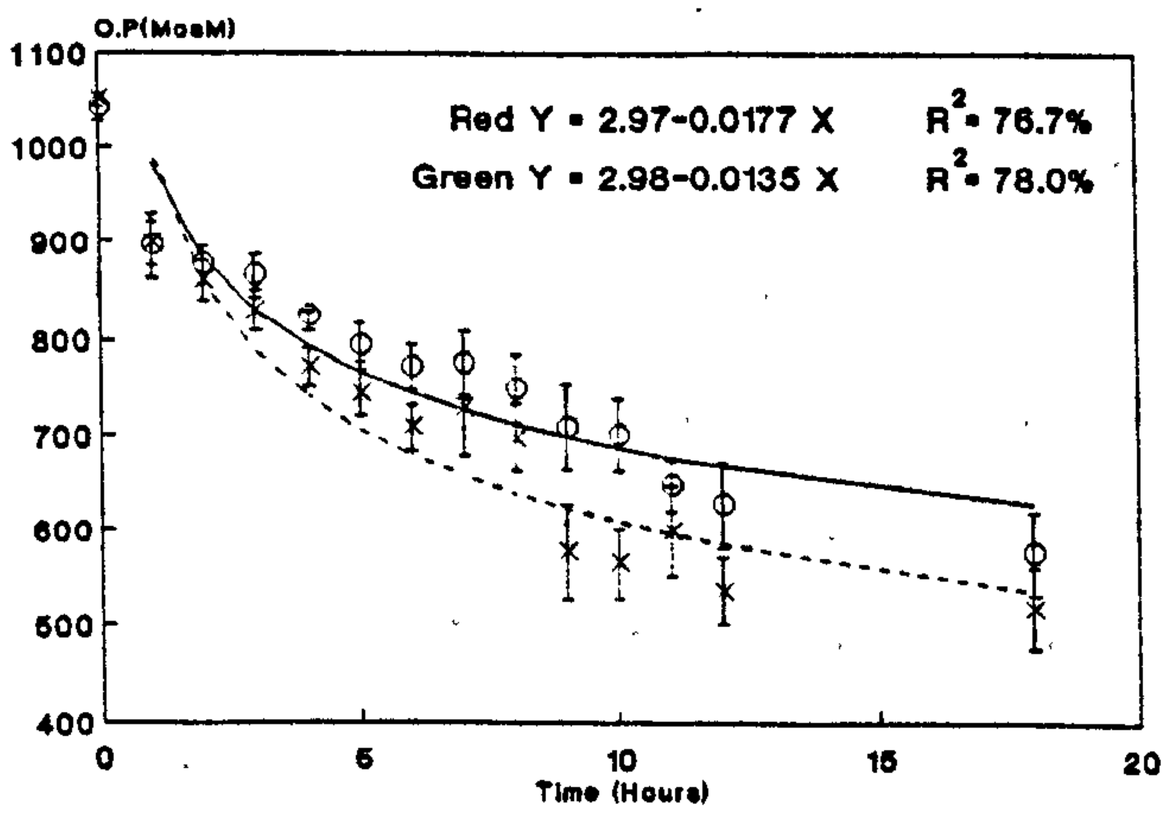
The decrease in haemolymph osmotic pressure of red and green crabs with time in a salinity of 5ppt., after previous acclimation to low (17ppt.), normal (34ppt.) and high (50ppt.) salinities. Points represent the mean value (with 95% C.L.) of samples taken from 5 individuals of each colour. Exponential curves were fitted to these points; regression equations and correlation coefficients (R^2 values) of the Log transformed data are also shown on the graphs.

Fig. 1.6.

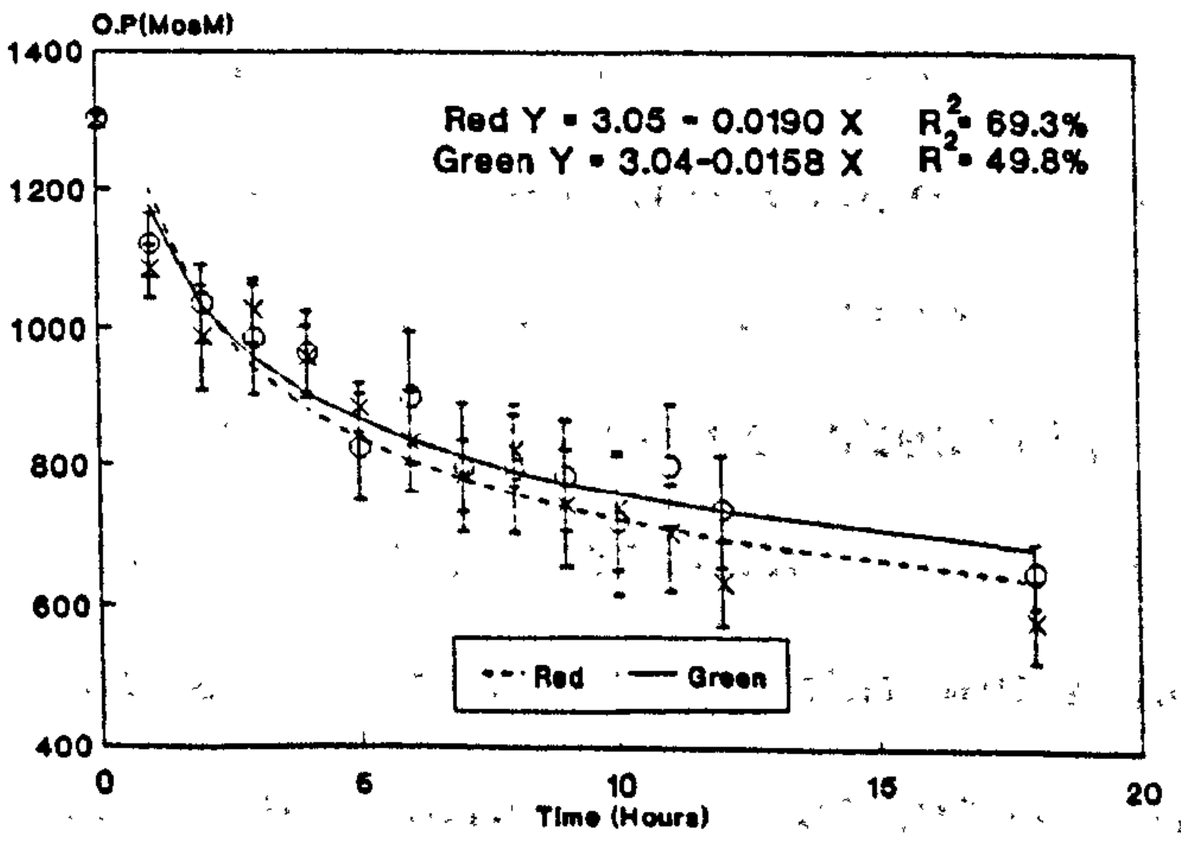
A)


Low (17ppt) acclimated
O.P(MoeM)
Red Y = 2.84-0.00872 X
R2= 42.6%
Green Y = 2.87-0.00749 X
R2= 35.4%
800
700
600
500
400
0
5
10
15
20
Time (Houre)

B)


Normal (34ppt) acclimated
O.P(MoeM)
Red Y = 2.97-0.0177 X
R2= 76.7%
Green Y = 2.98-0.0135 X
R2= 78.0%
1100
1000
900
800
700
600
500
400
0
5
10
15
20
Time (Houre)

C)


High (50ppt) acclimated
O.P(MoeM)
Red Y = 3.05 - 0.0190 X
R2= 69.3%
Green Y = 3.04-0.0158 X
R2= 49.8%
1400
1200
1000
800
600
400
0
5
10
15
20
Time (Houre)
Red
Green

HAEMOLYMPH OSMOLALITY AS RELATED TO SIZE AND TIME OF EXIT FROM LOW SALINITY.

This experiment was designed to assess the significance of the variables discussed in the material and methods section, and Figs. 1.7. to 1.9. show the results obtained. Spearman rank correlation tests were initially performed on the data sets to find if there was any relationship between the two variables under test. Samples were large (>30) permitting T-tests to be carried out, followed by regression analyses of significant correlations, the equations and R^2 values of which are shown on the graphs.

Figs. 1.7.A, 1.8.A and 1.9.A respectively, show that a negative correlation is obtained between the haemolymph osmolality and time of exit, after crabs had been acclimated to low (T-test, 60DF, $P<0.05$, R=2.729, G=1.790, S), normal (T-test, 60DF, $P<0.05$, R=10.764, G=5.629, S) and high (T-test, 60DF, $P<0.05$, R=8.34, G=7.11, S) salinities. If the crabs were exiting the low salinity when the haemolymph osmolality fell to a certain critical level then the slopes of the regression lines would be negligible. However the haemolymph osmolality was found to decrease steadily with time, in a similar fashion to the pattern observed in Fig. 1.6. A,B,C. This suggests that the crabs were not responding to changes in the haemolymph osmotic pressure when exhibiting a behavioural reaction. Comparisons of the

rate of change (slope) and insertion points (elevation) between the red and green crabs ($P>0.05$, 130 DF) at each acclimation salinity all proved not to be statistically significant.

Figs. 1.7.B, 1.8.B and 1.9.B respectively show time of exit in relation to the size of the animal. The only significant correlation obtained was for normal acclimated red crabs (60DF, $P<0.05$ R,T=2.357, S). In these there was a slight increase in size with increasing time of exit (Fig. 1.8.B), though even here the R^2 value of the regression analysis is low (5%), and there is a large variation within the data. In view of these results it can be concluded that there is no clear relationship between the crab size and its choice behaviour in either of the colour forms.

Figs. 1.7.C, 1.8.C and 1.9.C show the haemolymph osmolality as a function of the size of the crab. Significant correlations were obtained for low salinity acclimated red crabs (60DF, $P<0.05$, R,T=1.739, S) and high salinity acclimated green crabs (60DF, $P<0.05$, R,T=2.43 S). In both cases there is an increase in haemolymph osmolality with increasing size, but here again R^2 values obtained from regression analysis were only 2.8% and 4.5% respectively. These results suggest that there is no clear relationship between haemolymph osmolality and carapace width over the range studied.

The carapace width and haemolymph osmolality of red

and green crabs remaining in the 5ppt. salinity after 7 hours had elapsed were compared using a Mann Whitney U-test. There was found to be no difference in the size of red and green crabs remaining in the low salinity after acclimation to low ($P>0.5$), normal ($P>0.1$) and high ($P>0.1$) salinities. Red crabs remaining in the 5ppt. were found to have significantly lower haemolymph osmolalities when acclimated to low ($P<0.01$) and normal ($P<0.001$) salinities, as compared with green crabs. Although haemolymph osmolality was also lower in red crabs remaining in 5ppt. salinity after high salinity acclimation, this difference proved not to be statistically significant ($P>0.2$).

Fig. 1.7.

The relationship between haemolymph osmolality, carapace width and time of exit from a salinity of 5ppt. for low salinity (17ppt.) acclimated red and green crabs.

A) Haemolymph osmolality (milliosmoles) as a function of time of exit (minutes) from the low salinity.

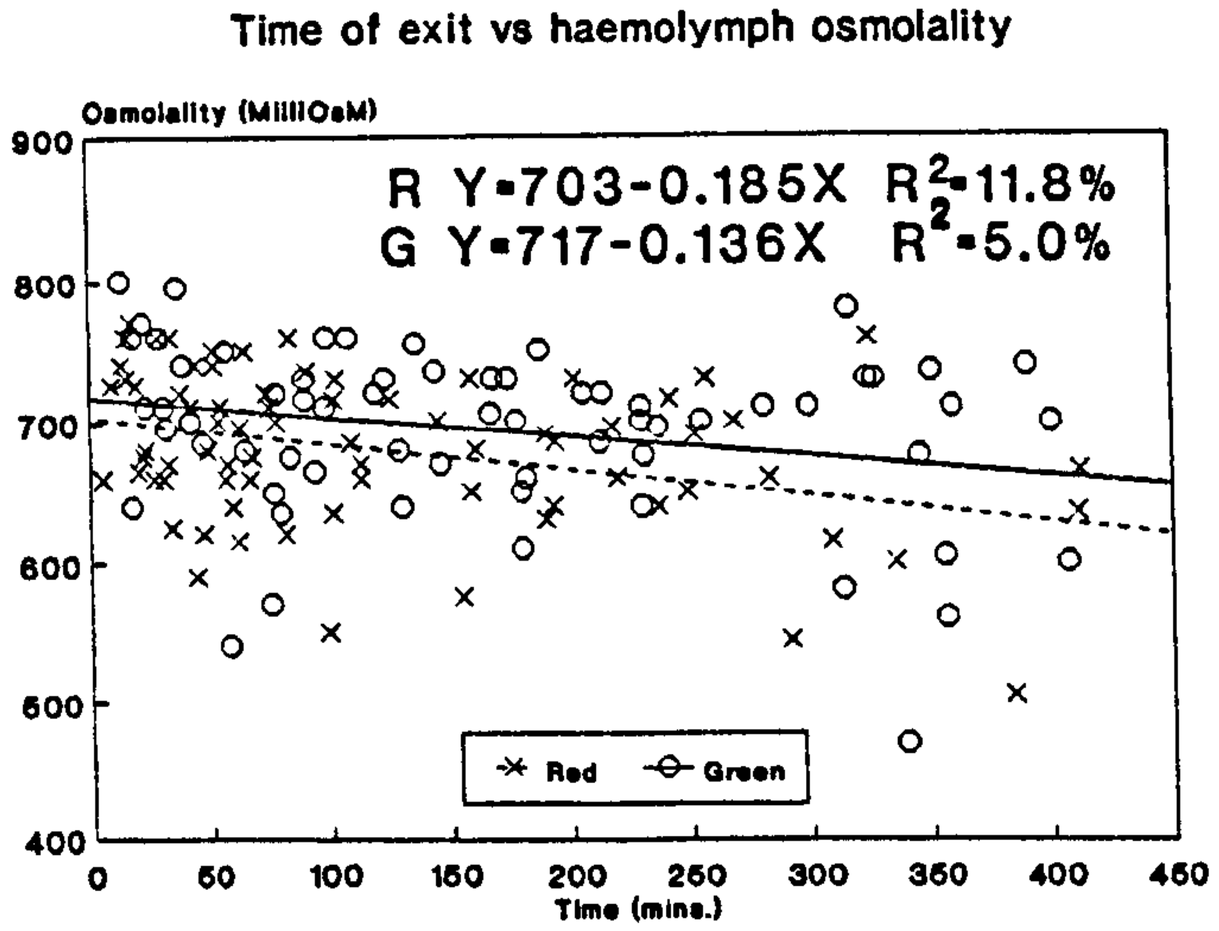
B) Size of the crab (mm) as a function of its time of exit from low salinity.

C) Haemolymph osmolality as a function of the size of the crab.

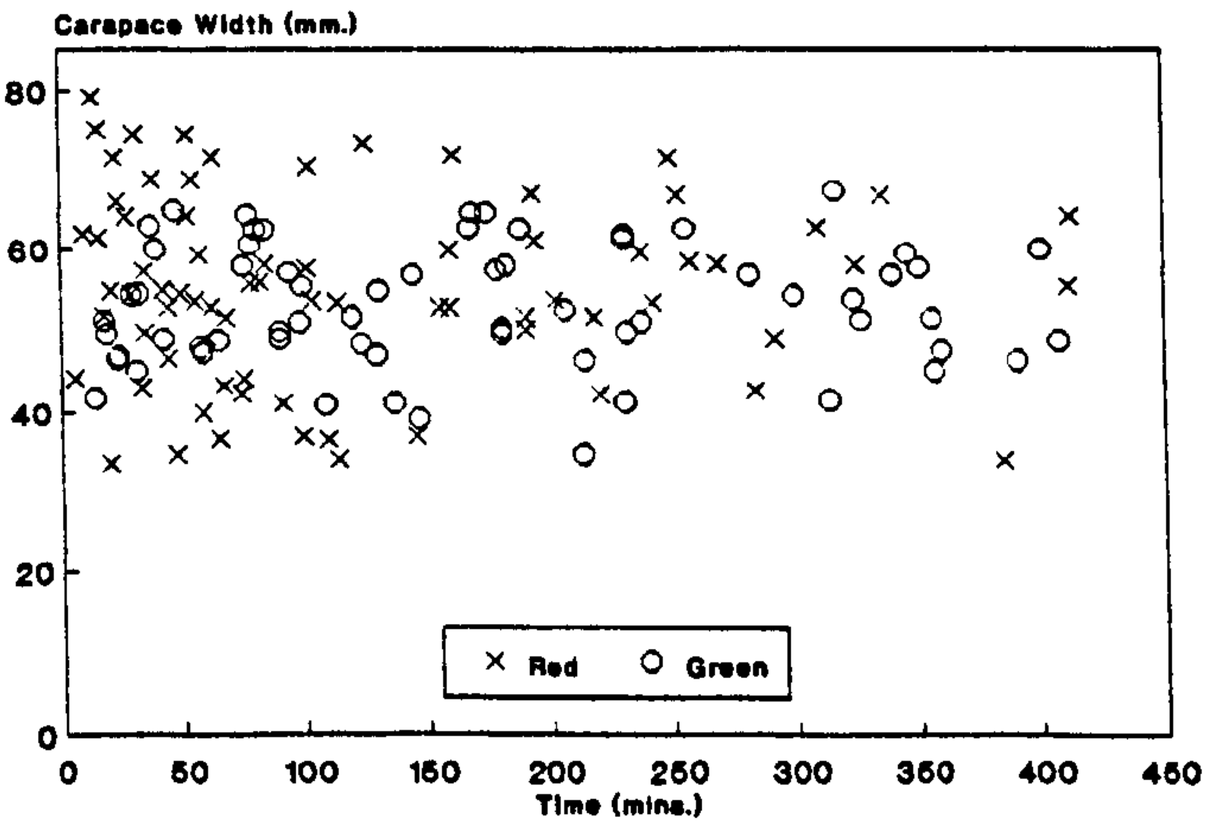
Graphs represent pooled data from 4 repeat experiments each with 25 crabs of each colour. Regression lines with equations and R^2 values are given where a significant correlation between the two factors occurs.

Fig. 1.7. Low salinity acclimated (17ppt.) crabs

A)



B)



C)

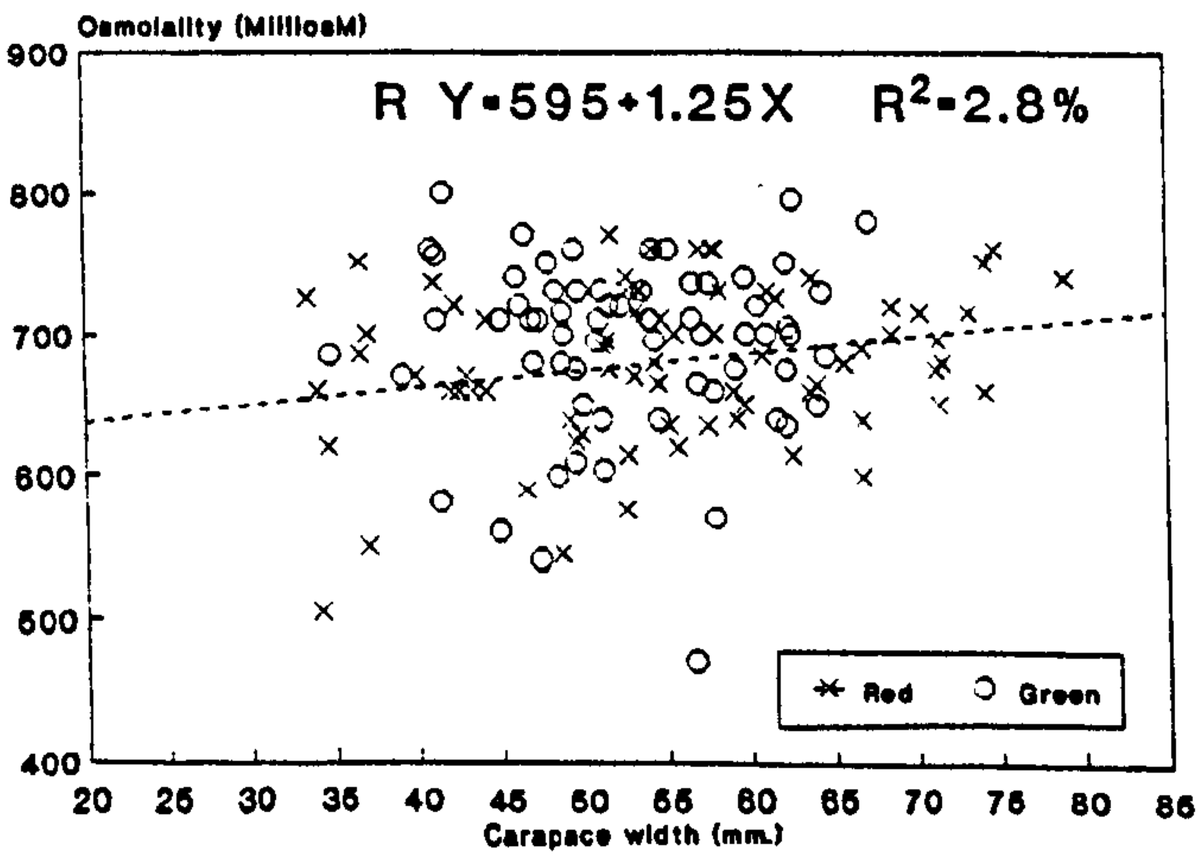


Fig. 1.8.

The relationship between haemolymph osmolality, carapace width and time of exit from a salinity of 5ppt. for normal salinity (34ppt.) acclimated red and green crabs.

A) Haemolymph osmolality (milliosmoles) as a function of time of exit (minutes) from the low salinity.

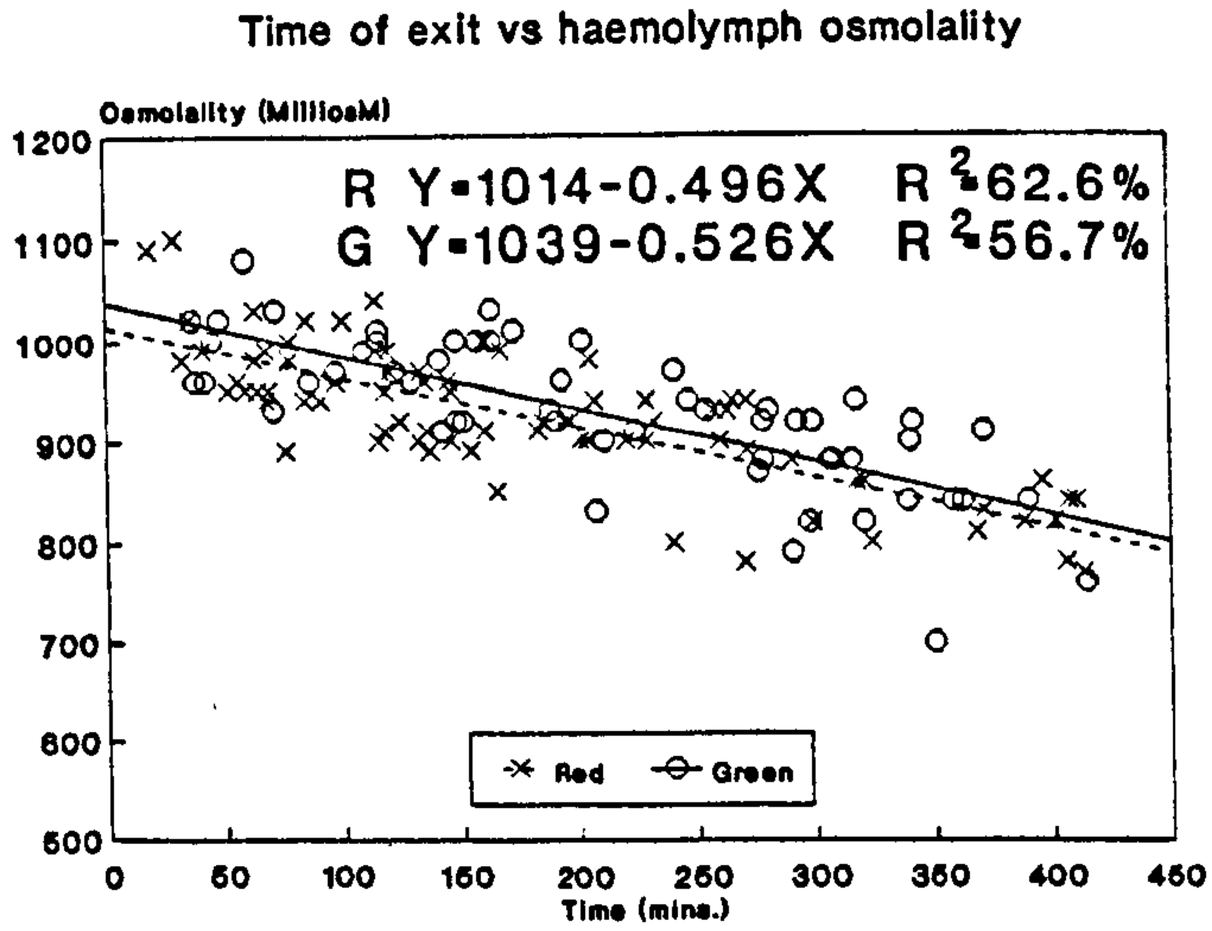
B) Size of the crab (mm) as a function of its time of exit from low salinity.

C) Haemolymph osmolality as a function of the size of the crab.

Graphs represent pooled data from 4 repeat experiments each with 25 crabs of each colour. Regression lines with equations and R^2 values are given where a significant correlation between the two factors occurs.

Fig. 1.8. Normal acclimated (34ppt.) crabs

A)



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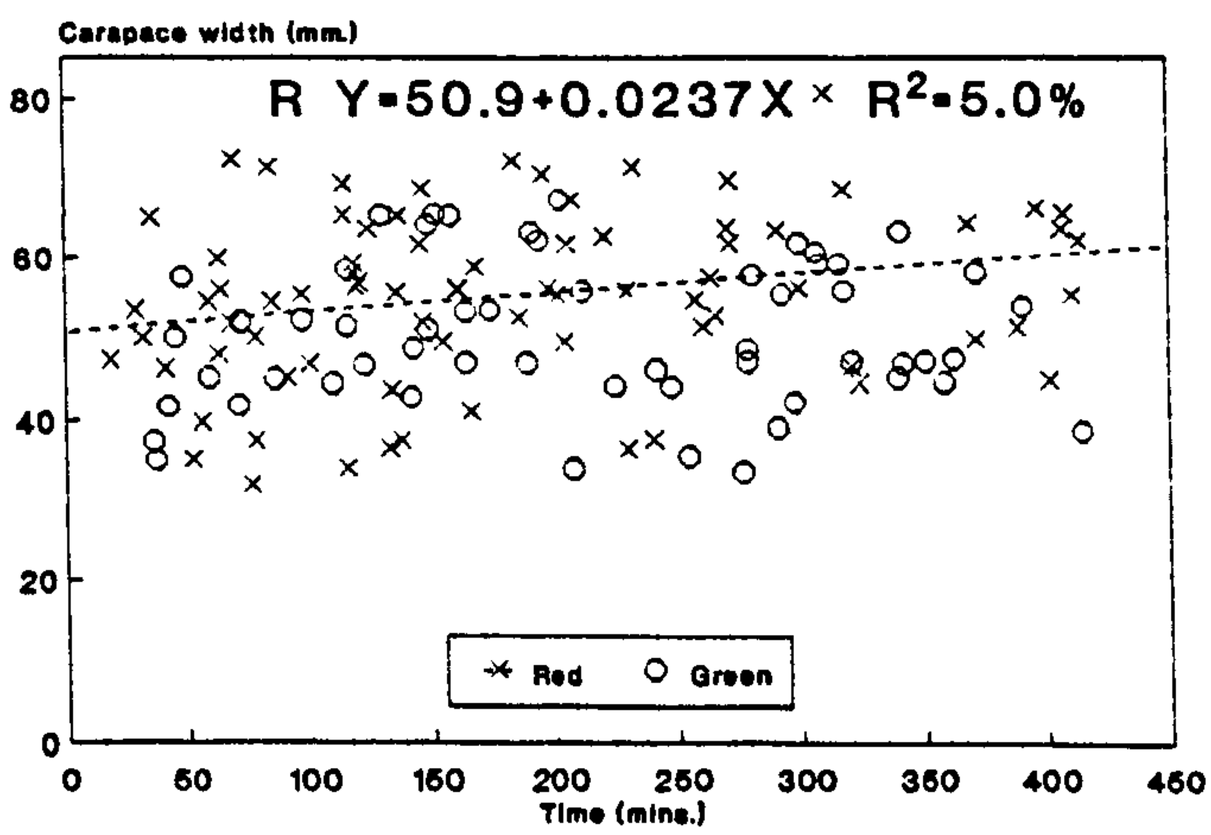


C)

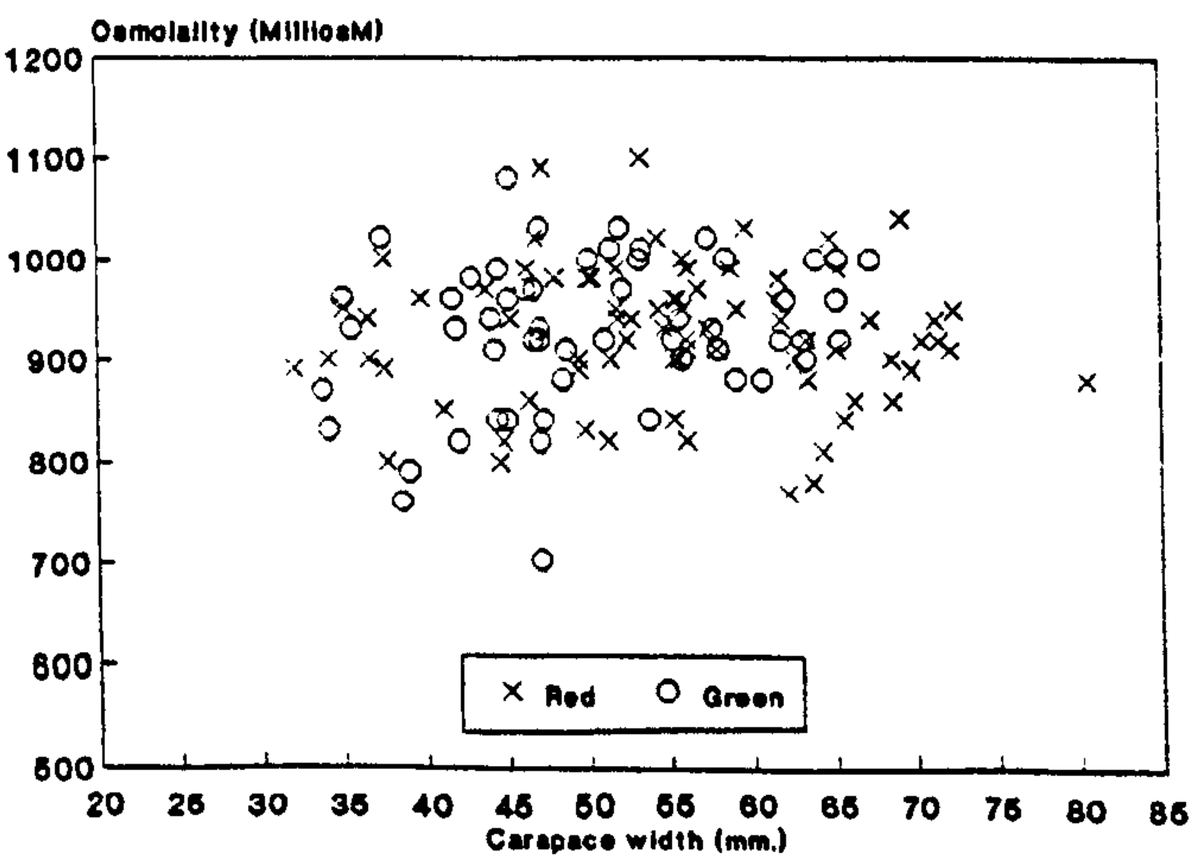


Fig. 1.9.

The relationship between haemolymph osmolality, carapace width and time of exit from a salinity of 5ppt. for high salinity (50ppt.) acclimated red and green crabs.

A) Haemolymph osmolality (milliosmoles) as a function of time of exit (minutes) from the low salinity.

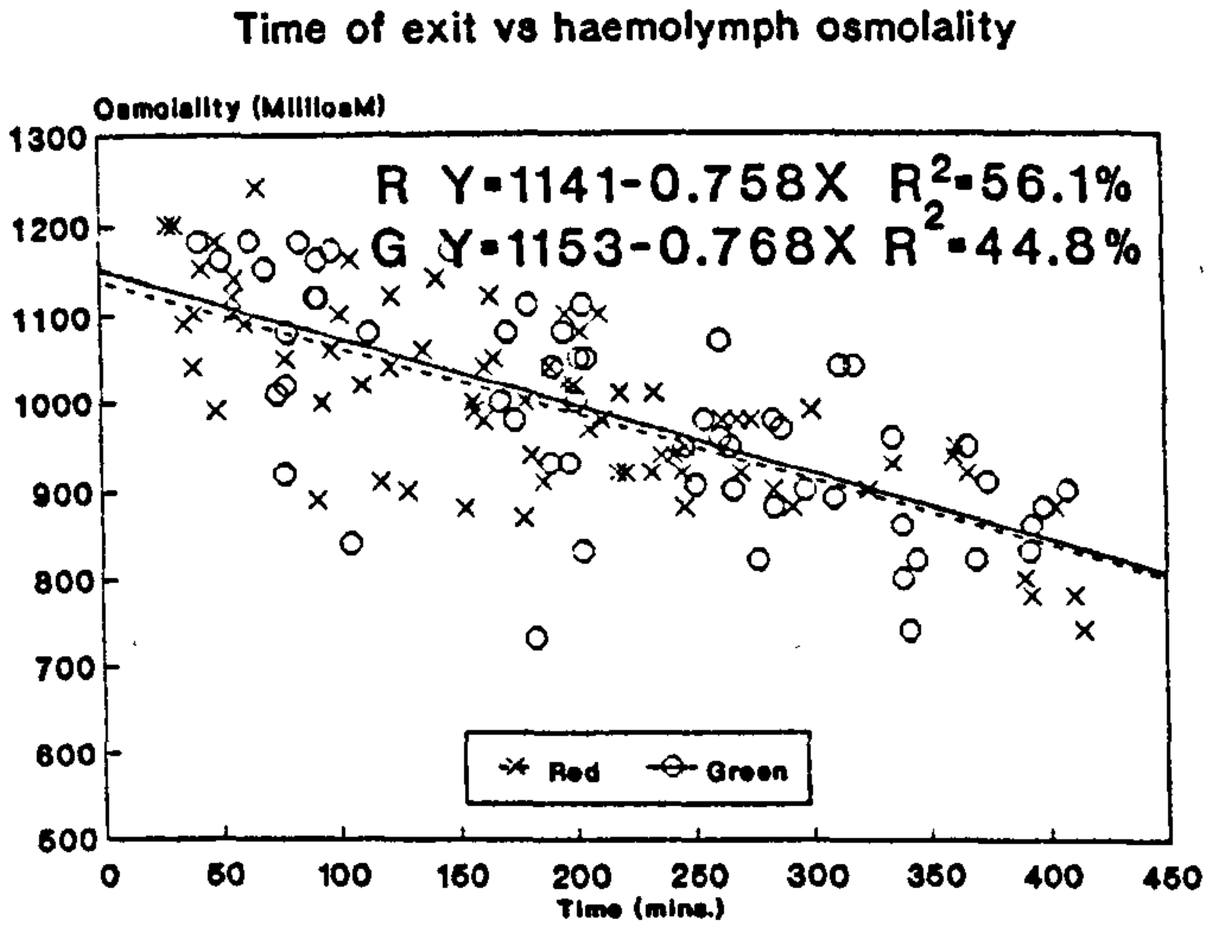
B) Size of the crab (mm) as a function of its time of exit from low salinity.

C) Haemolymph osmolality as a function of the size of the crab.

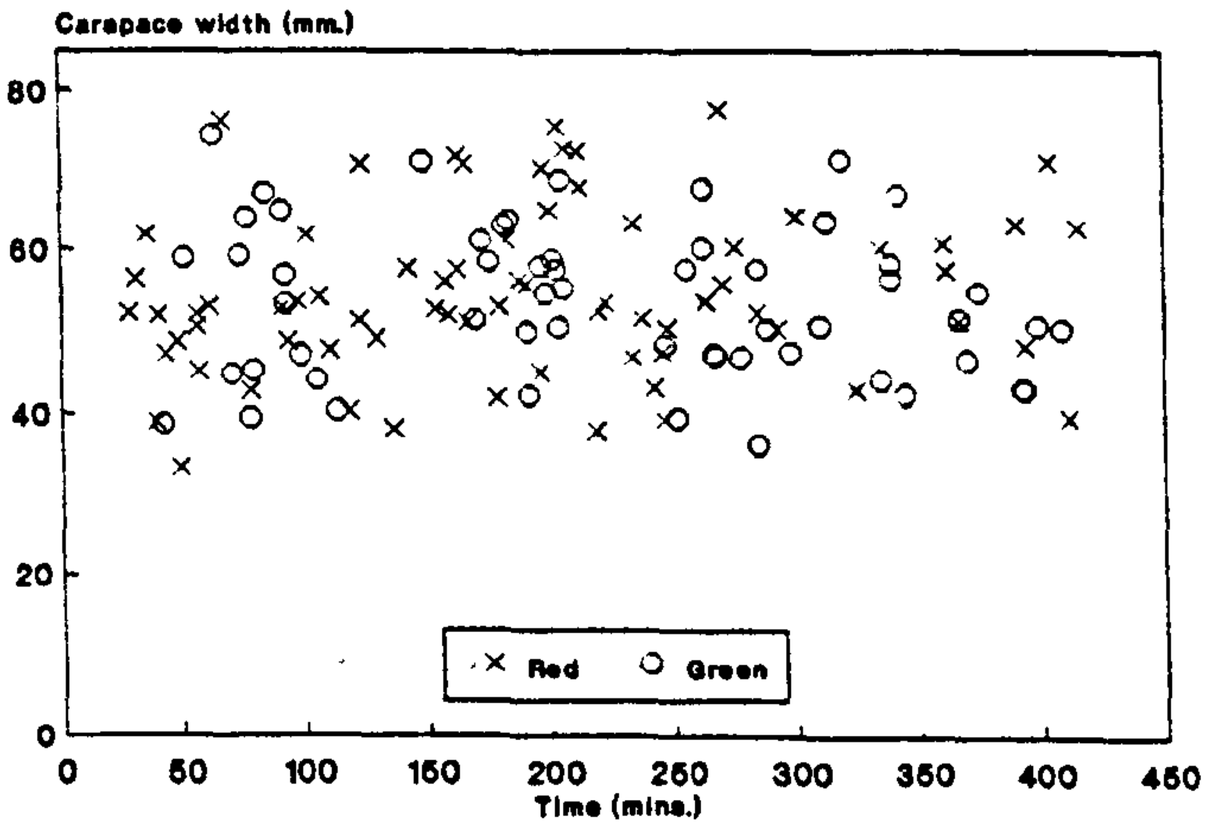
Graphs represent pooled data from 4 repeat experiments each with 25 crabs of each colour. Regression lines with equations and R^2 values are given where a significant correlation between the two factors occurs.

Fig. 1.9. High salinity acclimated (50ppt.) crabs

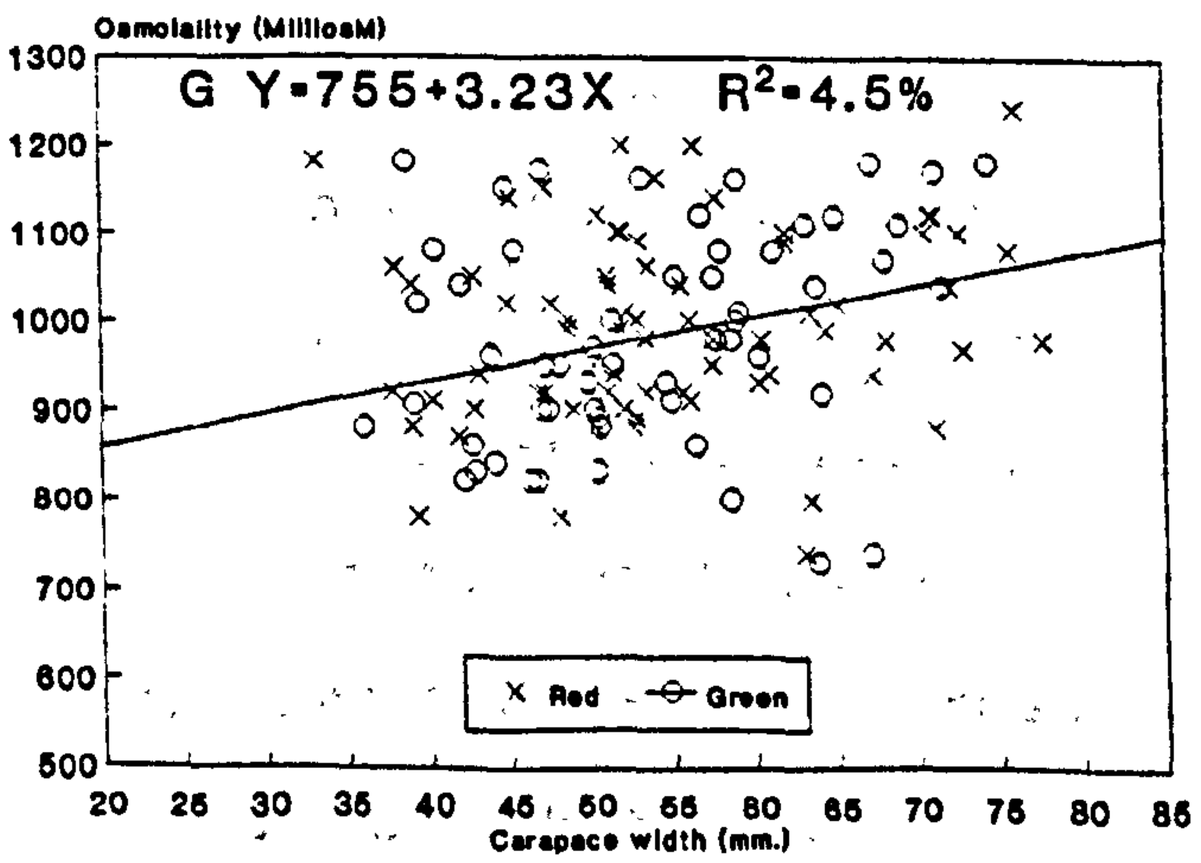
A)



B)



C)



HEART-RATE

Fig. 1.10.A shows the change in heart-rate of red and green crabs after lowering the salinity from 34ppt. to 5ppt. A large variation in heart-rate occurred between individual crabs even in full seawater. The mean heart-rate in 34ppt. salinity was similar for the red and green crabs at between 80 and 90 beats/min. An increase in heart-rate occurred upon lowering the salinity, and the heart-rate of red crabs reached a mean maximum value of about 115 beats/min. after 1 hour in low salinity. From then onwards the heart-rate of red crabs declined at a steady rate, reaching a mean value of around 50 beats/min. after 12 hours in 5ppt. salinity. The heart-rate of green crabs also rose sharply on transfer from 34ppt. to 5ppt. to a value of about 110 beats/min. after 1 hour in low salinity. The heartbeat reached its maximum rate of 117.3 beats/min. after 3 hours in 5ppt. Thereafter there was again a downward trend in the pattern of heartbeat rate, but the rate of decline was lower than that of the red crabs. After 12 hours in 5ppt. salinity the heart-rate of green crabs had fallen to a mean rate close to that in full seawater at the start of the experiment. The larger variation in red crab heart-rate after 7 hours is accounted for in that heart-rate decreased substantially in one individual at that time, when also another individual died. A hiloglinear test SPSS-X (see

Appendix) was carried out on the data and Table 1.5. shows the results obtained. Clearly significant changes in heart-rate occurred in each colour form with time, and the changes were significantly different from each other when comparing the red and green crabs.

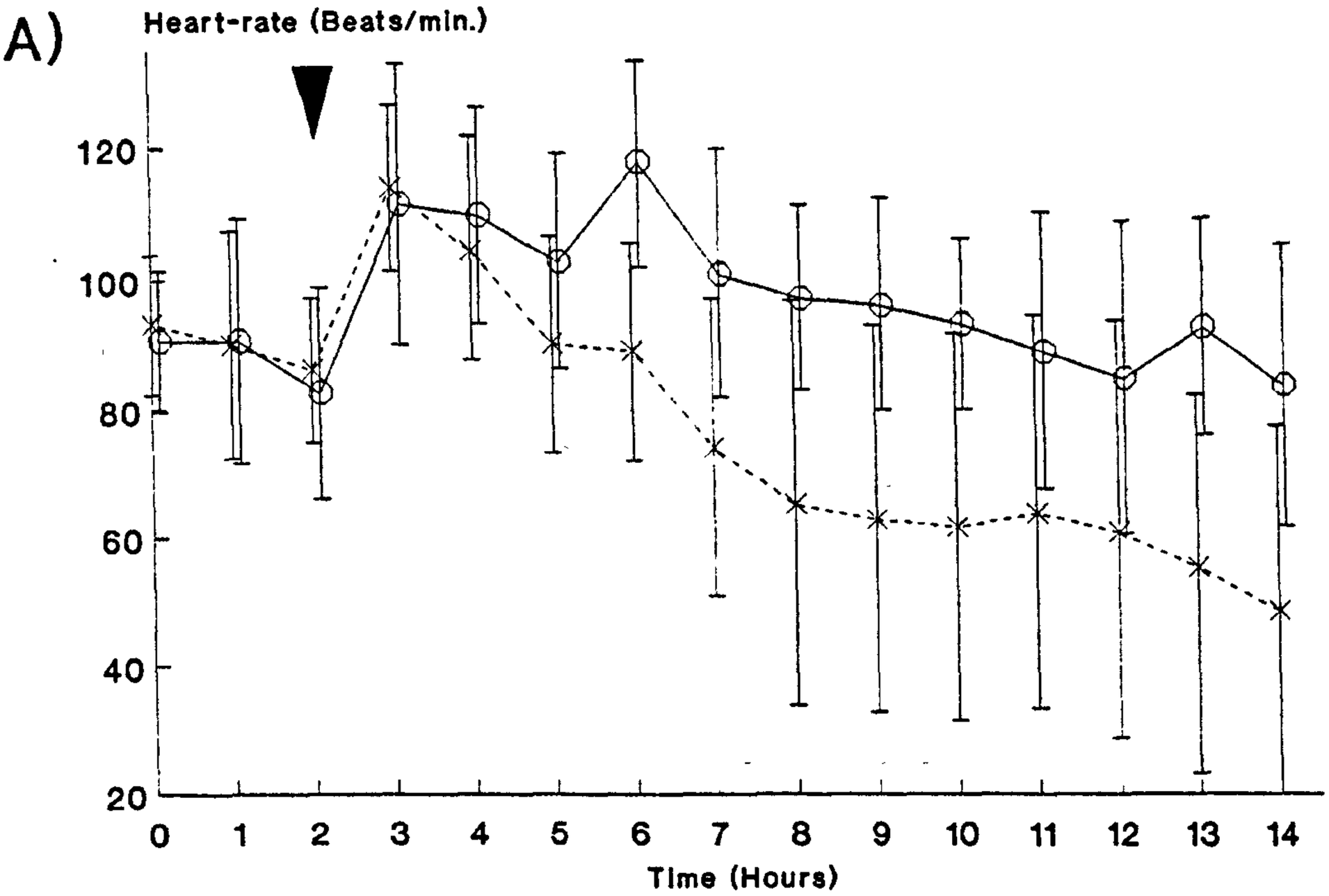
Fig. 1.10.B shows the change in heart-rate, expressed as a percentage of the preceding value. This confirms that the largest percentage increase in both colour forms occurs immediately after addition of the freshwater, and that subsequently the hourly percentage changes were more frequently lower in red crabs than in green crabs.

Fig. 1.10

A Heart-rate (Beats/min.) of seven red and seven green crabs (with 95% C.L. of the mean) recorded for 2 hours in seawater and subsequently for 12 hours in salinity of 5ppt.

B Percentage change in heart-rate, expressed as a percentage of the previous value (mean values with 95% C.L.)

Fig. 1.10. Heart-rate



Percentage change in heart-rate

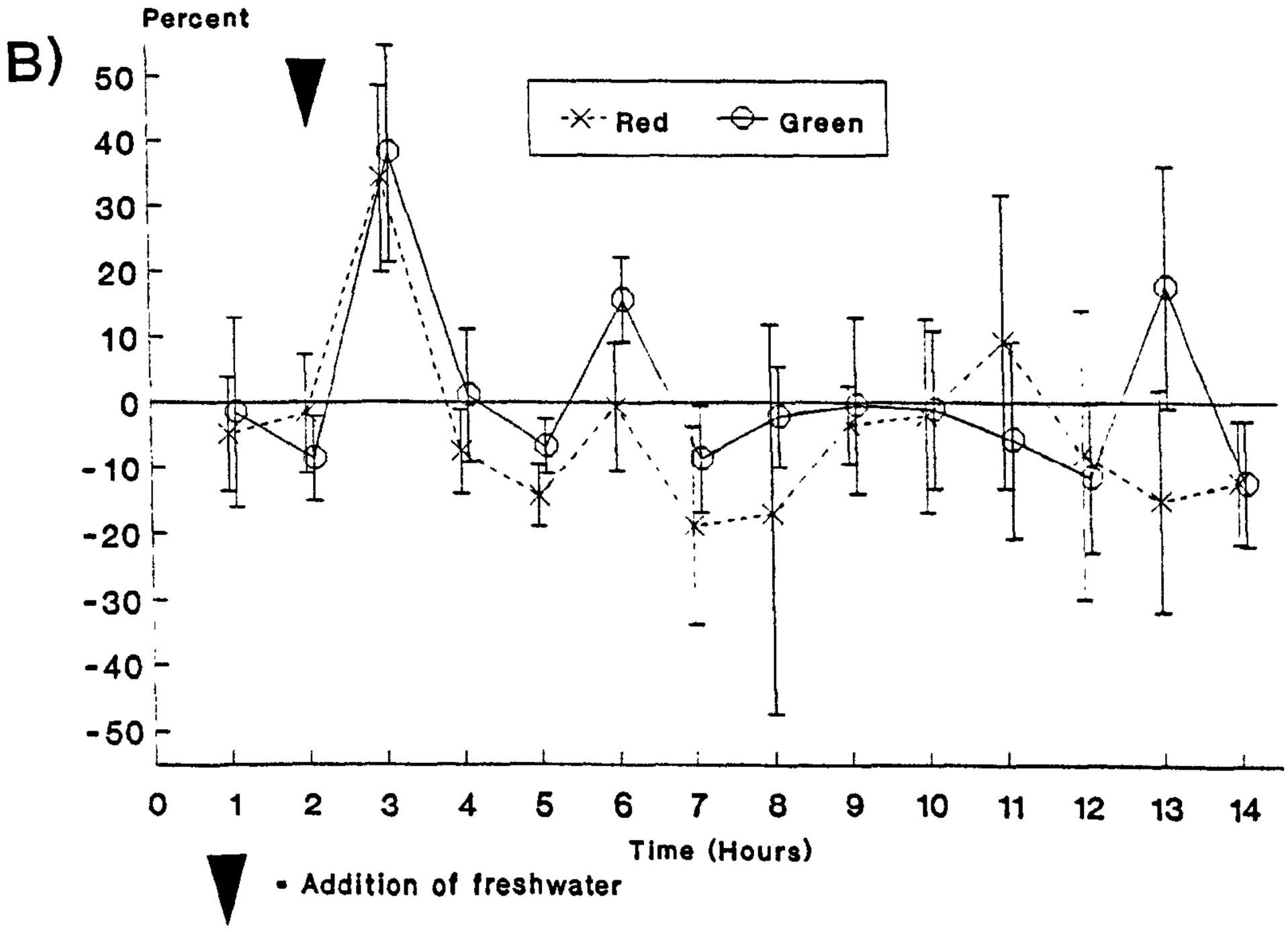


TABLE 1.5. Hiloglinear test for change in heart-rate in response to salinity change.

Interaction	DF	Partial Chisqu.	Prob.
TIME	14	415.007	(**)
COLOUR	1	213.135	(**)

(**= P<0.01)

DETERMINATION OF MOULT STAGE

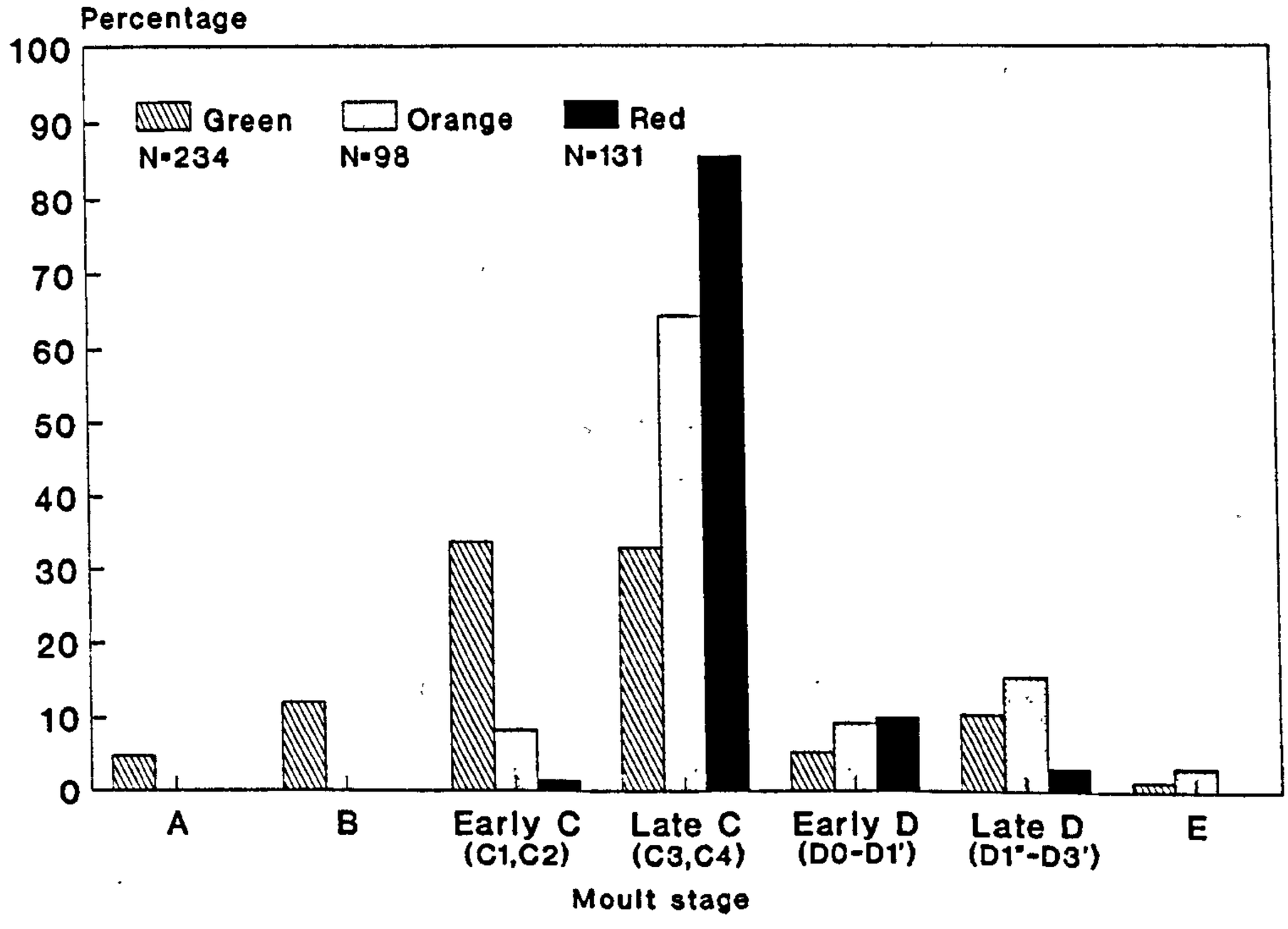
Fig. 1.11.A shows the moult stages of green, orange and red crabs. There is a progressive change in colour from postmoult (stages A and B) to late intermoult (late C). All crabs that moult, irrespective of their colour, become green at immediate postmoult. This coloration is maintained throughout moult stages A and B. In early C stage the majority of crabs are still green, but there is now a small percentage of orange and red crabs. The majority of orange and red crabs were found to be in the Late C stage, by which time there were more than twice as many red crabs as green crabs, with orange crabs of intermediate abundance. Relatively equal percentages of red and green crabs were found in premoult (D stage), when the relative percentage of orange crabs was greatest. Only a small number of crabs were found actually moulting (stage E), as this stage lasts only for a few hours.

Fig. 1.11.B shows the percentage of crabs of each colour at each stage that were encrusted with epibionts, mainly barnacles, spirorbid worms and bryozoans. This shows that epibionts first became apparent in late C stage and that there was an increase in percentage of crabs encrusted with epibionts from late C through early D and late D stages. There was also a progressive increase in the percentage of crabs with epibionts from green through orange to red at each of the moult stages.

Almost twice as many red crabs were encrusted with epibionts as compared to green crabs. The overall percentage of animals with epibionts were: green crabs 15.8%, orange crabs 32.7% and red crabs 53.4%.

Fig. 1.11. Distribution of colours of *Carcinus* with respect to the moult stage.
(Male crabs 40-80mm. carapace width)

A)



Percentage of crabs in each moult stage covered with epibionts

B)

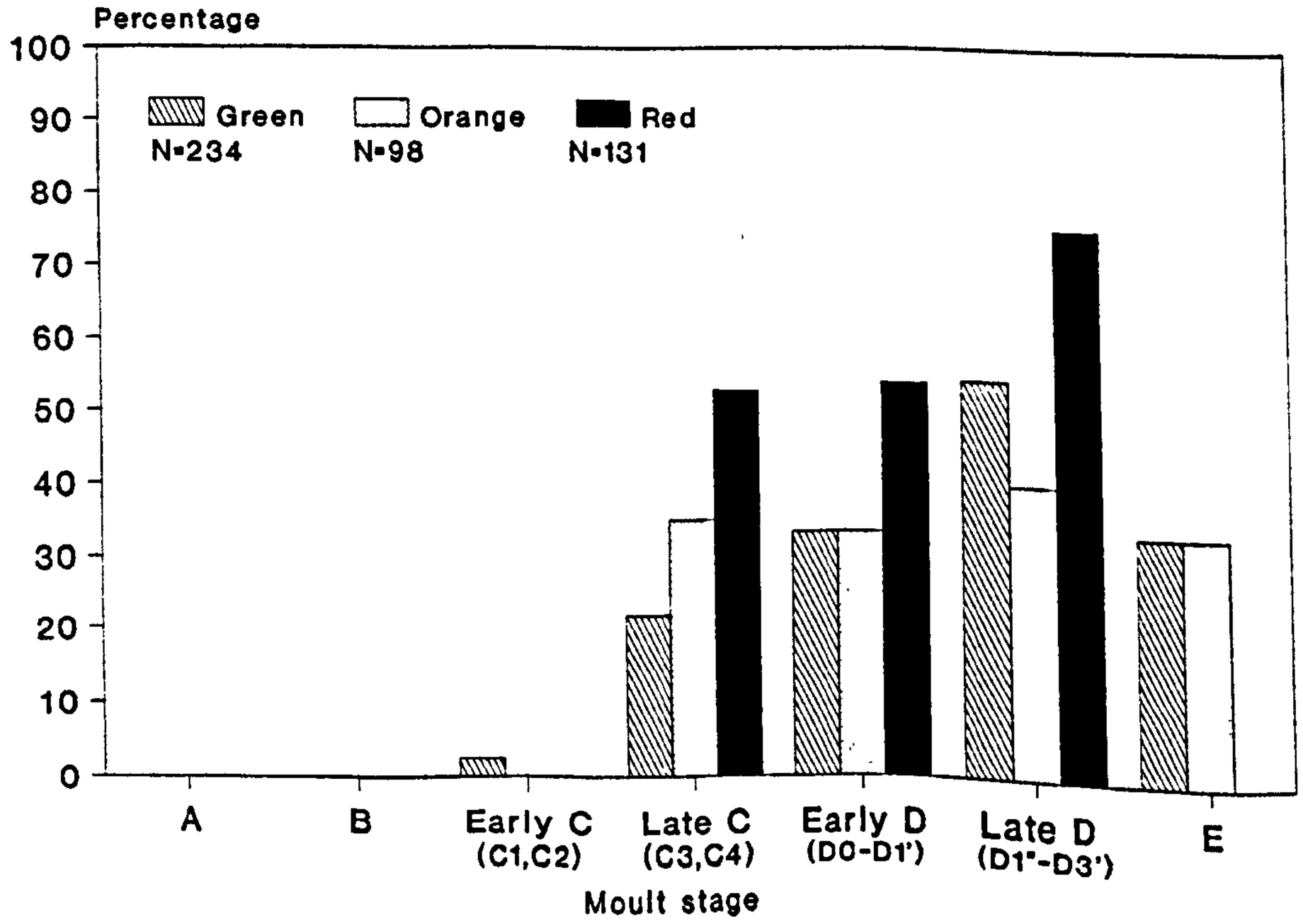
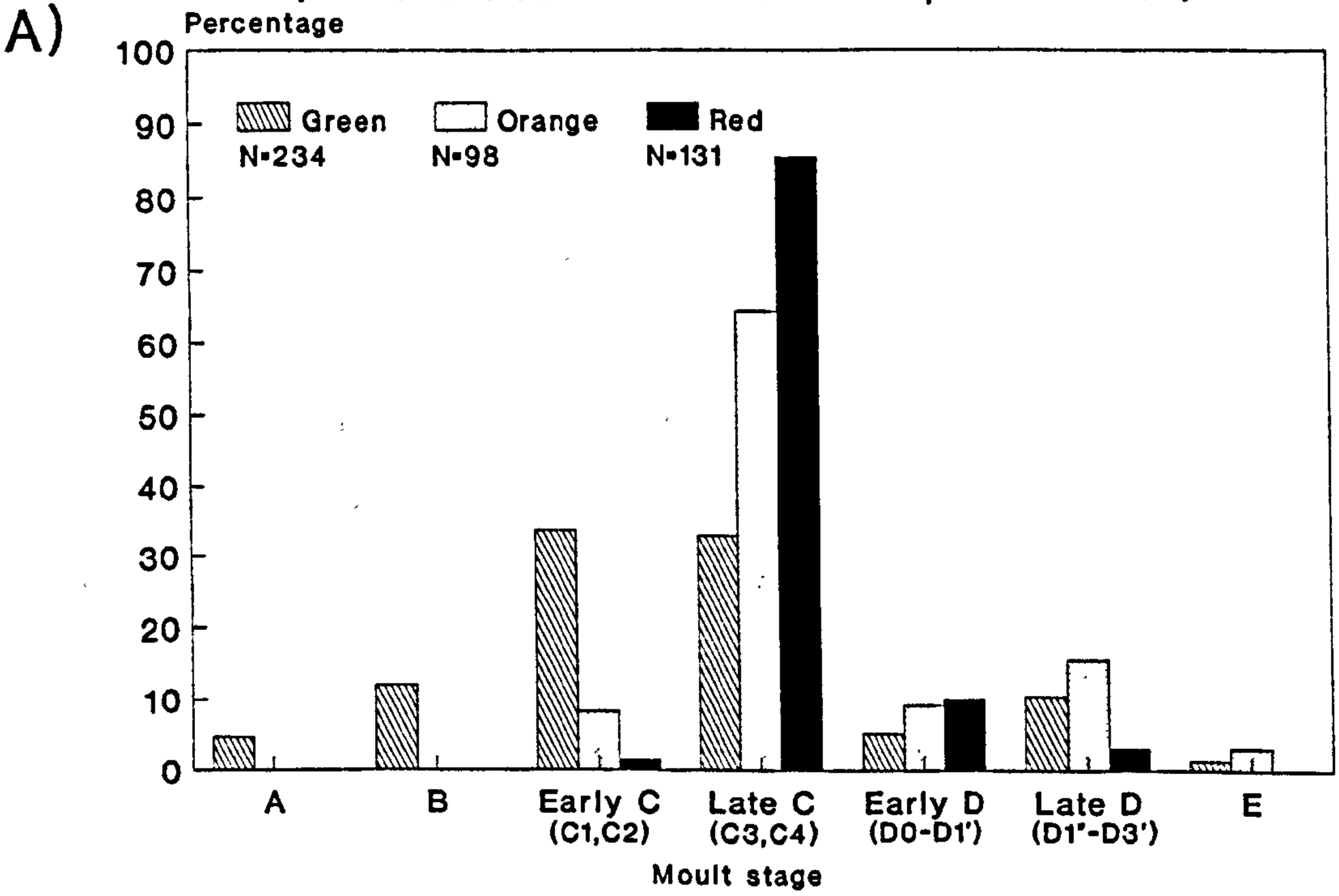
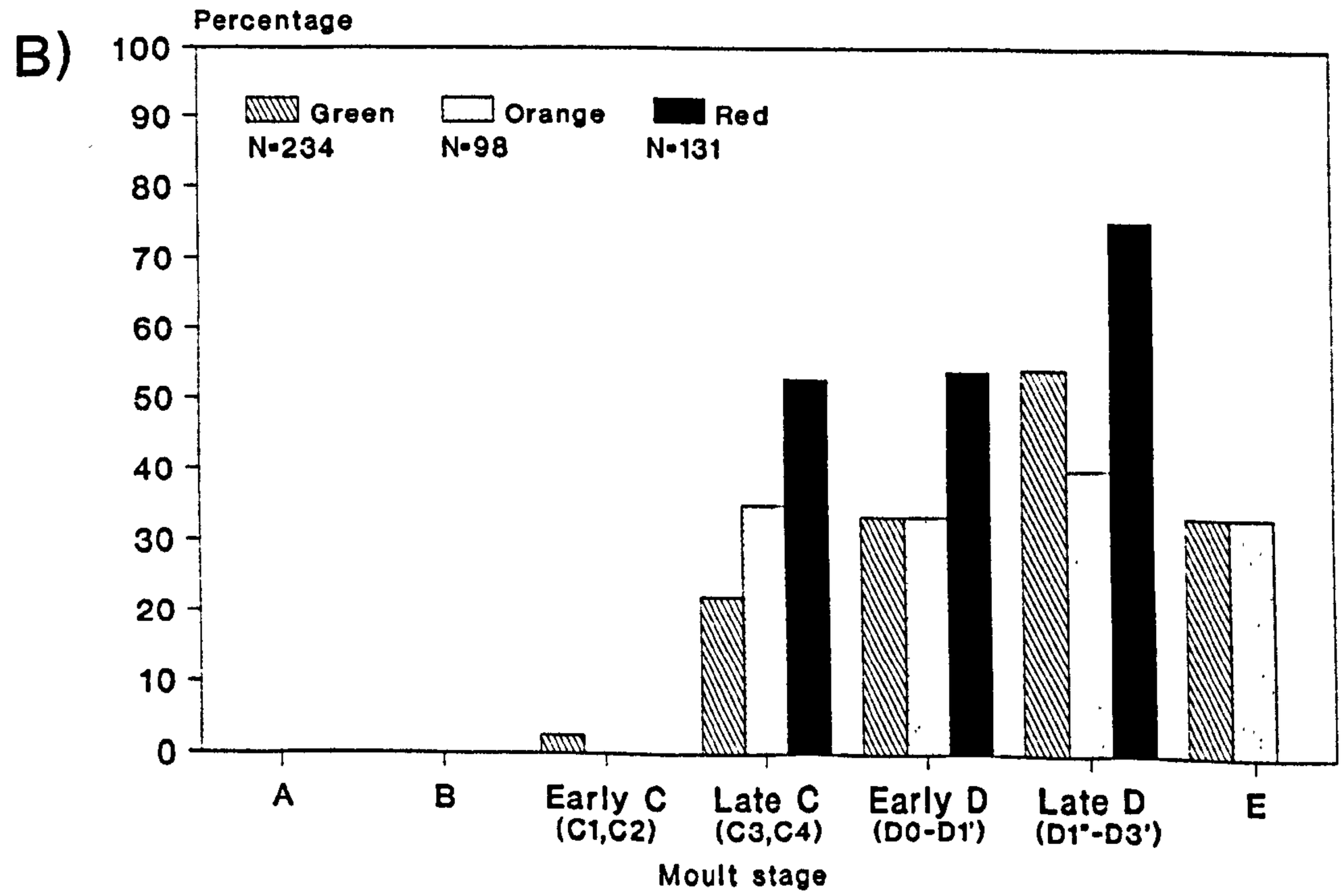


Fig. 1.11. Distribution of colours of *Carcinus* with respect to the moult stage.
(Male crabs 40-80mm. carapace width)



Percentage of crabs in each moult stage covered with epibionts



CARAPACE THICKNESS

The thickness of red and green crab carapaces was examined to determine if red crabs in addition to having a larger proportion of epibionts, also showed a greater degree of carapace calcification.

Fig. 1.12. shows the carapace thickness as a function of the size of the crab. Regression equations and R^2 values are included on the graph. There is, as expected, an increase in carapace thickness with increasing carapace width in both colour forms. Red crabs, however, tend to have a thicker carapace over the size range investigated. Comparison of the slope of the regression lines (Zar, 1974) shows no significant difference ($Z=0.021$, 104 DF, $P>0.05$, NS), but there is a statistically significant difference between the elevations of the two regression lines ($T=6.86$, 104DF, $P<0.05$, S). Thus at any given size over the range tested, red crab carapaces are approximately 0.09 mm thicker than those of green crabs of similar size.

Fig. 1.12.

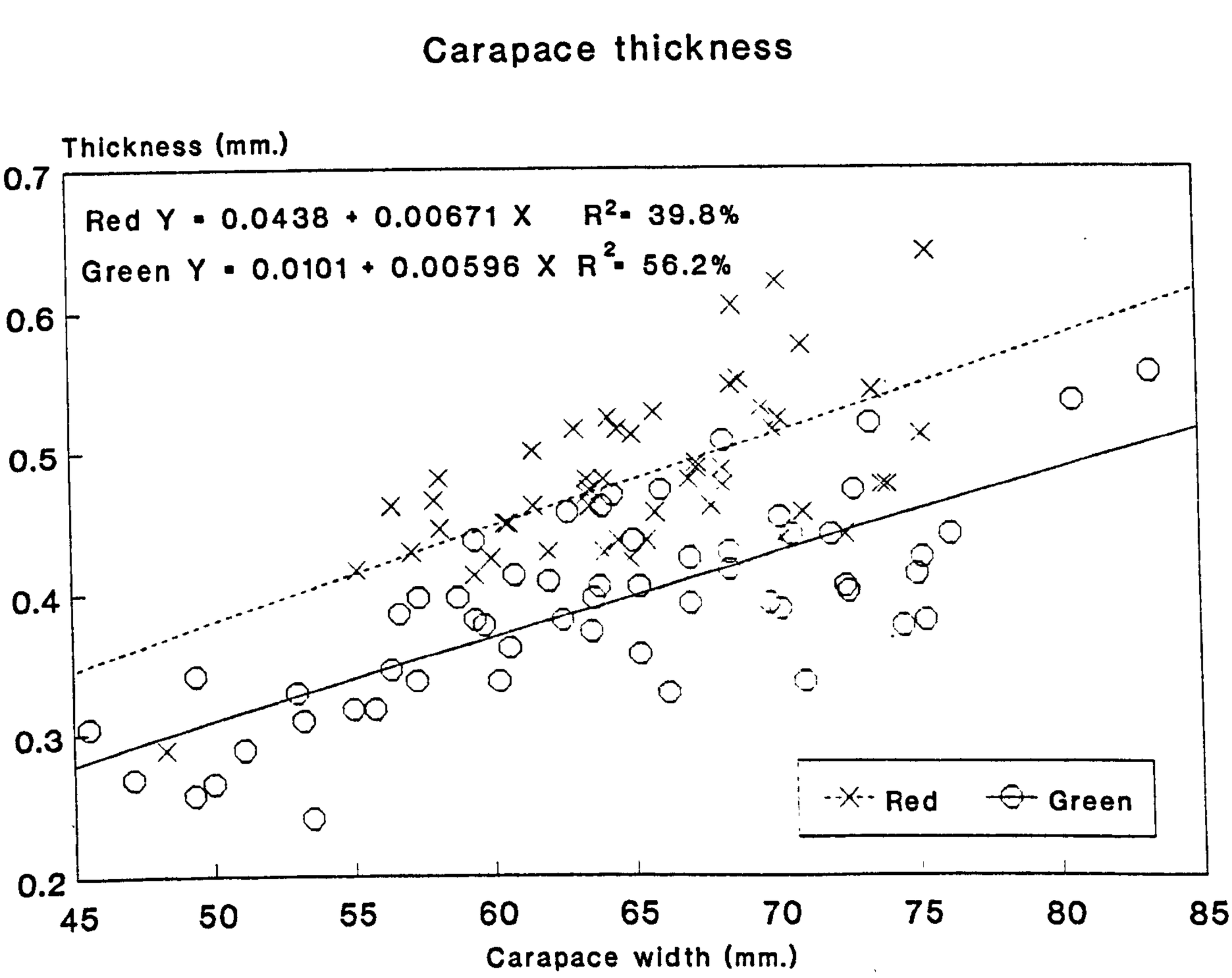


Fig. 1.12. Carapace thickness (mm) of red and green crabs as a function of the carapace width. Points shown on the graph are a mean thickness obtained from ten random measurements taken along the midline (antero-posteriorly) of the carapace. Equations and correlation coefficients (R^2 values) of the fitted regression lines are also given.

DISCUSSION

Carcinus has long been reported to survive indefinitely in salinities as low as 4ppt. (Poulsen, 1922, 1949, Broekhuysen, 1936), a large increase in survival time occurs between salinities of 2 to 3ppt. (Perkins *et al*, 1969). However, only recently (Reid *et al*, 1989) has attention been drawn to the need to consider different survival times of *Carcinus* of different colours. Present examination of mortality rates between the groups of crabs tested illustrates that large green male crabs exhibit a wider salinity tolerance range than any other group tested. Red male crabs have the narrowest range of salinity tolerance, their mortality rate being rapid in the lowest salinities tested. These findings confirm and extend those of Reid *et al*, (1989), though in that study the mortality rates of both red and green crabs were much greater than those of the present study.

The high mortality rate observed in the red crabs may also partly be due to the effects of hypoxia. Reid and Aldrich (1989) reported higher mortality rates of red crabs in hypoxic conditions, which might have been generated after some time in present experiments since oxygen uptake by *Carcinus* is reported to increase at lower salinities (Taylor, 1977, Taylor *et al*, 1977).

In the present experiments crabs were not fed because of the difficulty in ensuring that each crab

consumed equal amounts of food (McLusky and Heard, 1971). Many of the deaths after longer time periods could therefore be related to the effects of starvation rather than the salinity. Certainly it is known that energy requirements for metabolic processes are higher in low salinity (Spaargaren, 1974, 1975c, 1982, Siebers *et al*, 1972, Taylor, 1977), therefore future experiments should examine survivability of fed animals.

Red crabs were found to have a significantly lower haemolymph osmolality in salinities of 17ppt. and below compared with green crabs. These differences were paralleled by similar variations in the major cations in the haemolymph suggesting that these, rather than organic constituents are responsible for controlling the haemolymph osmotic pressure. The concentrations of haemolymph magnesium, calcium and potassium were higher in red crabs compared to the green crabs in a salinity of 40ppt. Reasons for this pattern are unclear, but may have arisen due to unequal food consumption, although both colours of crabs were not fed for two days prior to experimentation. Red crabs may be unable to cope as well with higher salinities, however, this would also be expected to have been reflected in the haemolymph osmolality and sodium levels. The concentrations of potassium were very variable over the range of salinities tested and no definite trend was apparent with respect to the colour of the individual. Robertson

(1960) also reports that this is the most variable ion in the haemolymph.

The balance of internal fluids is dependent on the rate of active uptake and the permeability (Spaargaren, 1974a). Differences between these mechanisms may account for the observed disparities of regulatory ability between the red and green crabs. The increase in oxygen consumption at lower salinities (Taylor, 1977, Taylor *et al*, 1977) is associated with active uptake and variations occur in relation to the rate of maximum uptake (Spaargaren, 1975a,b). The sodium uptake is dependent on Na-K-ATPase activity (Siebers *et al*, 1982), which is found to increase in low salinities (Towle, 1981, Siebers *et al*, 1983). It serves as a protective mechanism to prevent the haemolymph concentration falling to critical levels. However it is reported that ATP-ase activity takes 2 to 3 weeks to reach new stable levels (Towle, 1981, Siebers *et al*, 1983, Winkler, 1986) and it is not modified over a tidal salinity cycle (Winkler *et al*, 1988). Instant changes are brought about by activation/inhibition by sodium of a number of unchanged ATP-ase molecules. It reaches its maximum at about 6ppt., the lower range survived by crabs (Siebers *et al*, 1983).

Differences in permeability may also account for the lower osmolality. There are two responses to permeability, a slow change and a much faster change

occurring in a few hours even before the internal fluid concentrations have equilibrated (Spaargaren, 1974a, 1975a,b). The body surface and gills are found to be a major pathway for loss of ions (Shaw, 1961), although *Carcinus* may be able to reduce water influx across the integument (Smith, 1967, 1970). However, it would be expected that since the red crabs are more heavily calcified (Kaiser *et al*, 1990, Fig. 1.12.) that they would be less permeable. Clearly in future both the ATP-ase activity and the permeability of red and green crabs needs to be analysed to determine if these affect the osmoregulatory abilities.

Strong regulation of the internal fluids takes place in the 15ppt. to 25ppt. range (Spaargaren, 1974a). When offered a choice of salinities (Chapter 3) crabs tended not to discriminate between salinities greater than about 22ppt. (Figs. 3.5, 3.6, 3.8). It would suggest that they show faster avoidance of salinities where they have to expend energy regulating the internal fluid concentration. The red crabs also exit salinities of 17ppt. and below at a faster rate than green crabs, corresponding closely with the observed differences in osmoregulatory ability at these salinities.

The decrease in haemolymph osmolality was monitored over an 18 hour period to study differences between the red and green crabs and the effects of acclimation to low and high salinities. There was a

rapid fall in the haemolymph osmolality of both red and green crabs within the first few hours, as also reported by other workers (Margaria, 1931, Shaw, 1961, Blasco and Forward, 1988), new stable values becoming apparent after 12 to 24 hours (Theede, 1969, Siebers *et al*, 1972). After acclimation to high and normal salinities the haemolymph osmolality was similar for both red and green crabs at the start of the experiments. It was not until the osmolality fell below about 800 mosM that a difference between the red and green crab haemolymph osmolality started to become apparent. This is equivalent to a salinity of approximately 25ppt., the upper limit of the range at which strong regulation is initiated (Spaargaren, 1974a) and would suggest that the red crabs are not as efficient regulators as green crabs.

Spaargaren (1974a) and Winkler *et al* (1988) report that the concentration of the internal fluids of *Carcinus* did not change substantially over a sinusoidal tidal salinity cycle. Direct transfer to a low salinity, as used in present experiments, is equivalent to a square wave salinity cycle observed in the Foryd estuary. The results suggest that after six hours of freshwater, as experienced by crabs at the head of the Foryd estuary (Fig. 2.2.A), the osmolality would have fallen below 800mOsm (25ppt.) at which strong regulation occurs. The crabs may then be expected to

exhibit behavioural avoidance of such a salinity.

Experiments were performed to discover if *Carcinus* exhibited avoidance responses to low salinity when the internal body fluid concentration fell to a critical level. However, this appears not to be the case (Figs. 1.7-1.9.A), since over the time period tested the haemolymph osmolality decreased steadily and the animals did not exit the low salinity at a specific concentration. There is also no definite correlation between the size of the animal and the time of exit, suggesting that both small and large crabs behave similarly to salinity variation. This tends to contradict the results of Chapter 2 and those of Spaargaren (1989) that smaller crabs are better adapted to survive in low salinities. There is also no relationship between haemolymph osmolality and the size of the animals, contradicting the results reported by Gilbert (1959). The haemolymph osmolality, however, was recorded after different time intervals, and standardisation of the time of sampling may reduce the errors. These results suggest that low salinity avoidance behaviour is not triggered by changes in the haemolymph concentration, but that, some other physiological change may be the cue for such behavioural responses. Further work is required to analyse changes in the internal ionic concentration of red and green crabs exposed to square wave salinity changes in order

to determine which changes in internal concentrations may be causally related to behavioural reactions.

Variations in ion exchange cannot be wholly attributable to permeability or active uptake, but must partly arise from variations in the blood flow through the gills brought about by changes in heart-rate (Spaargaren, 1974b, 1982, Smith, 1970, Hume and Berlind, 1976). Changes in heart-rate are also associated with increased halokinesis and oxygen uptake and may lead to a more effective dispersion of effectors or hormones involved in regulation (Hume and Berlind, 1976). The heart-rate of red and green crabs was therefore monitored in a salinity of 5ppt. On lowering of the salinity there was an almost immediate increase in the heart-beat frequency. This was of similar magnitude in both red and green crabs. Hume and Berlind (1976) and Cumberlidge and Uglow (1977) report that the heart-rate takes about 30 minutes to reach its maximum and remains at this elevated state for up to 2 hours. The heart-beat frequency of crabs in the present study was very variable even before the hypo-osmotic shock. This has also been reported by Hume and Berlind (1976) for *Carcinus* in normal seawater and in water of reduced salinity, however, these workers found no explanation for the large variation. The stroke volume is less variable and tends not to increase upon lowering of the salinity (Spaargaren, 1974b). The heart-rate of crabs

is closely related to its metabolic rate (Spaargaren, 1982). Red crabs have a higher metabolic rate than green crabs (Reid and Aldrich, 1989), but this is not reflected in the data of the present study. The heart-rate of red and green crabs in full seawater and immediately thereafter on dilution of the seawater is similar. After the initial increase in heart-rate that of the red crabs decreases at a faster rate compared with the green crabs. This however, is related to the deterioration of two red coloured individuals (as mentioned in the results section), rather than a difference in a physiological mechanism. The results suggest that differences in heartbeat frequency are ultimately not responsible for the poorer osmoregulatory abilities of red crabs.

The present study has shown that various aspects of the osmoregulatory physiology differ according to the colour of the individual, but as yet reasons for these inconsistencies still remain speculative. Red crabs do not migrate into the intertidal zone (Crothers, 1968, Reid *et al*, 1989) and so are unlikely to come into contact with low salinity water. It has been reported that the tolerance limits are wider if animals experience a range of salinities (Kinne, 1964c,d,). However, such tolerance limits are reversible (Theede, 1969), and since both red and green crabs were held in similar conditions prior to experimentation, it is

unlikely that resistance adaptation affects osmoregulatory ability in this case. It is more likely to be a difference in the rate of active uptake or permeability between the red and green crabs. These physiological differences cannot be attributed to size (since animals of a similar size were used) but may be age related as trends show that older animals have lower haemolymph osmolalities (Gilbert, 1959, Lynch *et al*, 1973), and red crabs might be expected to be older than green crabs of a similar size since they have spent longer in intermoult. Ionic regulatory abilities vary during moult (Robertson, 1960), whilst Adelung (1971) reported differences during the intermoult period in crabs maintained in full strength seawater. As all crabs used in the present study were in late C or early D stages then this probably does not account for the differences. It is also possible that the differences are part of a continuous pattern of variation observed in *Carcinus* (Aldrich, 1983, 1986, 1989, Aldrich and Reid, 1989).

Red crabs are characterised by a greater load of epibionts and are more heavily parasitised than green crabs (Crothers, 1968, Kaiser *et al*, 1990). The red coloration is thought to be due to a prolonged intermoult (Reid *et al*, 1989, Kaiser *et al*, 1990). The majority of red crabs are in C4 stage of the moult cycle, which suggests that the coloration builds up

during the intermoult from green through orange to red. The amount of epibionts also increases in a similar fashion through the colour range. A percentage of red individuals may be in terminal anecdysis but some red crabs are found to have limb buds suggesting that they will moult again (Kaiser *et al* 1990). The moult stages of green, orange and red crabs were analysed over the summer period, and similar percentages of red and green crabs were found in premoult. Many orange crabs were found in premoult or moulting, but this coloration was due to colour changes associated with moulting itself rather than to the occurrence of true orange crabs (Webster, pers. comm.). Kaiser *et al* (1990) failed to find red crabs in premoult, however analyses were carried out in autumn (Kaiser pers. comm.) when few crabs would be moulting anyway. During the prolonged intermoult period the exoskeleton of red crabs becomes more heavily calcified, and averaged 0.09 mm thicker compared to that of green crabs of a given size. Kaiser *et al*, (1990) reported that the red crabs were stronger and able to exert greater forces with thicker chela. This enabled them to open larger mussels and dominate green crabs in aggressive interactions.

Aldrich (1983) proposed that there were separate phases where energy was channelled into either growth or reproduction. Reid, Abello and Naylor (in prep.) reported that red crabs were more successful in

obtaining a mate, however it is not clear whether this is simply because they are stronger or that they are reproductively more viable.

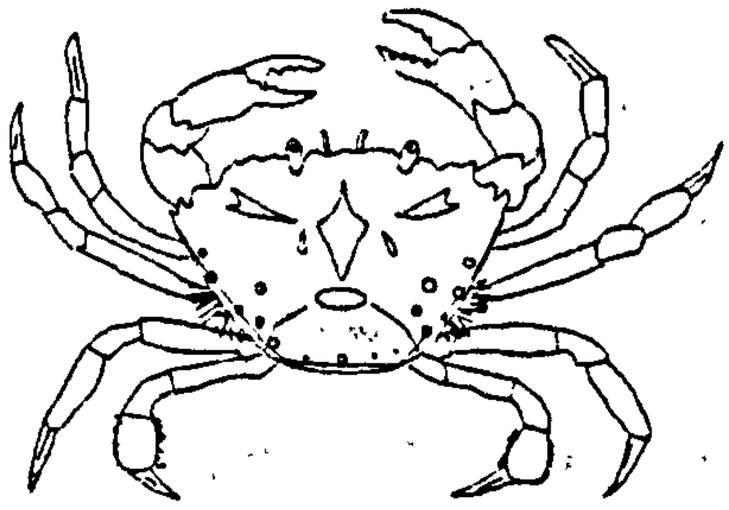
At present the physiological variability has often been attributable to size, sex or life history strategies of *Carcinus*. It is now possible to explain some of the observed variations as a result of the colour of the individual. The red coloration is at least partly correlated with the time spent in intermoult but is not simply due to the onset of terminal anecdysis. All red crabs that moult become green but it is not clear if all green crabs of a given size turn red before they next moult. The actual mechanism of colour differentiation, therefore, needs to be elucidated. Future work needs to address the problem of determining the physiological mechanisms that account for the osmoregulatory differences between the red and green colour forms. In particular active uptake and permeability changes should be investigated. This opens up many new pathways for study of physiological differences between the red and green crabs in relation to their behaviour and ecology.

CHAPTER 2:

Field studies on the distribution of *Carcinus maenas* in a tidally mixed estuary

SUMMARY

In the tidally mixed estuary male and female crabs occurred in roughly similar proportions, but red and orange crabs of both sexes were virtually absent. The majority of estuarine crabs were green and were, on average, smaller than their open shore counterparts. Migration out of the estuary occurred in the colder months, the animals returning to the upper reaches of the estuary in late spring.



INTRODUCTION

Carcinus maenas occurs in estuaries and areas of low salinity such as the Baltic, as well as on fully marine coasts (Poulsen, 1922, 1949, Broekhuysen, 1936, Muus, 1967, Wolff and Sandee, 1971, Warner, 1977, Ameyaw-Akumfi and Naylor, 1987). However, whereas the species is well known to make excursions into the intertidal zone of open coasts on each flood tide (Edwards, 1958, Naylor, 1958, Dare and Edwards, 1981) less is known of its detailed movements within estuaries. Tidal migrations of some individuals within estuaries have been reported by Ameyaw-Akumfi and Naylor (1987), who found that *Carcinus* was able to move up to 2 km in 6 hours, but these workers suggested that most *Carcinus* remain within the estuary for extended periods of time. Similar findings are reported for *Scylla serrata* (Hill, 1978) which, although capable of moving up to 800 metres in a night, tended to remain for extended periods within a limited area of an estuary.

Open coast populations of *Carcinus* exhibit seasonal migration offshore in December (Naylor, 1962, Crothers, 1968), but some small individuals tend to remain intertidally (Atkinson and Parsons, 1973). Their consequent return in March and April is related to temperature change (Naylor, 1963). Similar findings are reported for *Macropipus holsatus* in an area influenced by strong tidal currents (Verwey, 1958, Venema and

Creutzberg, 1973) and for *Carcinus* in estuaries and in the Baltic (Broekhuysen, 1936, Wolff and Sandee, 1971, Rasmussen 1973, Klein Breteler, 1976).

The distribution of mobile crustaceans within an estuary may also vary with sex, male *Crangon crangon* usually being found in waters of higher salinity than females (Havinga, 1930), and vice versa in *Eriocheir sinensis* (Peters *et al*, 1933). Broekhuysen (1936) and Wolff and Sandee (1971) found no differences in the relative distributions of male and female *Carcinus* in estuaries with respect to salinity. However McVean and Findlay (1979) found that females predominated over males within the Yealm estuary over the year, as was also found for intertidal populations on an open shore (Naylor, 1962).

Reports of variations in estuarine populations of Crustacea in relation to body size are more common and, in general, organisms in estuaries tend to attain a smaller size than their marine counterparts (Gunter, 1945). An exception to this rule is *Callinectes sapidus*, larger specimens of which and those in terminal moult are better adapted to live in lower salinities (Haefner and Schuster, 1964). A gradation in size of *Carcinus* with respect to salinity has been reported by Crothers (1968) and by Wolff and Sandee (1971). The first author found that crabs from salt marshes were small and nearly always green in colour. Spaargaren (1989) suggested that

despite their larger surface area to body volume ratio, smaller *Carcinus* were better adapted to survive in areas of fluctuating salinities. However Broekhuysen (1936) and McVean and Findlay (1979) found no size variation within the estuary or between crabs from an estuary and those of the open shore.

The degree of penetration of an estuarine system is not only dependent on phenotypic characteristics of the organism, but also on the physical parameters of the estuary itself. The salinity regime encountered and, in particular, the availability of suitable substrates and habitats for crabs, may be of paramount importance (Teal, 1958, Barnes, 1967).

The aim of the present study was to investigate variations in the size, sex, number and colour of a population of *Carcinus maenas* from a tidally mixed estuary. The rationale was to examine distribution patterns occurring within an estuary, on a monthly and an annual basis and to compare these with open shore populations of the same species.

MATERIAL AND METHODS

INTRODUCTION TO FORYD ESTUARY

The area chosen for study was Foryd Bay which is situated 3km West of Caernarfon, North Wales (53′ 06′N 4 22′W) O.S map 115. The estuary is formed from the confluence of the rivers Gwyrfai and Carrog. It is approximately 2.5km in length by 1km wide. The bay completely empties of seawater at low tide and the river flows over a sandy bed into the most westerly end of the Menai Straits. Seven sites were chosen covering a range of habitats, six along or adjacent to the estuary itself and one some 15 kilometres along the Menai Straits away from the influence of freshwater.

Site 1 - Ynys Faelog on the eastern end of the Menai straits at Menai Bridge, grid reference 722560. This was considered as an open shore, control site. The rocky substrate was heavily covered with macroalgae, mainly *Ascophyllum* and *Fucus* species.

Site 2 - The open shore, 0.5km East of the mouth of the Foryd Estuary, (Grid reference 618458). The area consisted of large stones and boulders with similar macroalgal growth to site 1.

Site 3 - The mouth of the Foryd estuary (grid reference 609451). The bottom substrate consisted entirely of sand with very little cover; surface flora and fauna were sparse.

Site 4 - This site was situated 0.75km from the mouth of the estuary approximately 1/3 way towards the limit of tidal influence at grid reference 603447. The substratum consisted of sand, with a number of large stones to which Fucoid algae were attached.

Site 5 - Situated 1.5km from the estuary mouth, a little more than half way up the estuary this was sited at grid reference 597452. The substratum consisted of mud and small stones but also with numerous larger stones providing shelter and attachment surfaces for algae.

Site 6 - This site was 1.75km from the mouth of the estuary, at grid reference 594453. The bottom consisted of mud and small stones; a number of large boulders were present densely covered in *Ascophyllum nodosum*.

Site 7 - The head of the estuary 2.25km from the mouth, at grid reference 590453. The bottom was mud and gravel with many stones. Fucoid algae were abundant.

Fig. 2.1.

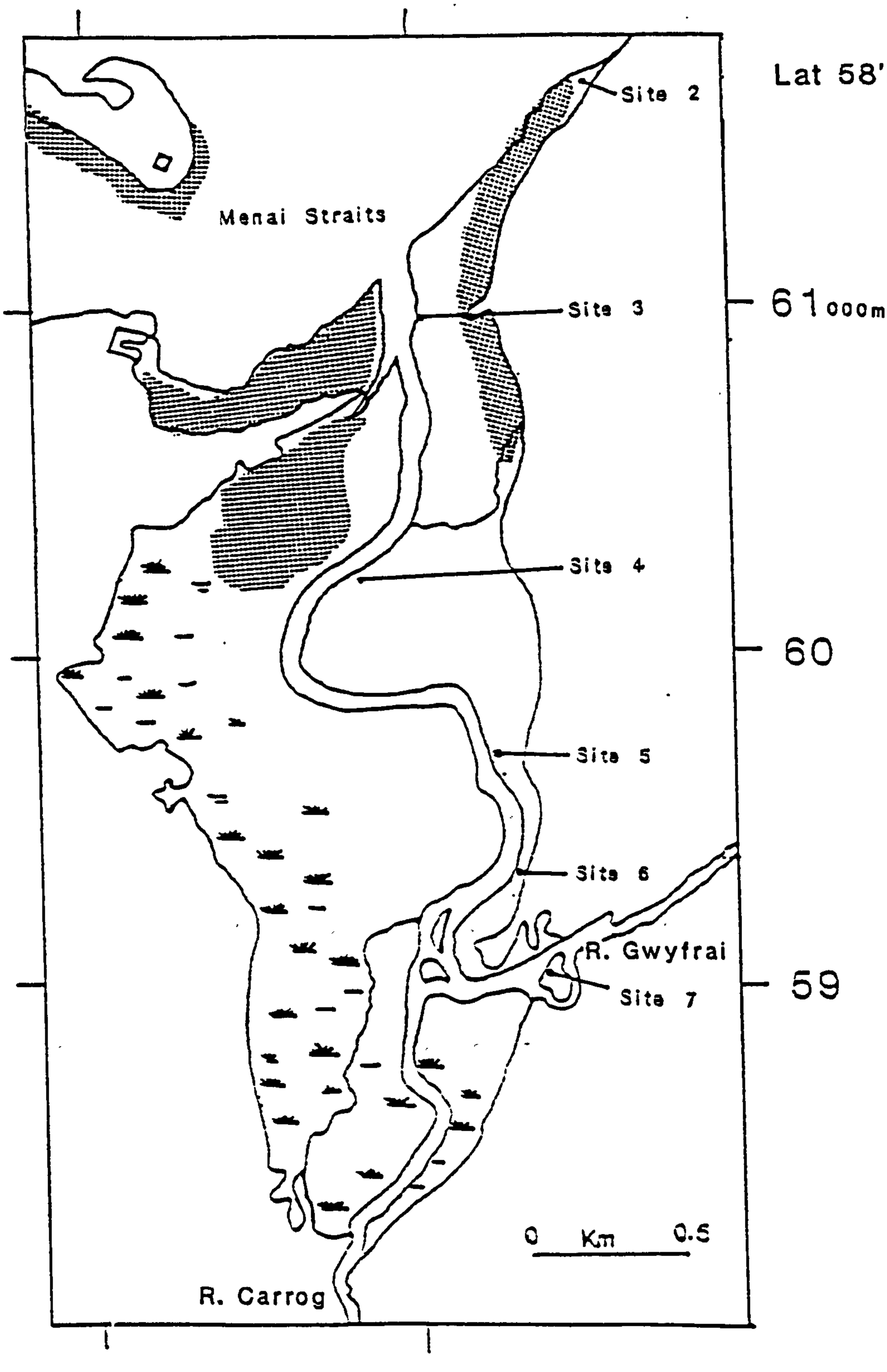


Fig. 2.1. Map of Foryd Bay, Gwynedd showing positions of sampling sites. Adapted from Ordinance Survey map 115.

COLLECTION OF CRABS

Single entrance cylindrical netlon traps 75 cms x 20 cms in diameter baited with mackerel (*Scomber scombrus*) were used to capture the crabs. Two traps per site were placed close to low water mark, over a five day period during a falling spring tide. Traps were emptied and re-baited once each day. The number, carapace width, sex and colour of the crabs were recorded each day, and the results were pooled for each five day period. Trapping was carried out during April, May, June, September and December 1989 and in February 1990. In addition to trapping on a monthly basis, monitoring of animals within the estuary was carried out to ascertain whether or not the crabs migrated from the estuary on the ebb tide. This was achieved by trapping in the main channel for one hour before and after low water on nocturnal low tides. Three sites were chosen, the mouth (site 3), half way up (site 5) and the head of the estuary (site 7) during June.

SALINITY PROFILES

Salinity profiles were determined using a Salinity temperature bridge salinometer type M.0.5. This was fixed to a pole pegged in the bed of the river at low water. The salinometer head was held clear of the bed to prevent it silting up and becoming entangled with debris. Salinity in the main channel was monitored over a complete tidal cycle from the time of one low water to the next, with measurements taken every fifteen minutes. Changes in salinity were monitored at the mouth (site 3), half way up the estuary (site 5), and the head (site 7). Observations were made on a neap cycle in the summer (12.7.89-14.7.89) when there was low freshwater input, and on a spring tidal cycle in the winter (11-13.12.89) when freshwater runoff was high.

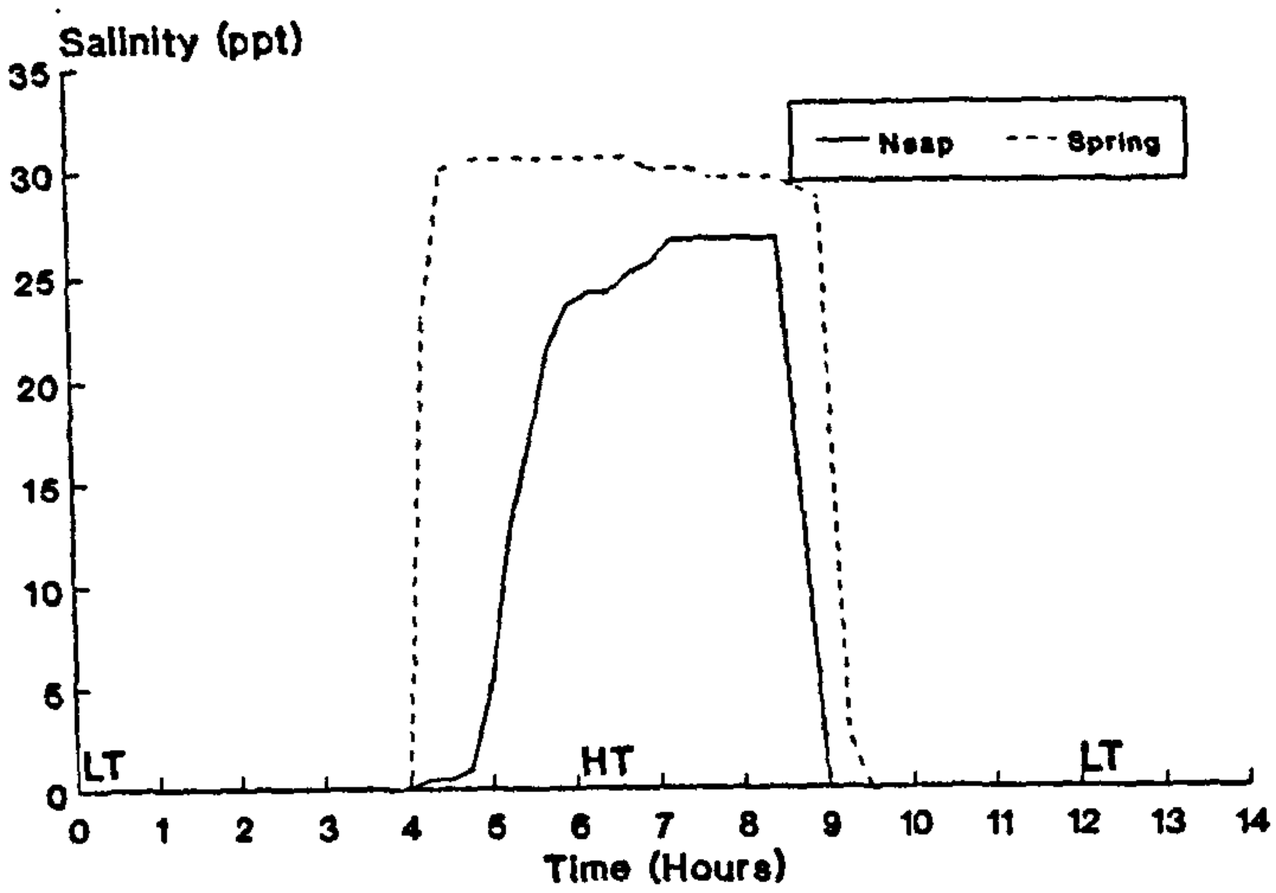
RESULTS

SALINITY PROFILES

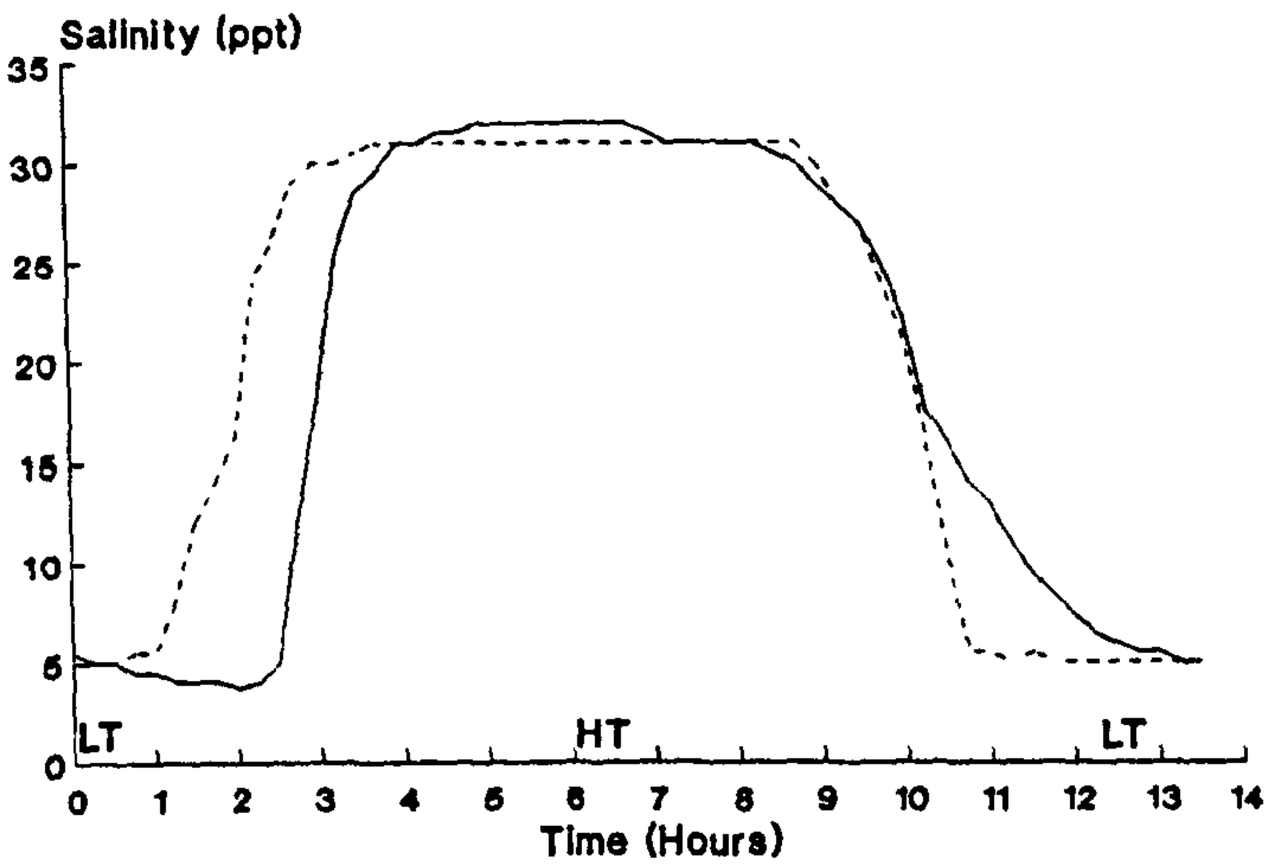
Fig. 2.2. represents salinity profiles under certain tidal and meteorological conditions at sites 3, 5 and 7 in the Foryd estuary. The salinity clearly changed very rapidly as the tide flooded into or ebbed from the estuary, varying rapidly between full strength seawater and freshwater. The greatest salinity variation clearly occurred at the estuary head (Fig. 2.2.A.), where crab inhabitants would experience freshwater for more than half the complete tidal cycle. An abrupt change between seawater and freshwater occurred in under an hour. Salinities half way along the estuary (Fig. 2.2.B.) decreased to 5ppt. for 2 hours on each side of low water. Salinity changes at the estuary mouth were of the magnitude of 8ppt. and these occurred only for an hour before and after low water.

Fig. 2.2.

A)



B)



C)

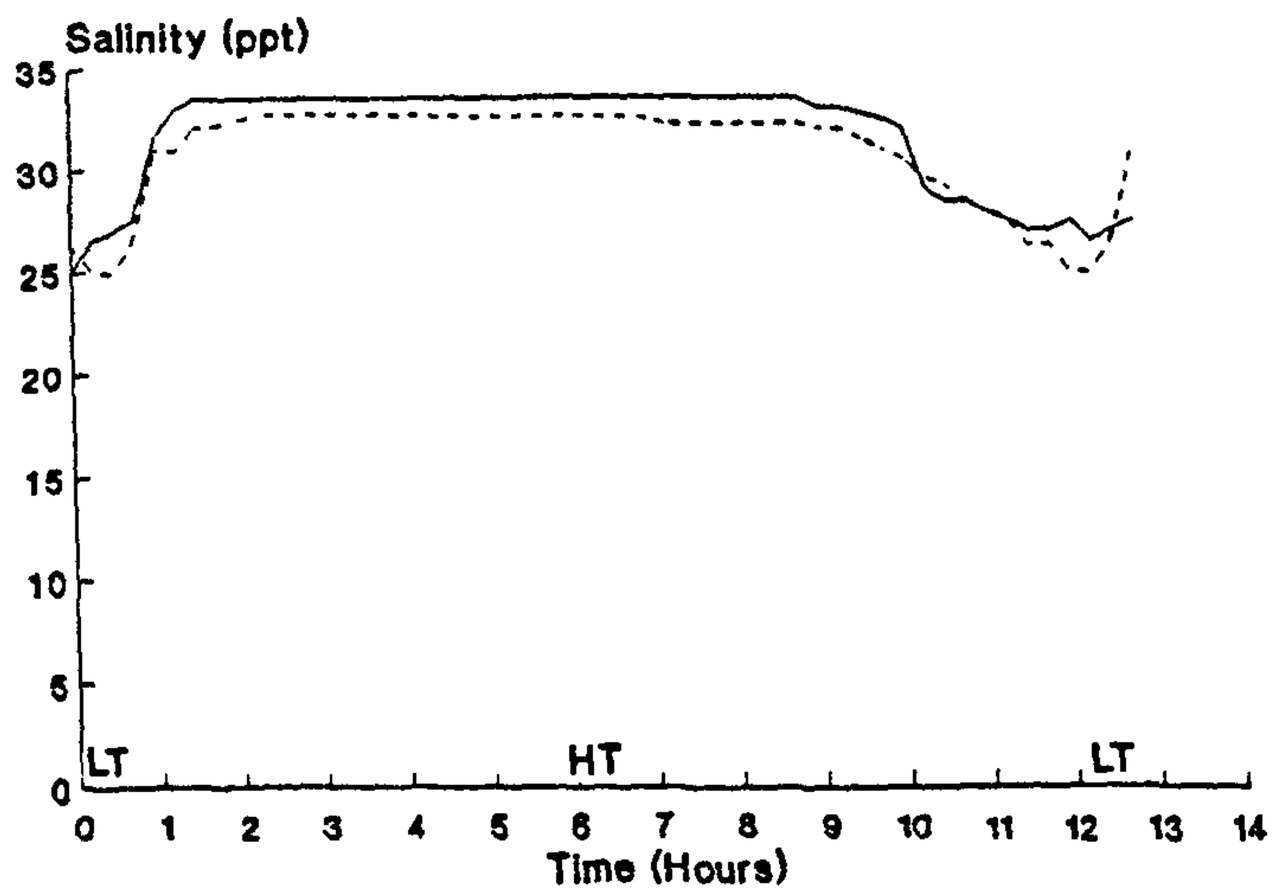
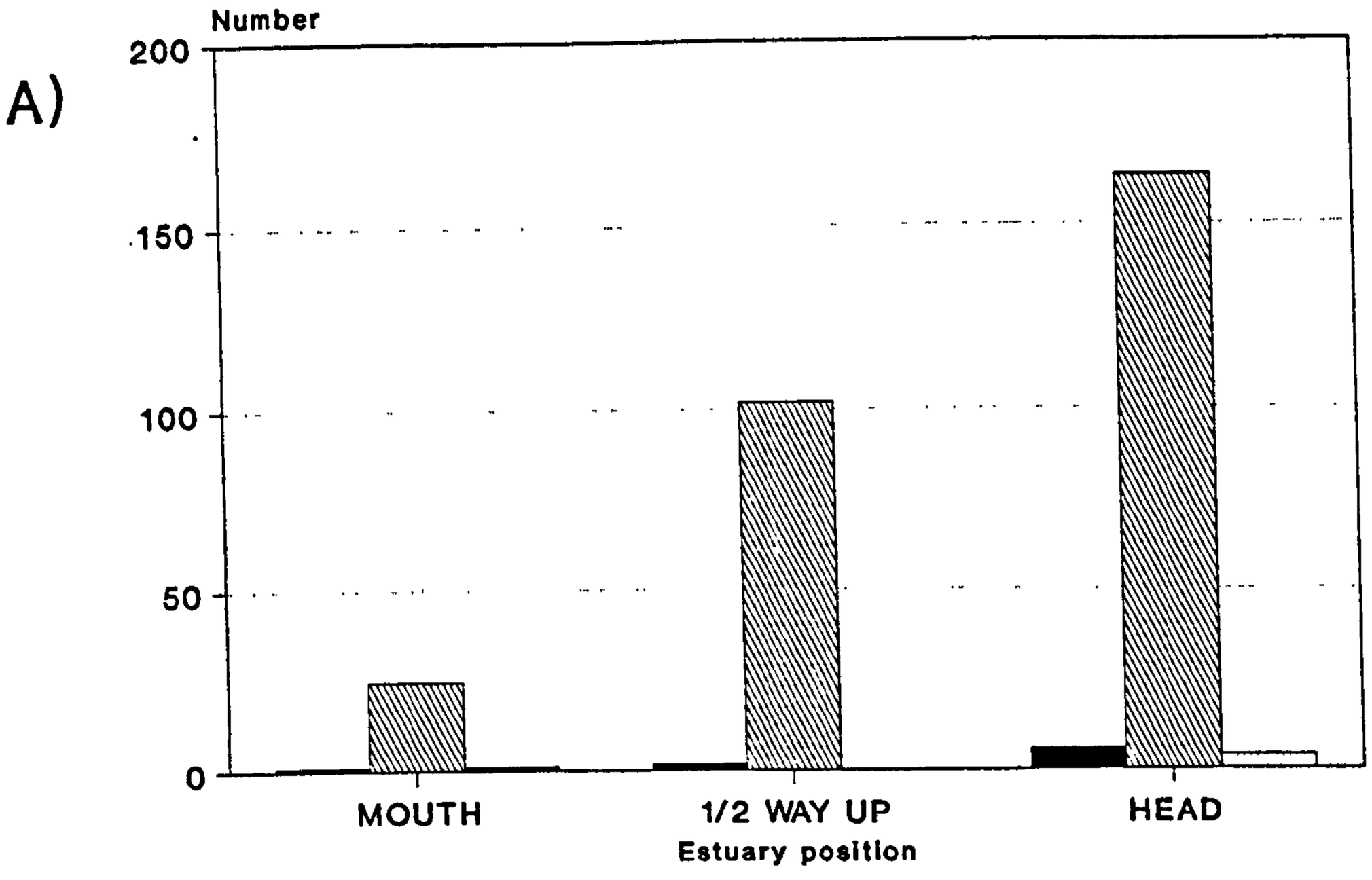


Fig. 2.2. Salinity profiles at sites 7(A), 5(B), 3(C) in the Foryd estuary, recorded on neap tidal cycles in the summer with low freshwater runoff, and spring tidal cycles in the winter when freshwater input was high.

TRAPPING OVER LOW WATER

Trapping for 1 hour before and again after low water produced relatively large numbers of crabs. Fig. 2.3.A and B. shows the number of males and females caught in two nights trapping. The vast majority of both males and females caught were green in colour, orange and red crabs being largely absent. The results suggest that there is a population of *Carcinus* that remains within the estuary, over the low tide period, with little evidence that crabs are moving seawards at that time.

Fig. 2.3.



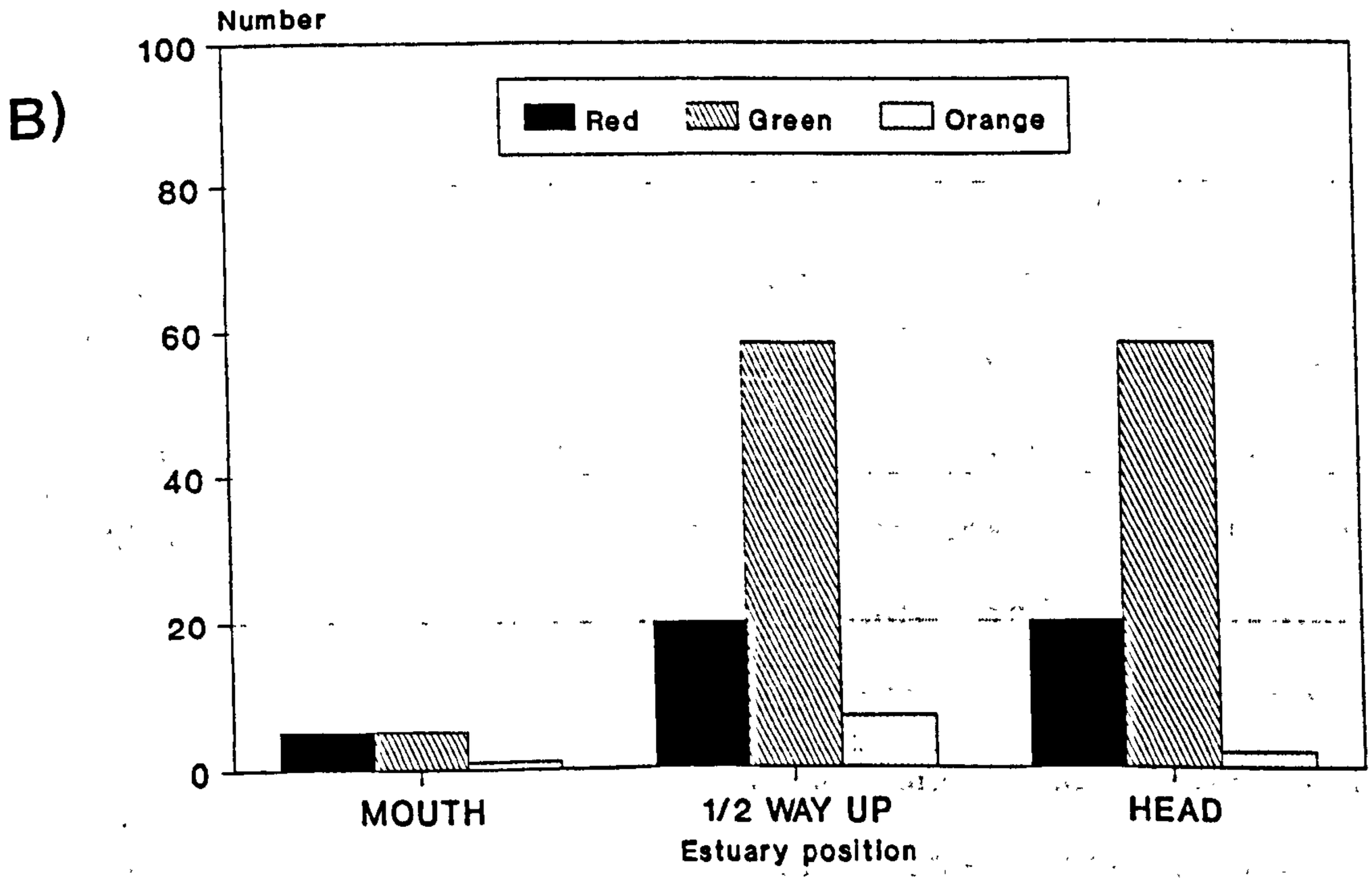


Fig. 2.3. Distribution of green,orange and red crabs in the Foryd estuary at low water. Results obtained over 2 nights in June, trapping 1 hour before and after low water, at the mouth (site 3), mid-estuary (site 5) and head of the estuary (site 7).

ANNUAL VARIATIONS IN ESTUARINE AND OPEN SHORE POPULATIONS OF *CARCINUS MAENAS*

Figs. 2.4. and 2.5. show the total number and distribution of green, orange and red males and females respectively, over the year. The number of crabs caught in February were low, especially in the upper reaches of the estuary. At that time, except at site 6, most crabs tended to congregate at the estuary mouth. During April the number of crabs started to increase both on the open shore and within the estuary, but no crabs were caught at the head of the estuary. From May onwards *Carcinus* were abundant at all sites throughout the summer months, and large numbers penetrated to the head of the estuary. By December the numbers of animals caught on the open shore, at the mouth of the estuary and at site 6 were still high, but crabs were essentially absent from the head of the estuary. A clear pattern emerges with respect to the colour of the crabs, especially in the males. Throughout the year the proportion of red, orange and green crabs caught on the open coast remained relatively constant, with green crabs more or less as abundant as red and orange specimens combined. However, within the estuary both orange and red crabs were very uncommon , especially from the estuary head. The estuarine population of males consisted almost entirely of green coloured individuals. The pattern was similar for females, though red coloured individuals were

predominant on the open shore from April to June and relatively large numbers of red females moved into the estuary during May. On some days in May catches at some estuarine sites consisted almost entirely of red females, but they were not caught in appreciable numbers at the head of the estuary. These red females had largely disappeared by June, and green female crabs were predominant in catches in the remaining months.

SEX RATIOS

Fig. 2.6. shows the sex ratio of males/females over the year. In most months studied males predominated in trap catches especially in the colder months. Females were caught in greater numbers than males at several sites in May, when large numbers invaded the estuary. During June and September females were caught in slightly larger numbers in the upper reaches of the estuary. At site 1 on the open shore the sex ratio remained relatively constant over the study period, whereas at the other sites this ratio was more variable. The records of 100% females at site 7 in February and December are for single specimens in each case. No consistent pattern is apparent, and variations in the sex ratios are probably dependent upon the season and temperature changes rather than the salinity regime encountered.

Fig. 2.4.

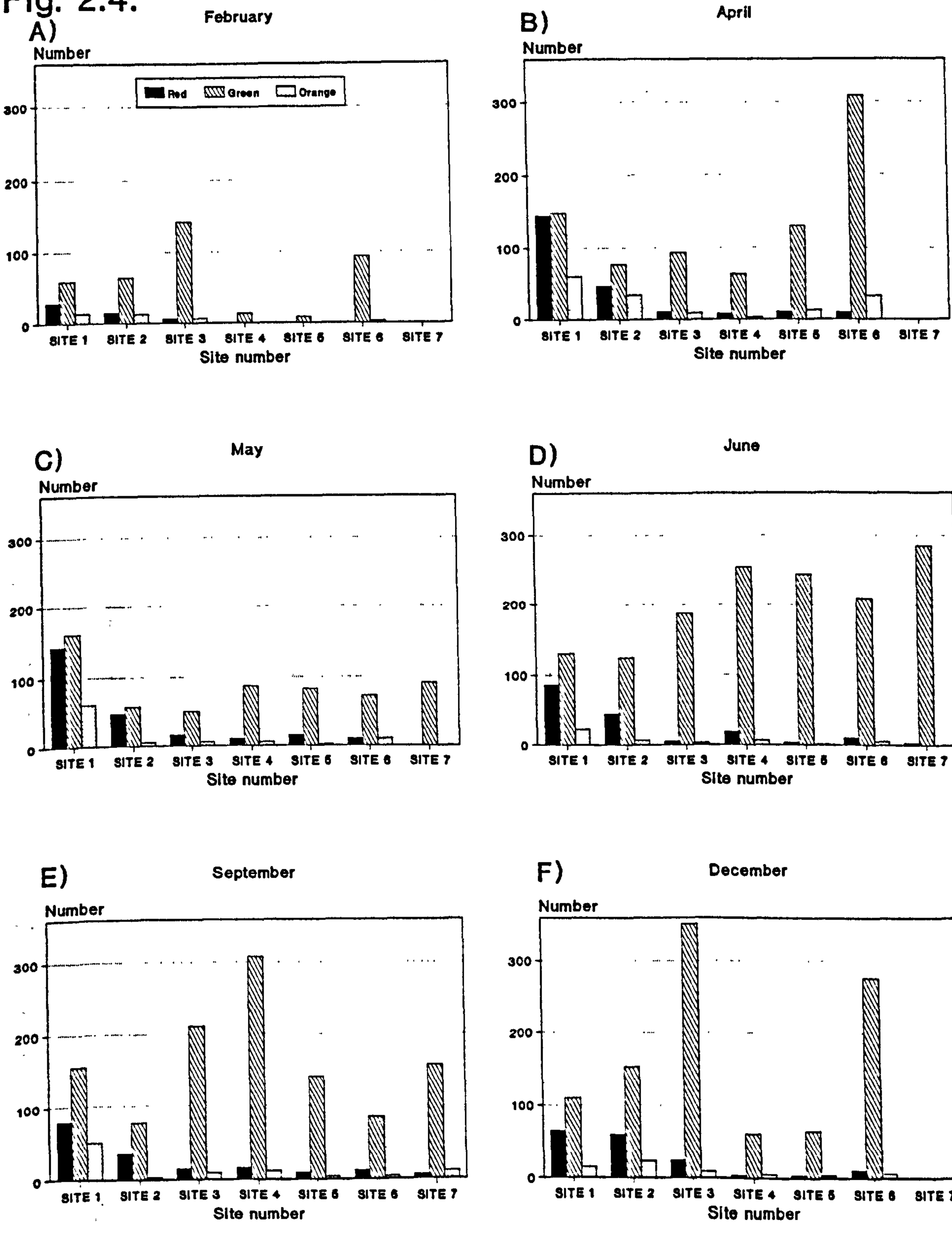


Fig. 2.4. Distribution of numbers of green, orange and red males at each site during the months of study. Numbers per site are totals from two traps over a five day sampling period, traps being emptied daily.

Fig. 2.5.

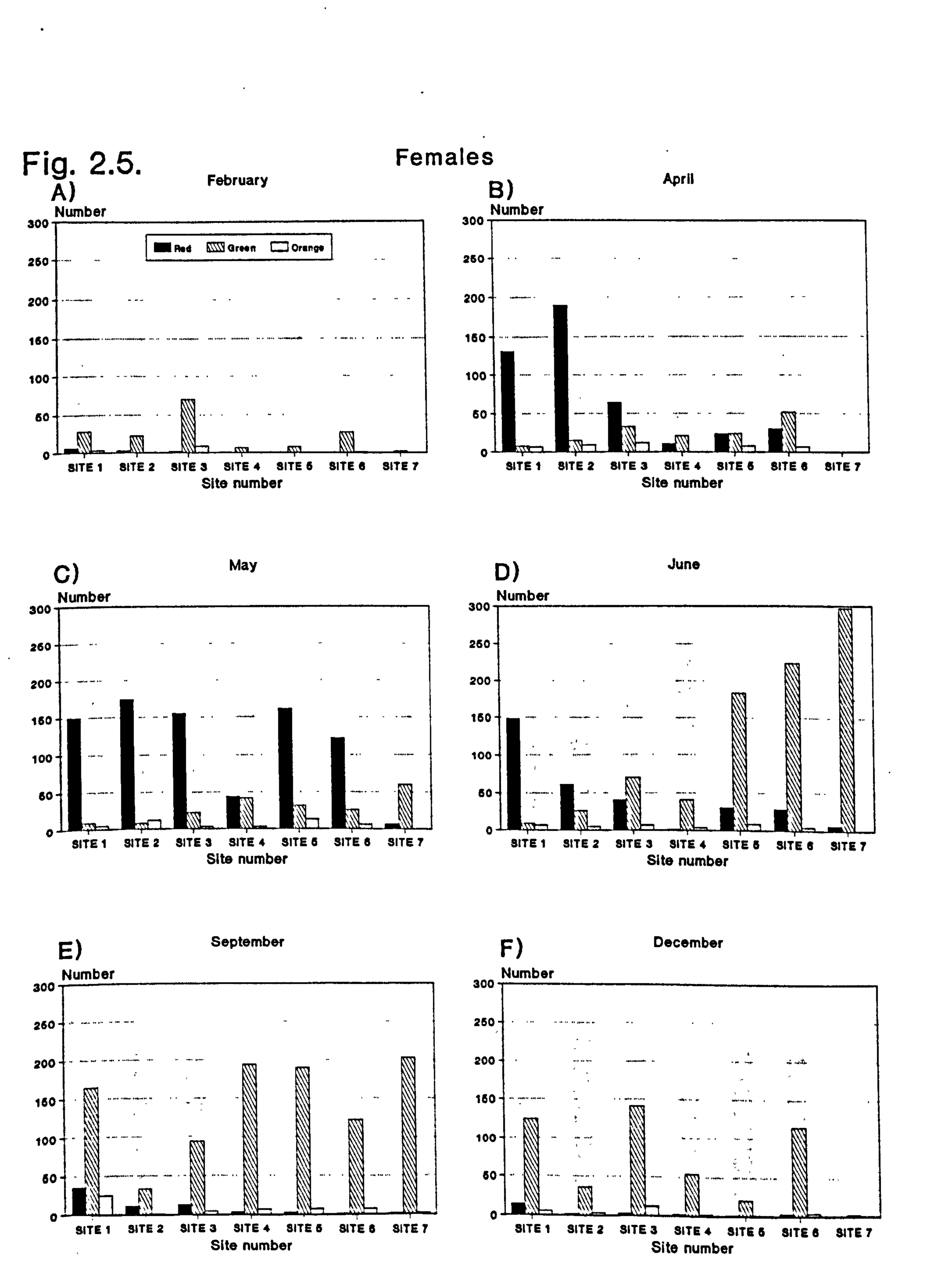


Fig. 2.5. Distribution of numbers of green, orange and red females at each site during the months of study. Numbers per site are totals from two traps over a five day sampling period, traps being emptied daily.

Fig. 2.6.

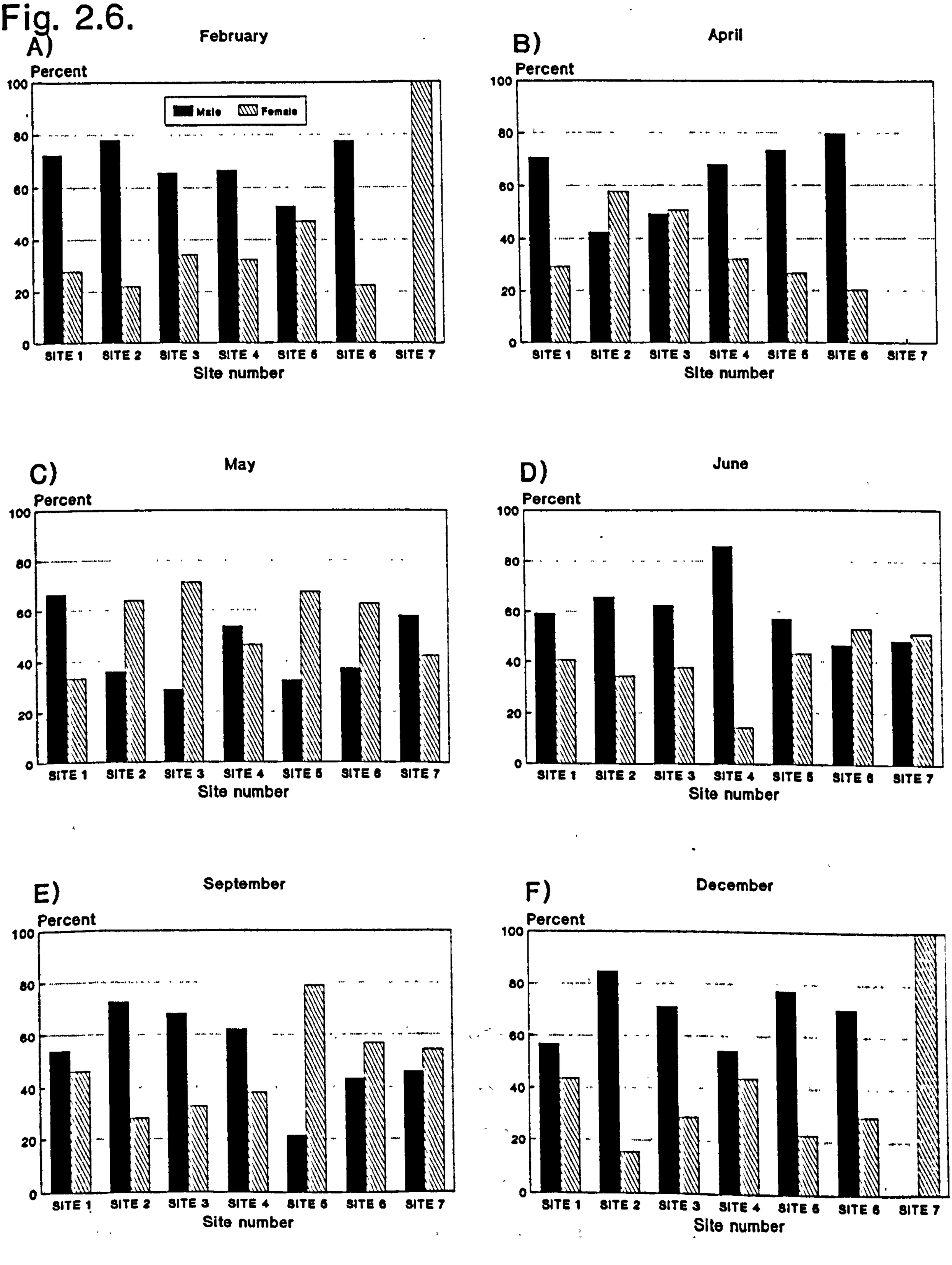


Fig. 2.6. Percentage of males to females at each site during the months of study. (The 100% values at site 7 in Feb. and Dec. are for single crabs only).

SIZE VARIATION

Figs. 2.7. and 2.8. show the mean carapace widths (with 95% C.L. of the mean) of green, orange and red males and females, in which a number of general trends are apparent. Firstly there is a size gradation with respect to colour, with red crabs tending to be the largest and green crabs the smallest, particularly at sites within the estuary. In addition crabs in the estuary, especially green crabs, tend to be smaller than those caught on the open shore sites.

To determine whether size differences occurred along the estuary one-way ANOVA tests were applied to random samples of data from each site for May, June and September, using green crabs only, except in May when large numbers of red females were also present. Table 2.1. shows the results obtained, and Tables 2.2 to 2.4 illustrate Tukey pairwise comparison tests carried out to ascertain whether site differences occurred. The size of males and females varied significantly between the sites studied. These differences tended to be rather irregular, but some generalisations can be made. Male crabs found at the two open shore sites were of a similar size range, and those from site 1 in the Menai Straits were larger than the crabs from the estuarine sites. In addition crabs captured at site 2 were larger than crabs found at sites 4, 5 and 7. However, crabs from site 4 were on average smaller than those found at all

the other sites. Female crabs were more uniform in size, those from site 2 and all estuarine sites being of a similar size. Female crabs collected from site 1 in the Menai Straits were frequently found to be larger than those from Foryd Bay.

A more effective representation of size variation was accomplished by dividing the crabs into three size classes: small, medium and large. Figs. 2.9. and 2.10. illustrate the changes in abundance of green crabs within these size classes over the year. Estuarine crabs were mainly small or medium in size, and the numbers of those found within the estuary were low during the winter months but rose during the summer. The number of small males reached a peak in June, but the majority of medium sized crabs were found in September. Small males were virtually absent from the open shore sites, which were characterised by medium and large size classes present all year round, with a peak in the summer months. Green female crabs were uncommon on the open shore, but small and medium sized individuals were widespread in the estuary during the summer. Large females were caught in low numbers at all sites, except at site 1 in September when females in the 51 - 75mm class were unusually abundant.

Fig. 2.7.

Males

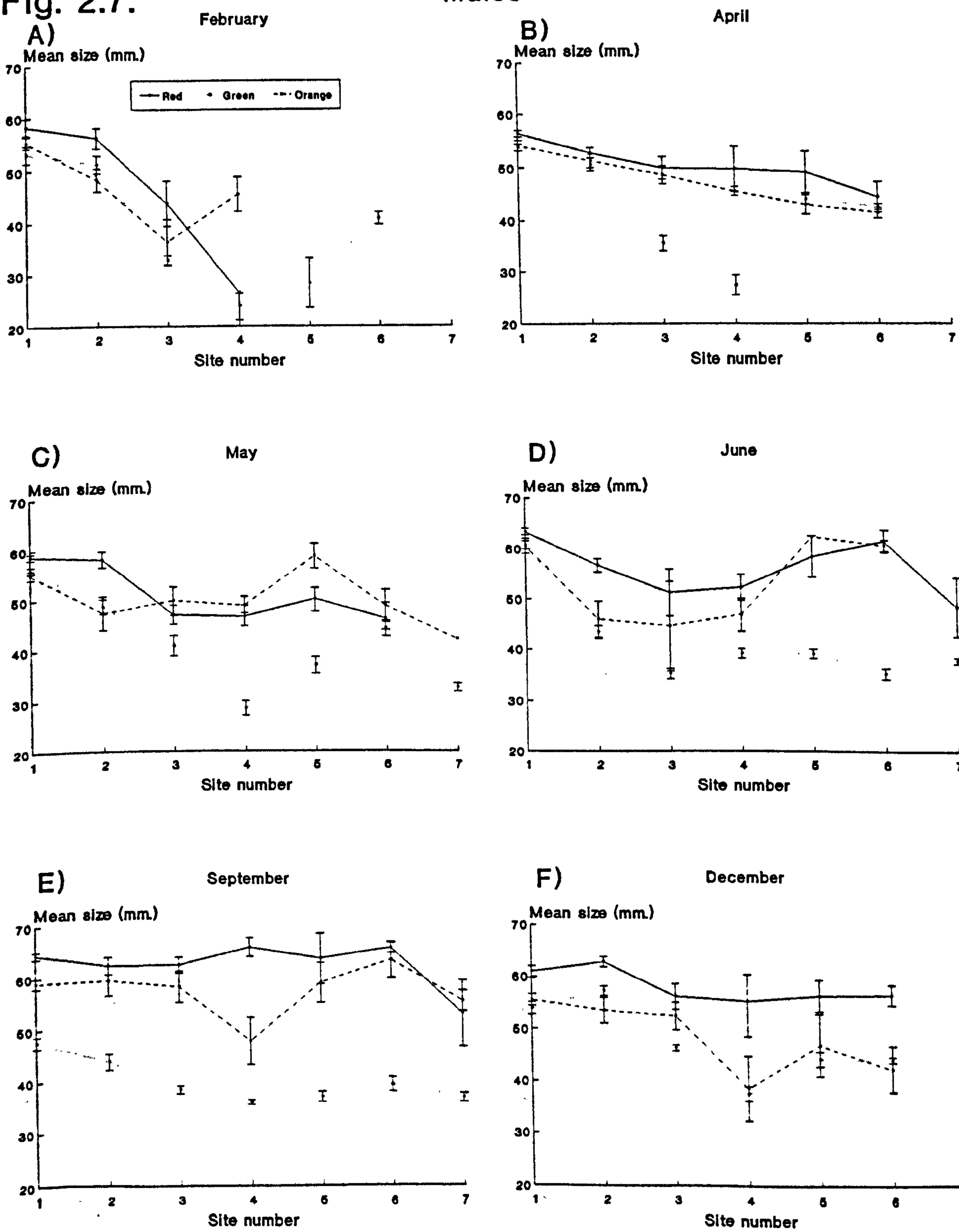


Fig. 2.7. Mean carapace width (mm) of green, orange and red males (with 95% C.L. of the mean) at each site during the months of study

Fig. 2.8.

Females

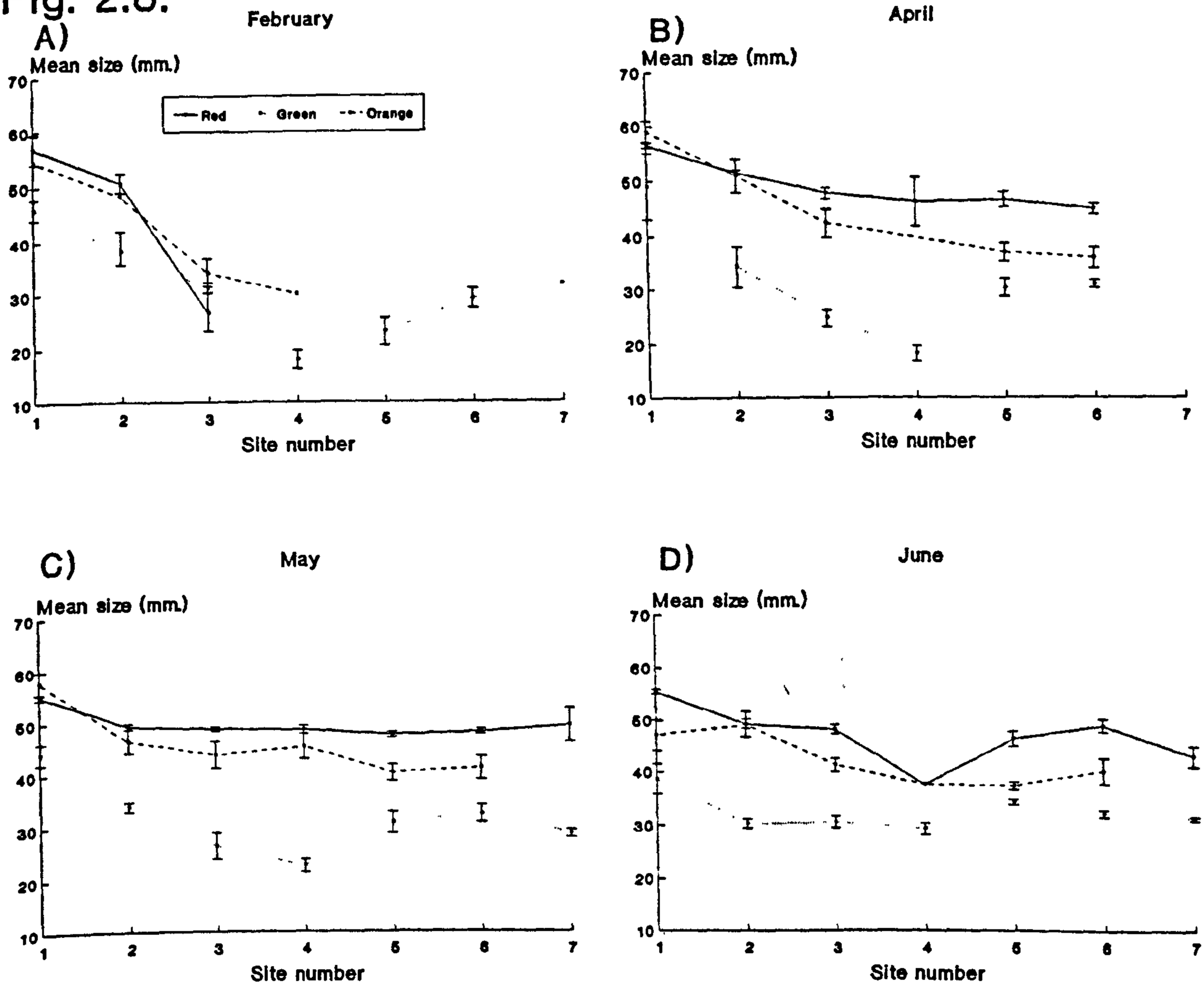
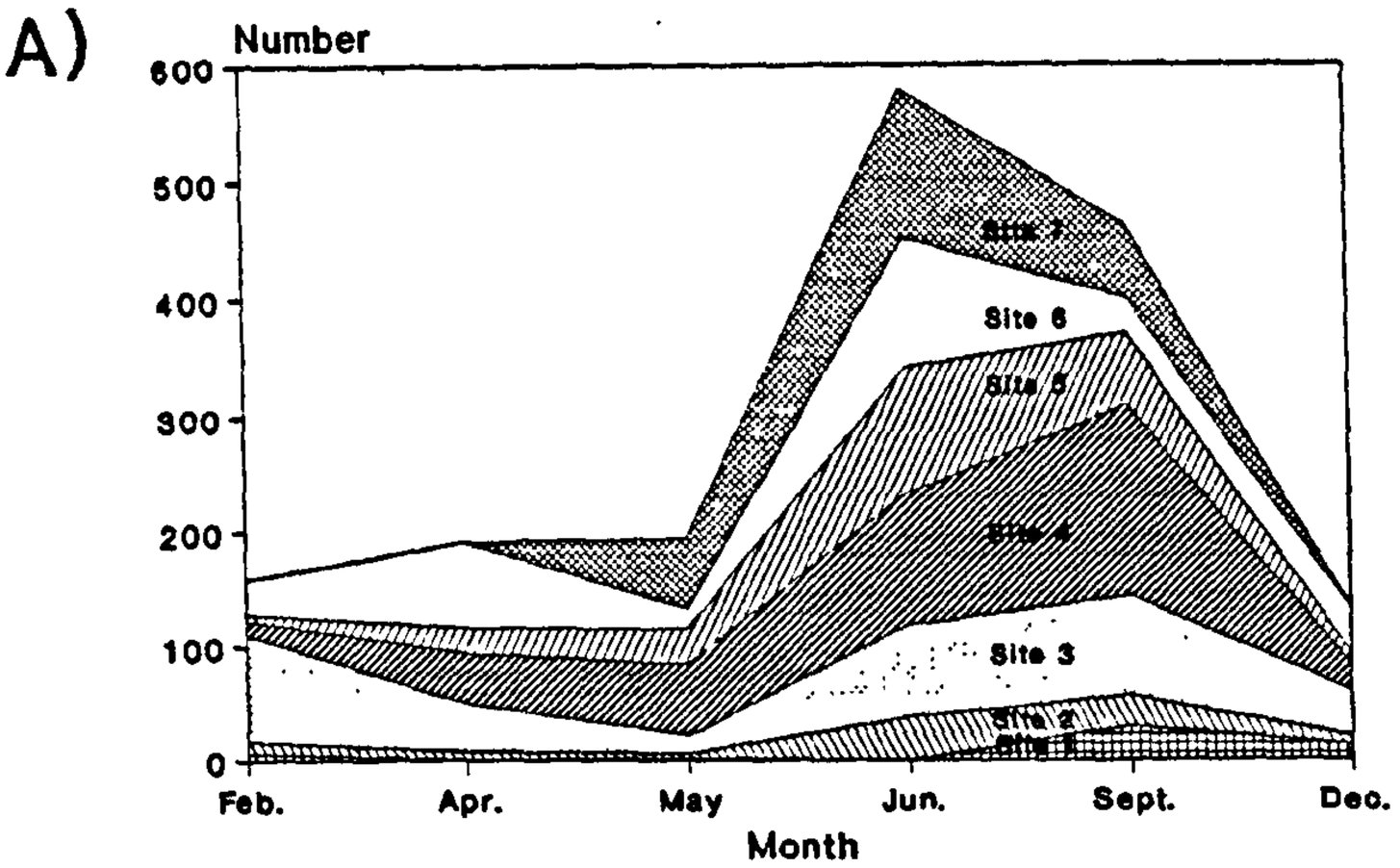
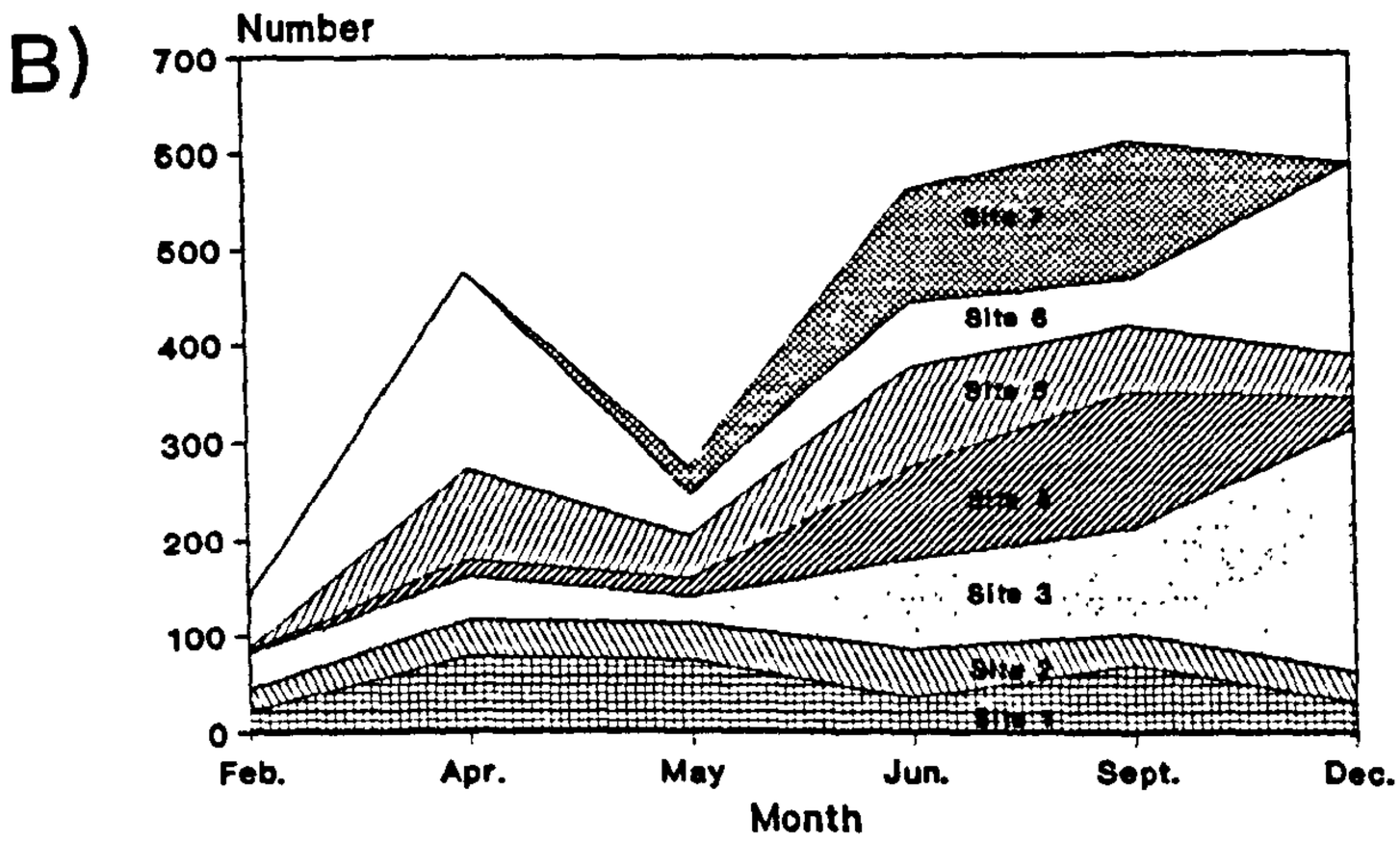


Fig. 2.8. Mean carapace width (mm) of green, orange and red females (with 95% C.L. of the mean) at each site during the months of study.

Fig. 2.9.





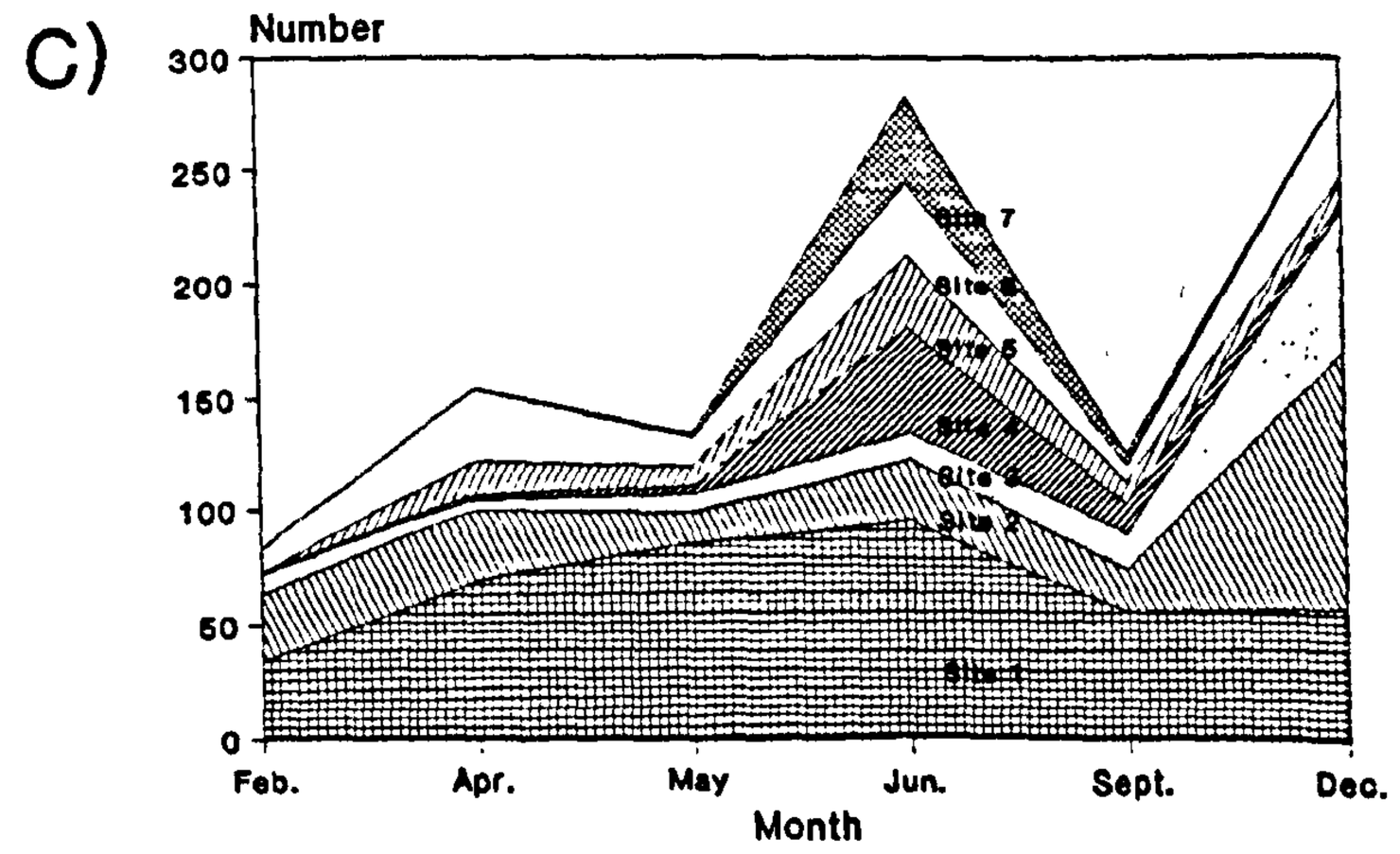
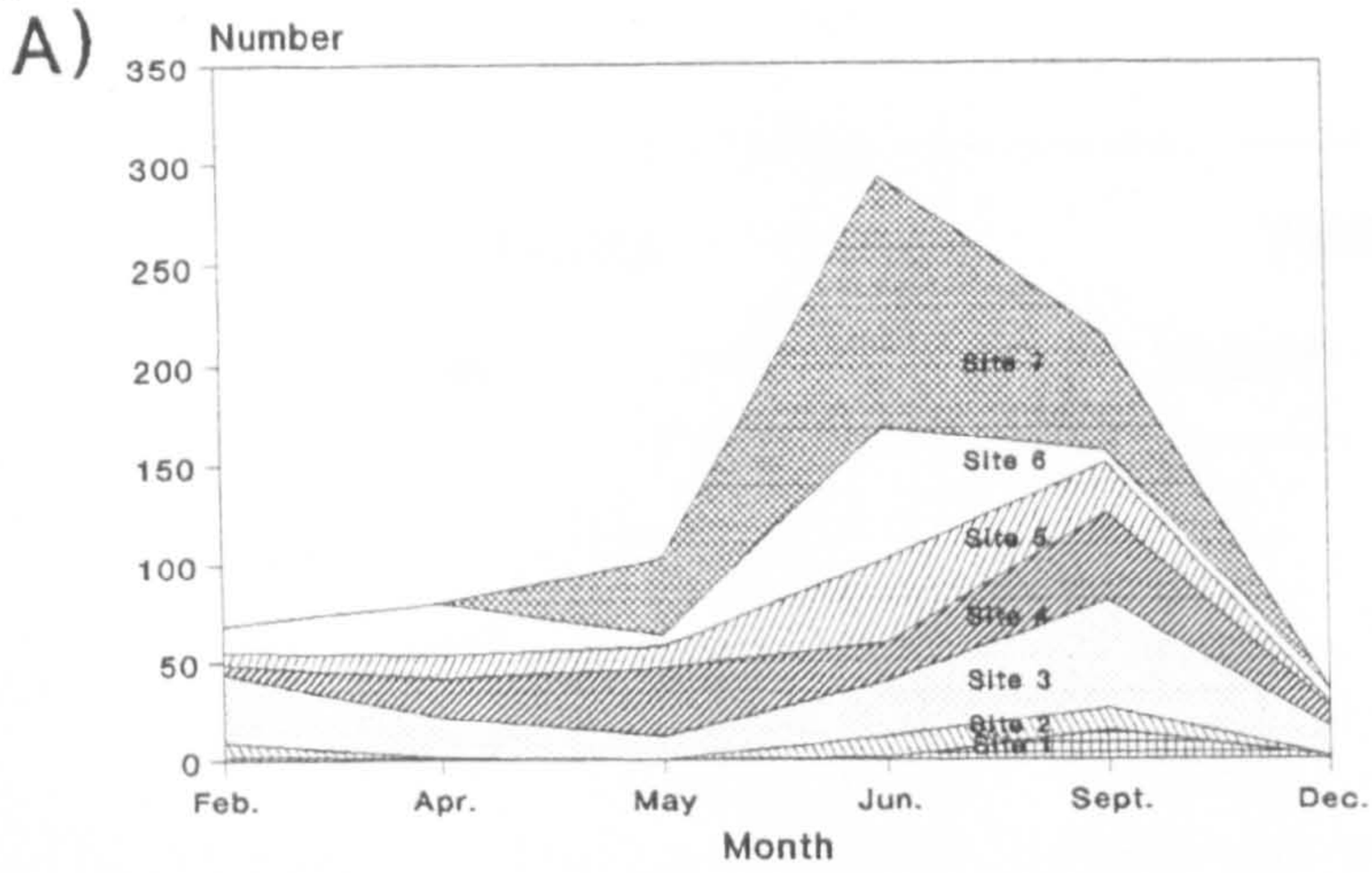


Fig. 2.9. Variations in the number of (A) small (0-35mm), (B) medium (36-55mm) and (C) large (56-85mm) size classes of males at each site during the months of study.

Fig. 2.10.



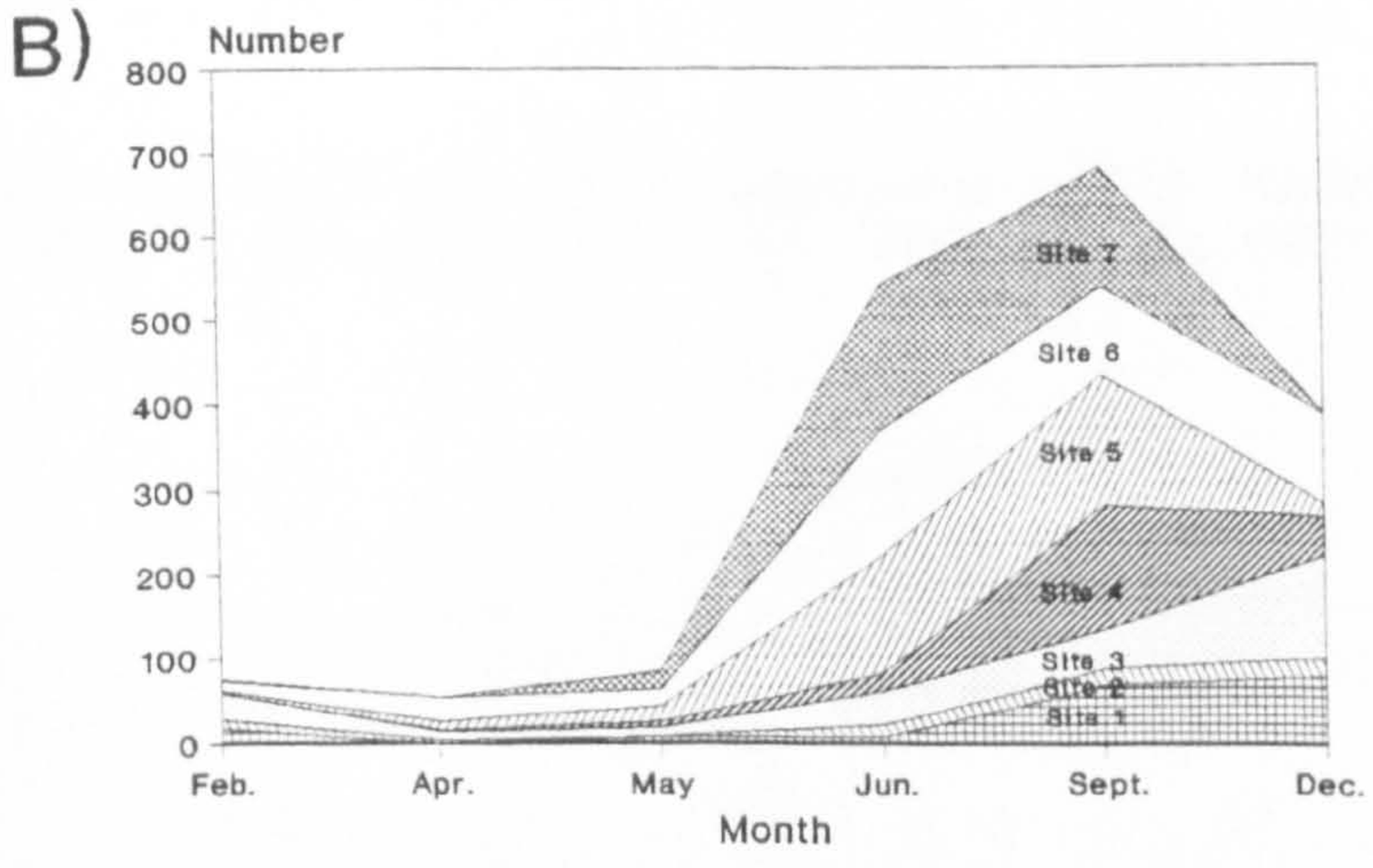


Fig. 2.10. Variations in the number of (A) small (0-35mm), (B) medium (36-55mm) and (C) large (56-85mm) size classes of females at each site during the months of study.

TABLE 2.1. Tests for differences in carapace width between sites during May, June and Sept., using 1-Way Anova tests

	MALES		FEMALES	
MONTH	F VALUE	PROB.	F VALUE	PROB.
MAY	15.84	(**)	6.26	(**)
JUNE	15.24	(**)	2.48	(*)
SEPT.	4.58	(**)	5.74	(**)

*= P<0.05, **=P<0.01

TABLE 2.2.Comparisons of carapace width between sites in May using the Tukey pairwise comparison test

MALES

SITE	1	2	3	4	5	6	7
1		NS	S	S	S	S	S
2	S		NS	S	S	NS	S
3	S	NS		S	NS	NS	S
4	S	NS	NS		NS	S	NS
5	S	NS	NS	NS		NS	NS
6	S	NS	NS	NS	NS		S
7	-	-	-	-	-	-	

FEMALES

- Denotes missing data. S= P<0.05

TABLE 2.3. Comparison of carapace width between sites in June using the Tukey pairwise comparison test.

	MALES						
SITE	1	2	3	4	5	6	7
1		NS	S	S	NS	NS	S
2	NS		NS	S	NS	NS	NS
3	NS	NS		NS	NS	NS	NS
4	S	NS	NS		NS	S	NS
5	NS	NS	NS	NS		NS	NS
6	NS	NS	NS	NS	NS		NS
7	NS	NS	NS	NS	NS	NS	
	FEMALES						

S= P<0.05

TABLE 2.4. Comparison of carapace width between sites in Sept. using the Tukey pairwise comparison test

	MALES						
SITE	1	2	3	4	5	6	7
1		NS	S	S	NS	NS	S
2	S		NS	S	NS	NS	NS
3	S	NS		NS	NS	NS	NS
4	S	NS	NS		NS	S	NS
5	S	NS	NS	NS		NS	NS
6	S	NS	NS	NS	NS		NS
7	S	NS	NS	NS	NS	NS	
	FEMALES						

S= P<0.05

ANNUAL VARIATION IN ABUNDANCE AND COLOUR BETWEEN SAMPLING SITES

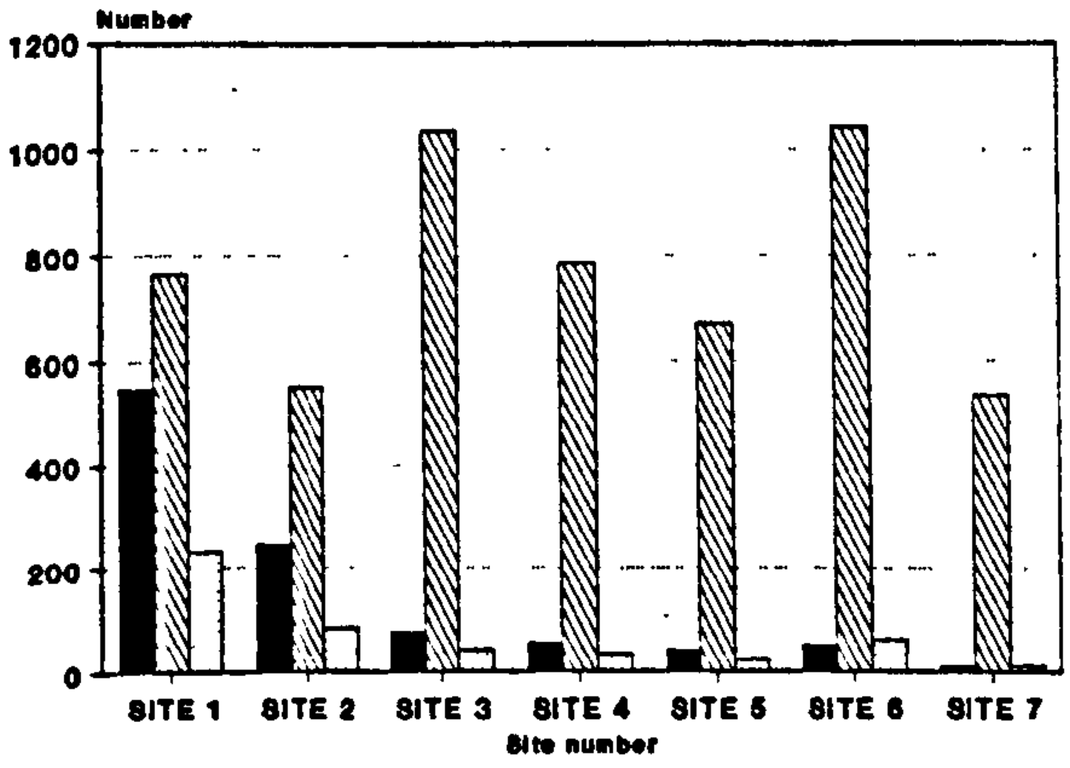
Variations in the numbers and size classes of crabs at each site were obtained by pooling data for each month sampled. The variation of the colour and number of individuals at each site is illustrated in Fig. 2.11. Crabs were abundant both on the open shore sites and in the estuary. Male crabs were found to decrease in numbers from the mouth of the estuary itself towards the head of the estuary (Fig. 2.11.A.), except at site 6, which appeared to be a particularly favourable habitat. Red and orange crabs were common on open shore sites, but few of these individuals were caught within the estuary. When the numbers of red, orange and green crabs are plotted as percentages at each site, a uniform trend is apparent. On the open shore sites green crabs total 50 - 60% of the population, red crabs comprise 30 - 35%, and orange crabs make up the remaining 10 - 15%. In the estuary the pattern is remarkably homogeneous. Red and orange crabs accounted only for 10 - 15% of the population at each site, whilst the percentage of green crabs at each site consistently comprised nearly 90% of the numbers caught in each case.

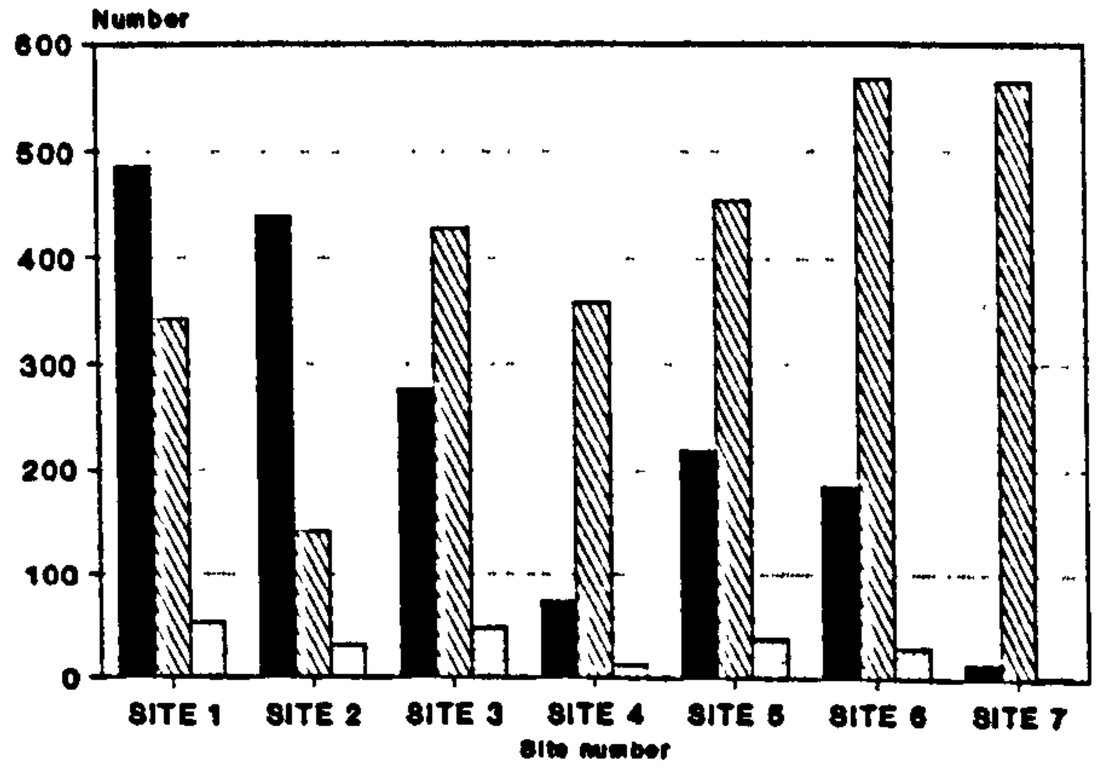
The population structure is similar for females, however, it less regular than for males (Fig. 2.11.C). Females were only about half as abundant as males at every site (Fig. 2.11.A, C), with red individuals

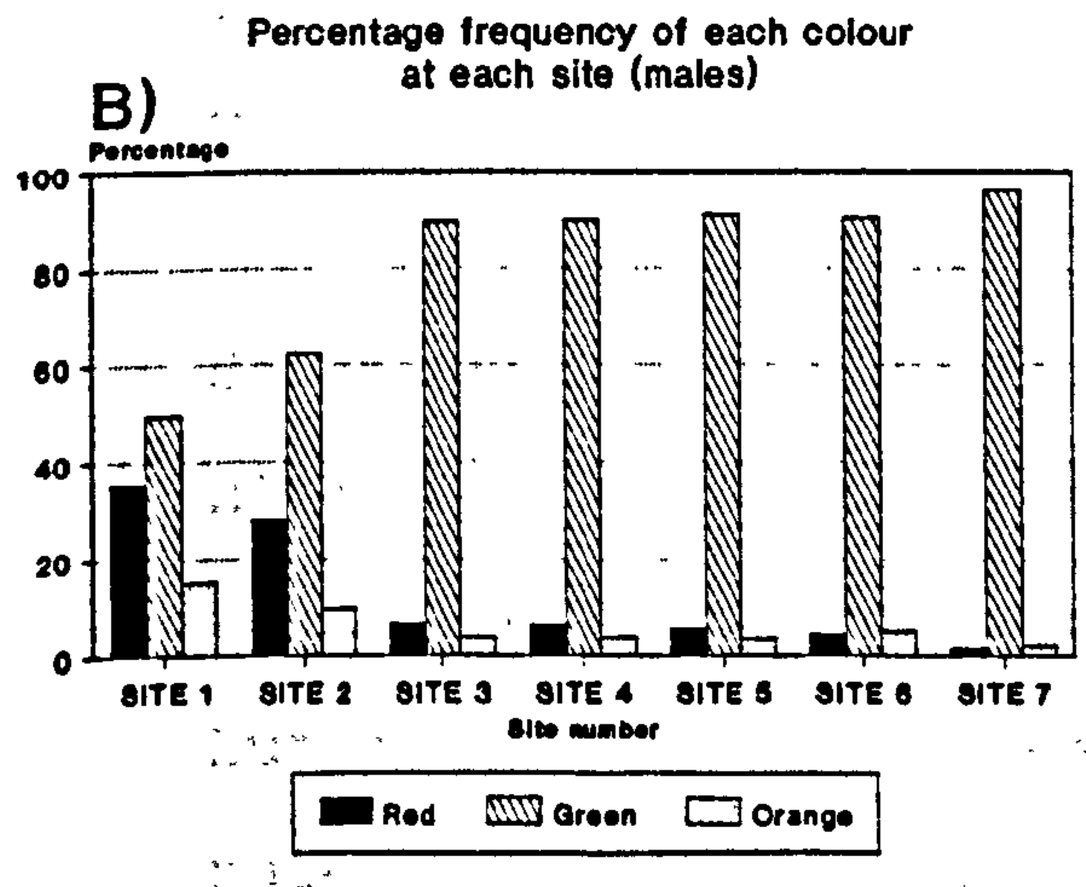
prevalent on the open shore sites and green crabs predominating in the estuary. Red females were caught in greater numbers in the estuary compared with red males.

The population of open shore sites comprises 55 - 70% red crabs, 20 - 40% green crabs and 7 - 8% orange crabs. The percentage of each colour in the estuary varied amongst the sites, but green crabs made up 60 - 90% of the catches, red crabs between 5 - 35%, and orange females accounted for less than 10% of the estuarine population (Fig. 2.11.D).

Fig. 2.11.







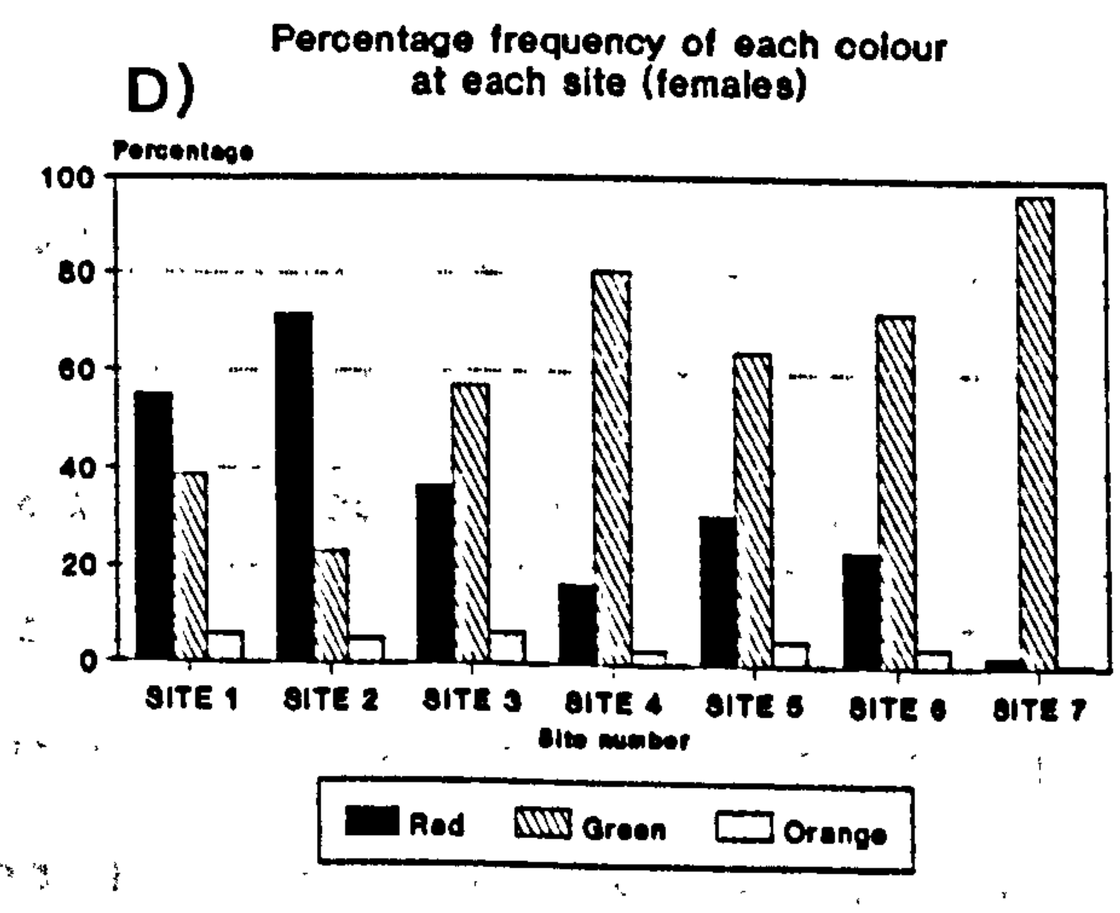


Fig. 2.11. A and C, Distribution of numbers of green, orange and red males and females at each site, obtained from pooled data from all months of study. Figs. B and D illustrate these results as percentage frequency of each colour at each site.

SPATIAL DIFFERENCES IN SIZE CLASSES

The number of individuals of each colour were further divided into 5mm size classes. Fig. 2.12. A to G illustrate the variation of males in these size classes at each site. Site 1 is dominated by large crabs, with specimens below 40mm carapace width being uncommon. Green, orange and red crabs, in the 50 - 70mm range were most common. At site 2 there was a wider size distribution, with greater numbers of smaller crabs than site 1, but still with a majority of crabs in the 50 - 70mm size classes. Red and orange crabs were virtually absent in the estuary, but the sizes present were similar to those of the red and orange crabs captured on the open shore (50 - 75mm). Green crabs in the estuary tended to be smaller than their open shore counterparts, sizes ranging predominantly between 25 - 55mm (Fig. 2.12. C-G) compared with crabs of around 60mm carapace width on the open shore (Fig. 2.12. A,B).

The size class distribution of females was dependent on the colour of the crab, rather than the sites where they were captured. The estuarine population tended to be slightly smaller than the open shore forms, but the size differences were not so pronounced as in male crabs (Fig. 2.13.). Red female crabs were present in moderate numbers at all sites except the head of the estuary. Unlike the males (Fig. 2.12. A, B) there was not as much overlap in the size class distributions of

the colours. There was a gradation in size from green through orange to red colour females. Green crabs were prevalent in the 30 - 50mm size classes, and red crabs in the 40 - 65mm range, with the distribution of orange crabs overlapping between these two ranges. Small crabs (< 15mm) of both sexes, although present at each site were virtually absent from the traps, which tended to select for the larger animals.

Fig. 2.14. illustrates the change in size class distribution as a function of the colour of the crab. Green males were found over the entire size range, greatest numbers being caught in the 40 - 45mm range. Red and orange males were only found in appreciable numbers in the larger size classes. Individuals smaller than 30mm carapace width were rarely captured. The modal class for red males was 60 - 65mm

Chi-square tests were applied to the data to determine differences in size classes. The distribution of the green crabs was wider compared with the orange (Chisqu.=307.4, 9DF, $P<0.05$, S) or the red crabs (Chisqu.=1302.2, 9DF, $P<0.05$, S). Although the distribution of red and orange crabs encompassed similar size ranges, their modes were found to be significantly different from each other (Chisqu.=132.31, 9DF, $P<0.05$, S).

Female crabs exhibited less overlap in size class distribution between the different colours compared to

the males (Fig. 2.14.B), and in general they were smaller. Red and orange crabs were seldom found below 30mm carapace width. The majority of green females were in the 30 - 40mm size class, whereas the modal class for red crabs was 50 - 55mm Relatively few orange females were caught. Chi-square analysis showed significant differences in size class distribution between green and orange (Chisqu=256.52, 7DF, P<0.05, S), green and red (Chisqu=1885.28, 7DF, P<0.05, S) and red and orange (Chisqu=140.83, 7DF, P<0.05, S) females.

Fig. 2.12.

Males

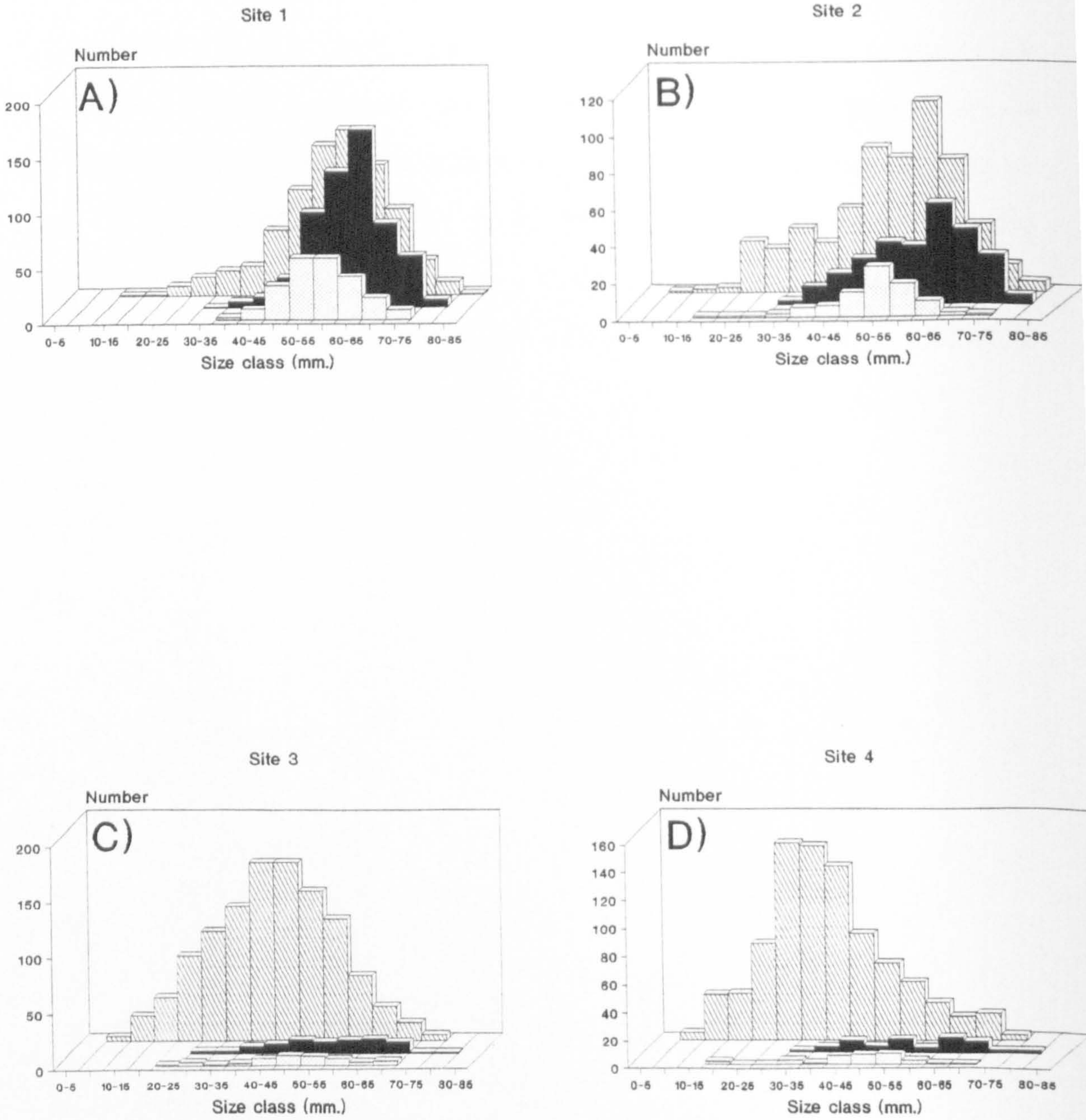


Fig. 2.12. Variation in the number and size class distribution (5mm size class increments) of green, orange and red males at each site, using pooled data from all the months of study.

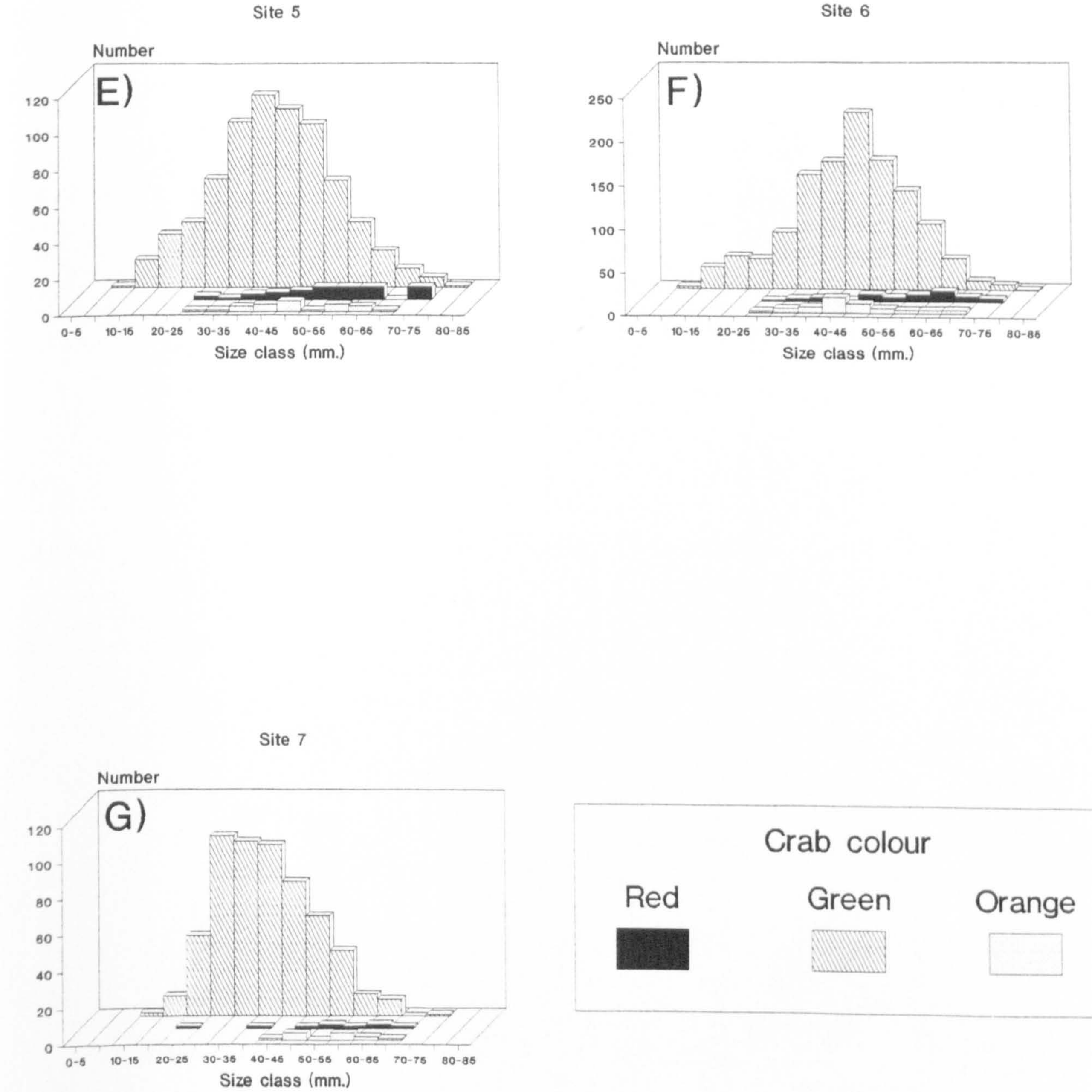

Site 5
Number
E)
120
100
80
60
40
20
0
0-5
10-15
20-25
30-35
40-45
50-55
60-65
70-75
80-85
Size class (mm.)
Site 6
Number
F)
250
200
150
100
50
0
0-5
10-15
20-25
30-35
40-45
50-55
60-65
70-75
80-85
Size class (mm.)
Site 7
Number
G)
120
100
80
60
40
20
0
0-5
10-15
20-25
30-35
40-45
50-55
60-65
70-75
80-85
Size class (mm.)
Crab colour
Red
Green
Orange

Fig. 2.13.

Females

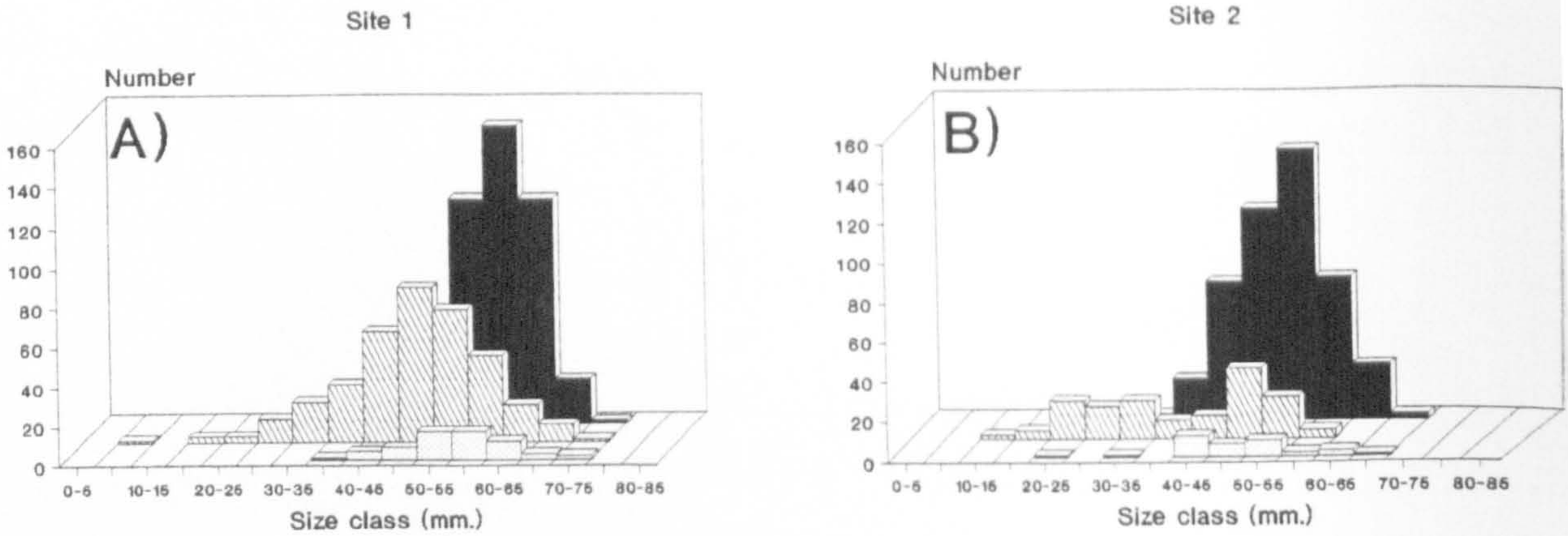


Fig. 2.13. Variation in the number and size class distribution (5mm size class increments) of green, orange and red females at each site, using pooled data from all the months of study.

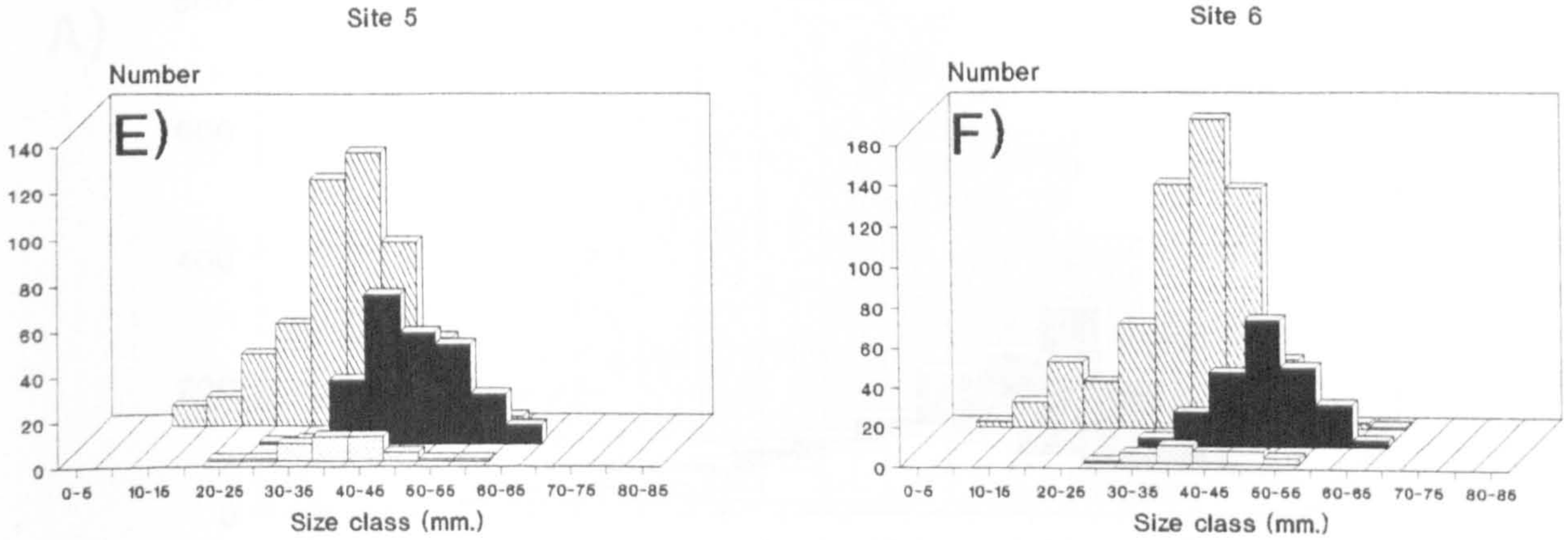
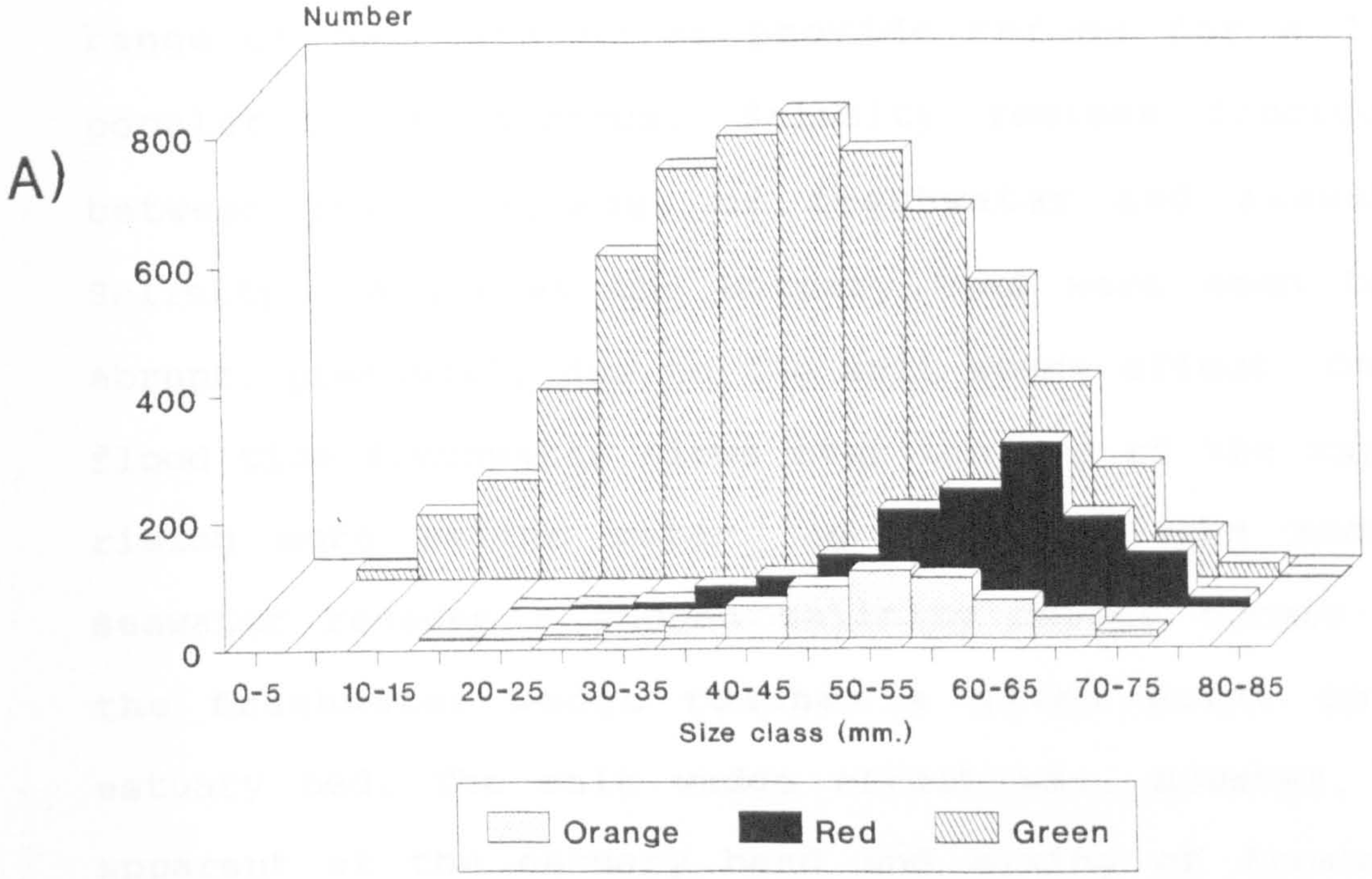


Fig. 2.14. Size class distribution (in 5cm size classes) of green, orange and red males and females, combined from pooled data of crabs captured from all the sites and for all months of study.

Fig. 2.14. Size class distribution of males



Size class distribution of females

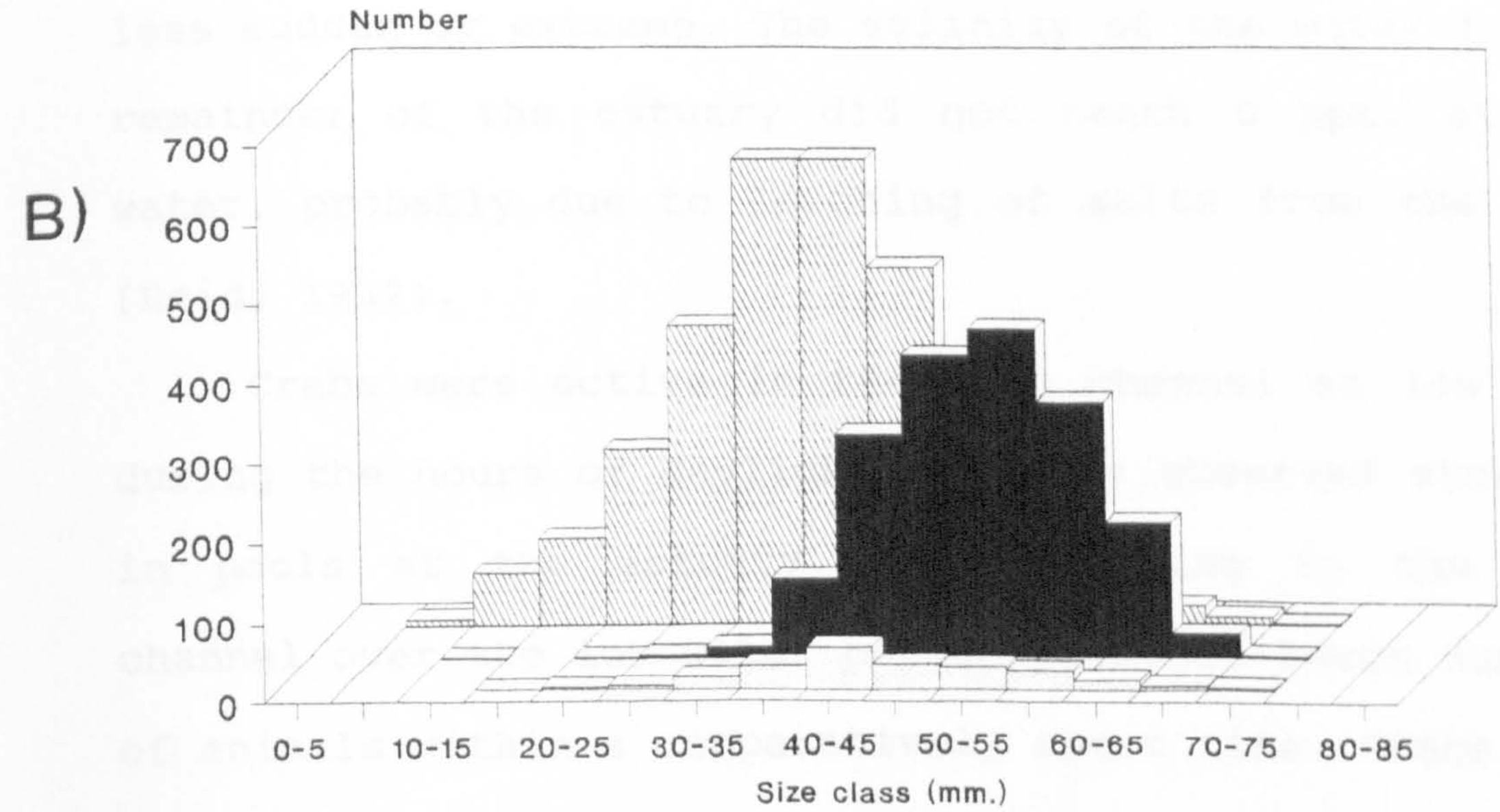


Fig. 2.14. Size class distribution (in 5mm size classes) of green, orange and red males and females, obtained from pooled data of crabs captured from all the sites and for all months of study.

DISCUSSION

The Foryd Bay is an estuary encompassing a wide range of habitats which provide refuge for a large population of *Carcinus*. Salinity regimes fluctuated between the extremes of freshwater and seawater. Salinity changes at the estuary head were seen to be abrupt, presumably due to the salt wedge effect. On the flood tide freshwater flows over the top of the rapidly rising more saline water. As the tide ebbs and the seawater recedes a sudden salinity change occurs when the freshwater wedge reaches a given point on the estuary bed. The salt wedge effect was, however, only apparent at the estuary head and mixing of freshwater and seawater occurred in the remainder of the estuary. Salinity changes downstream of the head were therefore less sudden or extreme. The salinity of the water in the remainder of the estuary did not reach 0 ppt. at low water, probably due to leaching of salts from the sand (Reid, 1932).

Crabs were active in the main channel at low tide during the hours of daylight and were observed stranded in pools at the estuary head. Trapping in the main channel over the low water period produced large numbers of animals within a comparatively short time. Crabs were obviously concentrated within the main channel at this time and as water flow was unidirectional this presumably explains why they promptly found the bait.

Ameyaw-Akumfi and Naylor (1987) also postulated from mark recapture experiments in the Foryd Bay that the majority of *Carcinus* remained in the estuary over low water.

Since crabs remain within the estuary those at the head will experience abrupt salinity changes and up to 6 hour periods of freshwater twice daily. These findings would seem to be in contradiction with the fact that *Carcinus* shows increased locomotor activity to hypo-osmotic shock (Taylor and Naylor, 1977, Bolt and Naylor, 1985), and exhibits a rapid escape response from low salinities (Thomas *et al*, 1981, Ameyaw-Akumfi and Naylor, 1987, see Chapter 3, Fig 3.2, 3.3, 3.8). Evasive behaviour however does not necessarily entail direct escape, since the effects of salinity may be avoided by burrowing into the sediment where the salinity is more stable (Barnes, 1974) or seeking shelter beneath stones where less adverse salinity conditions were presumed to occur (Perkins *et al*, 1969). Effective damping of internal concentrations coupled with the osmoregulatory abilities of *Carcinus* mean that over a tidal salinity cycle the osmotic pressure of the internal fluids only falls by about a third of that of the environment. This enables crabs to survive in fluctuating salinity conditions, down to values which if constant would be lethal (Spaargaren, 1974, Winkler *et al*, 1988).

Migration of crabs from the upper reaches of the

estuary occurred in December, and they did not return to the head of the estuary until late spring (April, May). Similar migrations have been reported from other estuaries (Broekhuysen, 1936, Wolff and Sandee, 1971, Rasmussen, 1973, Klein Breteler, 1976). These migrations are probably independent of salinity changes (even though salinity regimes may be lowered due to increased precipitation) since crabs can osmoregulate more efficiently at the lower temperatures that occur in winter (Dehnel, 1962, Weber and Spaargaren, 1970, Lynch *et al*, 1973, Taylor *et al*, 1977). The movements are more likely to be part of a seasonal, temperature-related, cycle of migration into the sublittoral zone during the winter months (Edwards, 1958, Naylor, 1962, Crothers, 1968, Atkinson and Parsons, 1973). The patchy distribution of crabs within the estuary throughout the year, is probably dependent upon the substrate (Barnes, 1967) and the availability of suitable refuge places (Perkins *et al*, 1969) as there appears to be no gradation with respect to salinity.

In the present study estuarine crabs were, on average, smaller than their open shore counterparts, as also reported by Crothers (1968) and Wolff and Sandee (1971). Small crabs may be less well adapted to cope with salinity fluctuations as their larger surface area to volume ratio renders them less capable of passive damping of internal fluid concentrations (Spaargaren,

1974, 1989). Gilbert (1959), however, found that small *Carcinus* had higher blood osmolalities in low salinities compared to larger individuals. Passive damping of internal fluids has now been shown to be ineffective, and Spaargaren (1989) has stated that area -specific permeabilities are important, particularly so with increasing size of the animal, suggesting that small animals, especially males would have a slight advantage in coping with salinity fluctuations of the external environment. The presence of smaller crabs within the estuary, however, contradicts earlier results (Chapter 1, Fig. 1.8.B) where no correlation was found under experimental conditions between the size of the crab and the time it exited from low the salinity.

Orange and red crabs were largely absent from all estuarine sites, but were abundant on the open shore. Red crabs have been shown in choice chamber experiments to avoid low salinities more quickly and at a higher threshold than green crabs (Chapter 3, Fig. 3.2, 3.3, 3.10) because they are unable to survive for extended periods in reduced salinities (Chapter 1, Fig. 1.2, 1.3). This would appear to be the dominant factor explaining their absence from estuarine areas, though size differences may also play a part. Crabs below 35mm carapace width were very rarely red in colour, so that if salinity imposes constraints on the occurrence of large crabs within the estuary, the predominant colour

will be green simply because the majority of such crabs are small.

No trends were apparent with respect to the sex ratio of males and females, either between estuarine and open shore sites or within the estuary. Over the year male crabs predominated in the trap catches by a factor of about 2 compared with females. In contrast Naylor (1962) and McVean and Findlay (1979) recorded a preponderance of females caught in the intertidal zone and an estuary respectively.

It is possible that the observed distribution of crabs in the Foryd estuary may depend merely on zonation differences related to tidal height, rather than on salinity. Small crabs are largely confined to the upper levels of the intertidal zone, whereas large crabs and those of red coloration are essentially subtidal in habitat (Crothers, 1968, Reid *et al*, 1989). Moreover extensive migration of the larger individuals into the intertidal zone of open coasts occurs on each flooding tide (Edwards, 1958, Naylor, 1958, 1962, Crothers, 1968, Atkinson and Parsons, 1973), and the majority of red crabs remain within the sublittoral zone (Crothers, 1968, Reid *et al*, 1989). As the intertidal zone of the estuary is exposed before that of the open shore, it may account for the smaller average size of crab and the lack of red crabs which do not migrate to such high a level on the shore. However, since river water is still

present within the estuary at low water these sites may be considered as essentially subtidal in nature. The distributional differences observed are, therefore, probably a result of interaction of varying osmoregulatory abilities of individuals and zonation with respect to tidal height, and neither can be considered in isolation.

The only other question remaining is how representative present samples were in relation to the population structure of *Carcinus* as a whole within the estuary. Baited traps provided the most practible method by which the population could be sampled, but such catches are dependent upon interactions between the crab, its environment and the trap and as such, may not be truly representative (Bennet, 1974). Traps tend to select for larger individuals (Williams and Hill, 1982), so any size frequency will be skewed. Also they only capture actively feeding crabs (Crothers, 1968, Williams and Hill, 1982, Robertson, 1989). Berried individuals and those in premoult or early postmoult do not feed (Adelung, 1971) so these as well as small crabs will tend to be precluded from catches. Crothers (1968) states that baited traps are only effective at catching about 80% of the population present at any one time. Clearly, most methods of collection introduce some form of bias, but as methods of trapping were standardised for all sites it is assumed that any error in present

samples was uniform at each site.

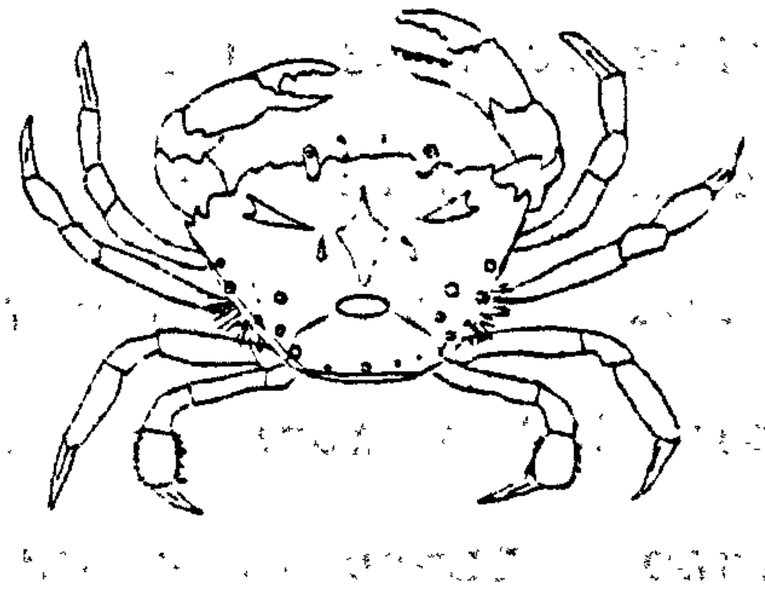
The present study has examined a population of *Carcinus* in one estuary. Further surveys of other estuaries and areas of continuous low salinity such as the Baltic are needed to determine if trends described here are consistent. Only then will it be possible to determine whether such distribution patterns arise solely as a result of the salinity regimes encountered, or whether other factors also intervene.

CHAPTER 3:

Behavioural responses of *Carcinus maenas* when offered a choice of salinities

SUMMARY

The differences in salinity tolerance of red and green crabs were reflected in their salinity preference behaviour. Green crabs persisted longer in the lowest range of salinities tested, especially if a shelter was available, the presence of which did not significantly affect the behaviour of red crabs. Prior acclimation affected the timing of choice behaviour; the lower the salinity of acclimation the earlier the time of exit from the lowest salinities tested, and vice versa.



INTRODUCTION

The physiological responses of crustaceans and other organisms with respect to changing salinities are well documented, whilst studies on the behaviour in response to this environmental variable are rather more fragmentary (Davenport, 1985). However, whereas physiological changes associated with alterations of an animals internal condition often take some time to complete (Lockwood, 1976), behavioural osmotic responses have advantages which enable the animal to avoid or delay exposure to adverse conditions.

Of the behavioural responses of Crustacea that have been investigated, the anomuran crab *Porcellana platycheles*, displays discriminatory behaviour, but only outside its limits of physiological tolerance. It showed rapid avoidance of salinities below 40% seawater, but could not distinguish differences when offered a choice between pairs of salinities above 40% seawater (Davenport and Wankowski, 1973). The amphipod *Corophium volutator* has been shown to survive in salinities from 2ppt. to 50ppt. (McLusky, 1967). However, its preferred salinity range was found to be 10ppt. to 30ppt., and it exhibited avoidance behaviour of salinities outside this range. *C. volutator* did not discriminate between pairs of salinities within its preferred range of tolerance (McLusky, 1970). Comparable behavioural reactions are reported for *Marinogammarus marinus* which had a

preferred salinity range of 80% to 100% seawater, although it was able to survive in more dilute concentrations (Bettison and Davenport, 1976). In contrast the mud crab *Scylla serrata* was able to survive in salinities between 1ppt. and 42ppt., but apparently showed no ability to discriminate between any of these salinities (Davenport and Wong, 1987). *Carcinus maenas* avoids both hyposaline and hypersaline media by increased locomotor activity, defined as halokinesis (Taylor and Naylor, 1977, Thomas, *et al*, 1981, Bolt and Naylor, 1985, Ameyaw-Akumfi and Naylor, 1987). Its preferred salinity range of 27ppt. to 41ppt. (Thomas *et al*, 1981), or 17ppt. to 40ppt. (Ameyaw-Akumfi and Naylor, 1987), as determined by behavioural studies, is well within the limits of its physiological tolerance, as *Carcinus* is known to survive indefinitely in salinities as low as 4ppt. (Poulsen, 1922, 1949, Broekhuysen, 1936).

Apart from preference for a medium within an organism's range of tolerance, behavioural selection of a medium may occur, influenced by the salinity to which the individual had previously been acclimated (Lockwood, 1976). The coconut crab *Birgus latro* was shown to control the osmotic pressure of its body fluids by behavioural selection of various seawater concentrations (Gross, 1955). *Pachygrapsus crassipes* chose salinities lower than 100% seawater after

acclimation to 150% seawater, but, salinity choice was unaffected by acclimation to 50% seawater (Gross, 1957) The hermit crab *Pagurus bernhardus* exhibited isolation behaviour, by retreating into its shell, at a lower salinity after acclimation to dilute seawater (Davenport *et al*, 1980). Prior acclimation to either hypersaline or hyposaline media, however, had no affect on the salinity choice behaviour of *Corophium volutator* (McLusky, 1970) or *Marinogammarus marinus* (Bettison and Davenport, 1976).

The aim of the present study was to investigate the salinity preference behaviour of *Carcinus maenas* in more detail, to examine any differences occurring in choice behaviour between the red and green colour forms and to study the effects of acclimation to both high and low salinities on the above parameters.

MATERIAL AND METHODS

DESCRIPTION OF TEST TANKS

Two types of choice chamber were used in salinity choice experiments.

1. A multiple choice chamber as used by Ameyaw-Akumfi and Naylor (1987) (Fig. 3.1.A) was constructed of waterproofed marine plywood 160 x 40 x 40cms high. Four V shaped chambers were constructed along its length using concrete. These were painted and sealed to prevent mixing between chambers. Each chamber sloped inwards to a maximum depth of 20cms and was separately aerated. Salinities were found not to vary by more than 0.5ppt. during the experimental period.

2. A two choice chamber was constructed within a fibreglass tank of 132 x 72 x 34cms high (Fig. 3.1.B). A divider was fibreglassed into the centre of the tank to prevent mixing of water between the two sides. Two boards of painted marine plywood sloped up at approximately 30^{o} to the centre of the tank and were held in place with concrete wedges. Three movable partition boards were placed along the length of the tank, effectively dividing it into four replicate channels approximately 17cms wide. Holes were drilled in each board to allow free circulation of water, and each channel was aerated. Perspex boards were placed over the tank to prevent crabs climbing from one channel to another.

A number of individual experiments were designed to test responses of crabs to varying salinities, and these are described in detail within the chapter. As mentioned in the general material and methods section all crabs were males of between 40 to 70mm carapace width, and unless otherwise stated red and green crabs were tested separately in experiments.

MULTIPLE SALINITY CHOICE

Two separately tested sets of differing salinities were arranged linearly in the multi-choice tank. The low salinity set consisted of 5,11,17 and 22ppt., and the high set of 22,28,34 and 40ppt. Batches of five crabs were introduced into each chamber. The number of crabs per chamber was recorded at half hourly intervals for the first 4 hours, since crabs tend to make choices for a particular salinity within 2-3 hours (Thomas *et al*, 1981). Thereafter the number of crabs was recorded at hourly intervals for 6 hours and subsequently at 2 hour intervals up to a total time of 12 hours. The crabs had been previously acclimated to salinities of less than, greater than or equal to that of seawater before being introduced to the choice chambers. The sums of 4 replicates for each colour and acclimation were used in the presentation of results and statistical analysis. Full strength seawater (34ppt.) in each chamber was used as a control to ensure there was no bias for a certain chamber. Experiments were carried out in constant light

provided by a fluorescent strip suspended approximately two metres above the tank.

TWO CHOICE SALINITY DETERMINATION

A more accurate determination of timing of salinity perception was conducted using the two-choice chambers. Salinities of 5,11,17,22,28 and 34ppt. were tested against full strength seawater (34ppt.). The results for twenty crabs of each colour, previously acclimated to low and normal salinities were individually analysed. The crabs were introduced into the lower salinity and time lapse video recording equipment was used to determine the initial time of exit from the lower salinity. Average time of initial exit could then be calculated. Experiments were performed in constant light provided by a single fluorescent strip suspended 3 metres above the tank.

SHUTTLING BEHAVIOUR

Determination of movements between the two chambers of different salinity was carried out using activity detection switches (whisker switches) described in more detail in chapter 4 (Fig. 4.1.C.). Two whisker switches were connected per channel of the choice chamber, one on each side of the mid partition. Each time a circuit was broken by movement of a crab from one side of the chamber to another it was recorded as an event by a BBC 32K microcomputer and datalogged. Data were analysed

using the Orpheus data analysis program which allowed the number and time of shuttles between chambers to be calculated.

Salinities of 5,17 and 34ppt. were tested against full strength seawater (34ppt.). Crabs were introduced into the lower salinity, and between 8 and 12 crabs from each acclimation salinity (low, normal and high) were used. Movements of individual crabs between each salinity were recorded over a period of 24 hours in constant darkness.

SALINITY CHOICE - GROUPS OF CRABS

The previous experiments conducted using the two choice chamber tank analysed the behaviour of individual *Carcinus* held in separate channels. Possible group effects of a larger number of animals on salinity choice behaviour were tested by using a number of group sizes. A salinity of 5ppt. was tested against full strength seawater, with equal numbers of both colours of *Carcinus* being introduced into the salinity of 5ppt. Groups of 50,25,10,5 and single animals with repetitions of 4,2,5,10 and 50 times respectively were used in the experiments. The number of crabs remaining in the lower salinity after 0.5,1,2,3,4,5 and 6 hours was recorded, the experiments being carried out in constant light.

EFFECT OF SHELTER ON SALINITY CHOICE BEHAVIOUR

Shelters were constructed of half pieces of plastic pipe 15cms x 12cms in diameter, weighted down by stones glued on top of the shelter. The two choice chamber tank was used with a salinity of 5ppt. tested against full strength seawater (34ppt.). One shelter per channel of the tank was placed in the 5ppt. chamber and one crab was introduced into each channel, twenty five crabs of each colour being used. The number of animals remaining in the 5ppt. chamber after 0.5,1,2,3,4,5 and 6 hours was recorded. Additional experiments were conducted using a salinity of 5ppt. in each chamber with shelters provided in one of the chambers only. Twenty crabs of each colour were initially placed in the shelter-containing chamber, and this was repeated with another set of crabs introduced into the open side. The number of crabs in the sheltered and open sides were recorded over the same time period in constant dim light.

Fig. 3.1.
(Not to scale)

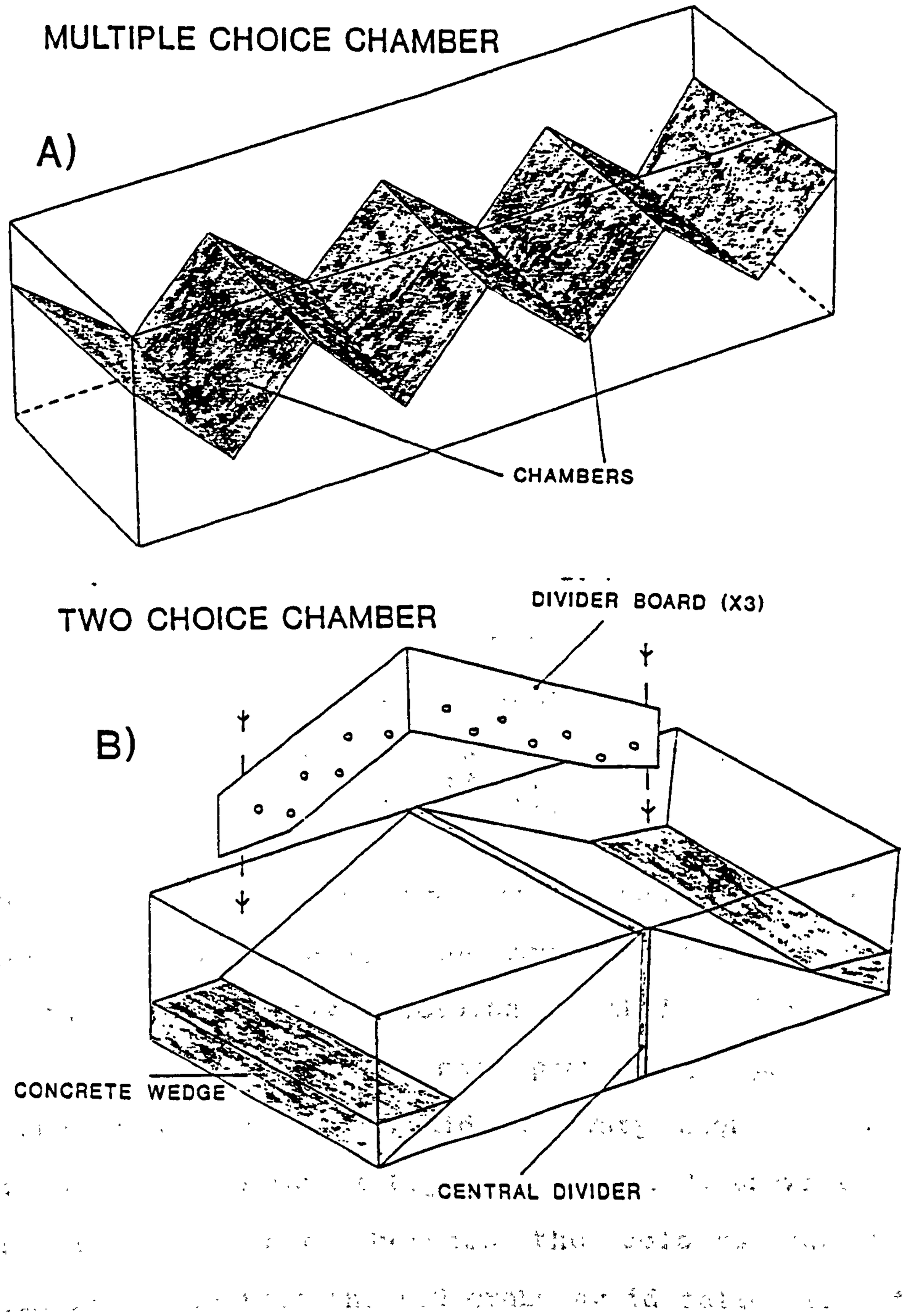


Fig. 3.1. A) Mutiple choice and B) Two-choice chambers used in salinity choice experiments. See text for dimensions

RESULTS

MULTIPLE SALINITY CHOICE

Figs. 3.2. A,B,C and 3.3. A,B,C show movements of red and green crabs between chambers of salinities ranging from 5ppt. to 22ppt. separated by a 6ppt. difference. The graphs show that movement occurs away from the lower salinities towards the higher salinities.

A hiloglinear test on SPSS-X (see Appendix) was used to test for significant differences of salinity choice between green and red colour forms and the salinity they had been previously acclimated to. Table 3.1. shows the interactions of the contingency table.

Various models were fitted, always including Time*colour*acclimation because of the design of the experiment. It was found that the only acceptable model (other than the saturated model) was :-

(Time*colour*acclimation)
Colour*acclimation*chamber
Time*acclimation*chamber

These 3 way interactions are interpreted as follows.

<u>Colour*acclimation*chamber</u> -The number of animals are distributed differently according to their colour and the salinity that they had been previously acclimated to, and this distribution did not vary significantly with time. Examination of Figs. 3.2. and 3.3. shows that this main difference between the colours can be accounted for in that the red crabs avoid salinities of 5ppt. and 11ppt. The numbers in 17ppt. stay relatively

constant and migration towards the highest salinity (22ppt.) occurs. In contrast green crabs avoid only 5ppt., the number of animals in the chambers containing 11ppt. and 17ppt. remaining relatively constant. However, as with red crabs there is migration towards the 22ppt. salinity. The distribution of the red and green colour forms in each of the chambers also varies with the acclimation salinity, but a consistent pattern in not discernible.

Time*acclimation*chamber - When the distribution of the differently acclimated crabs is considered as a function of time, a pattern becomes apparent. There is a variation in the numbers of crabs in each chamber with time according to the salinity of acclimation, but this distribution was similar for both the red and green crabs. Fig. 3.4. A and B shows this pattern clearly. The Cumulative Index shows directional movement, i.e. avoidance of low salinities and migration towards the higher salinities. The steeper the slope the faster the rate of movement towards the higher salinities, and a horizontal line indicates no preference for a particular chamber. Red and green crabs exit low salinities at a faster rate when they have been previously acclimated to low salinity (17ppt.) as compared with those acclimated to normal or high salinities. Crabs acclimated to normal salinities (34ppt.) exit low salinities at a faster rate compared to crabs previously acclimated to high salinity

(50ppt.) (Fig. 3.4.A,B). In other words given a choice of salinities in the range 5-22ppt. the higher the salinity of acclimation the longer the crabs were able to tolerate the lower salinities.

Fig. 3.5. A,B,C and Fig. 3.6. A,B,C show movements of 'red and green colour forms of *Carcinus* between chambers of salinities in the 22ppt. - 40ppt. range. Unlike the lower salinity set (Fig. 3.2. and 3.3.) there does not appear to be movement toward the optimum salinity (in this case 34ppt.). Table 3.2. shows the significant interactions obtained after carrying out a hiloglinear test on SPSS-X. The only significant 3 way interaction obtained is :-

(Time*colour*acclimation)

Colour*acclimation*chamber The number of animals in each chamber varies with their colour and the acclimation salinity, but analysis of the graphs shows no consistent trend with either colour or acclimation and no directional movement towards the optimum salinity. The derivation of the significant interaction is therefore probably explainable by chance.

To ensure there was no bias for a certain chamber, movements of red and green crabs were observed amongst chambers when salinity choice offered in each chamber was 34ppt. Fig. 3.7. A and B shows the results obtained, although movement occurs there is no preference for a particular chamber. This is confirmed by a hiloglinear

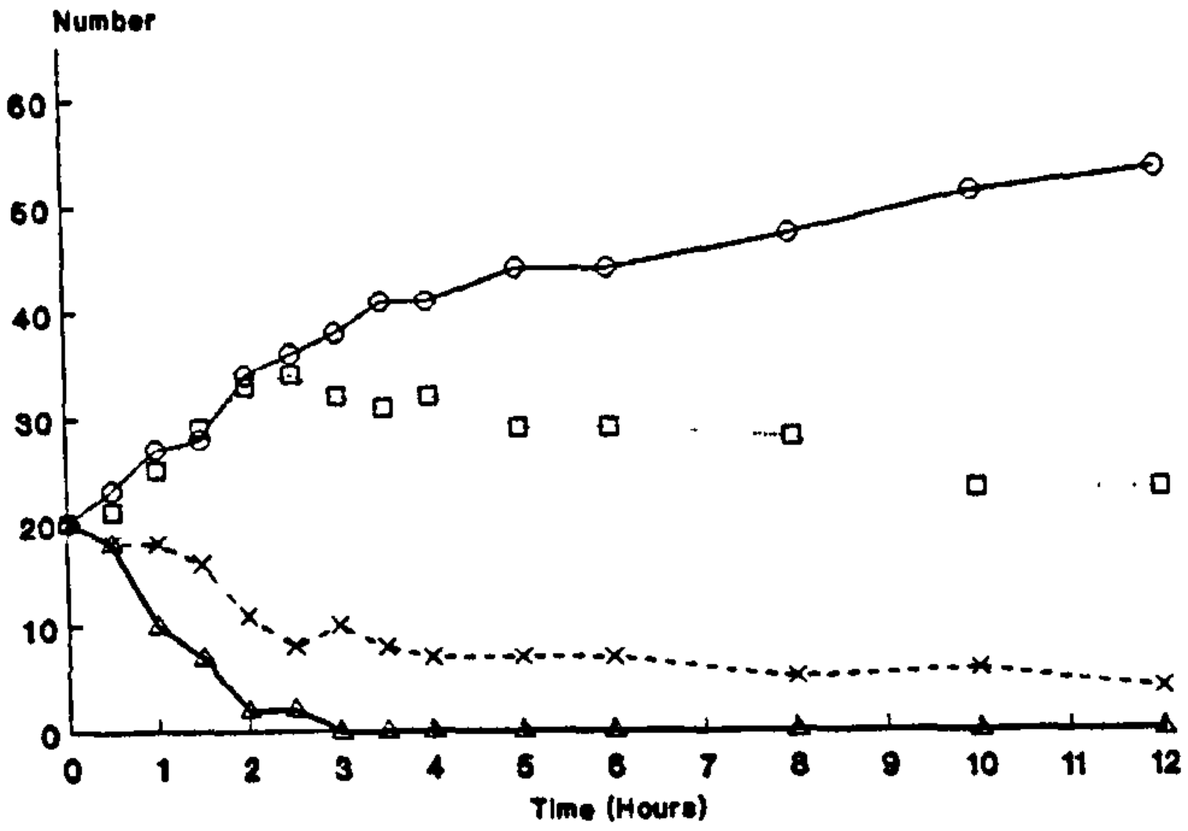
test, in which all interactions were found to be non-significant. The choices made by the crabs in the two previous experiments are therefore a result of the salinity experienced and not the preference for any particular chamber.

Fig. 3.2.

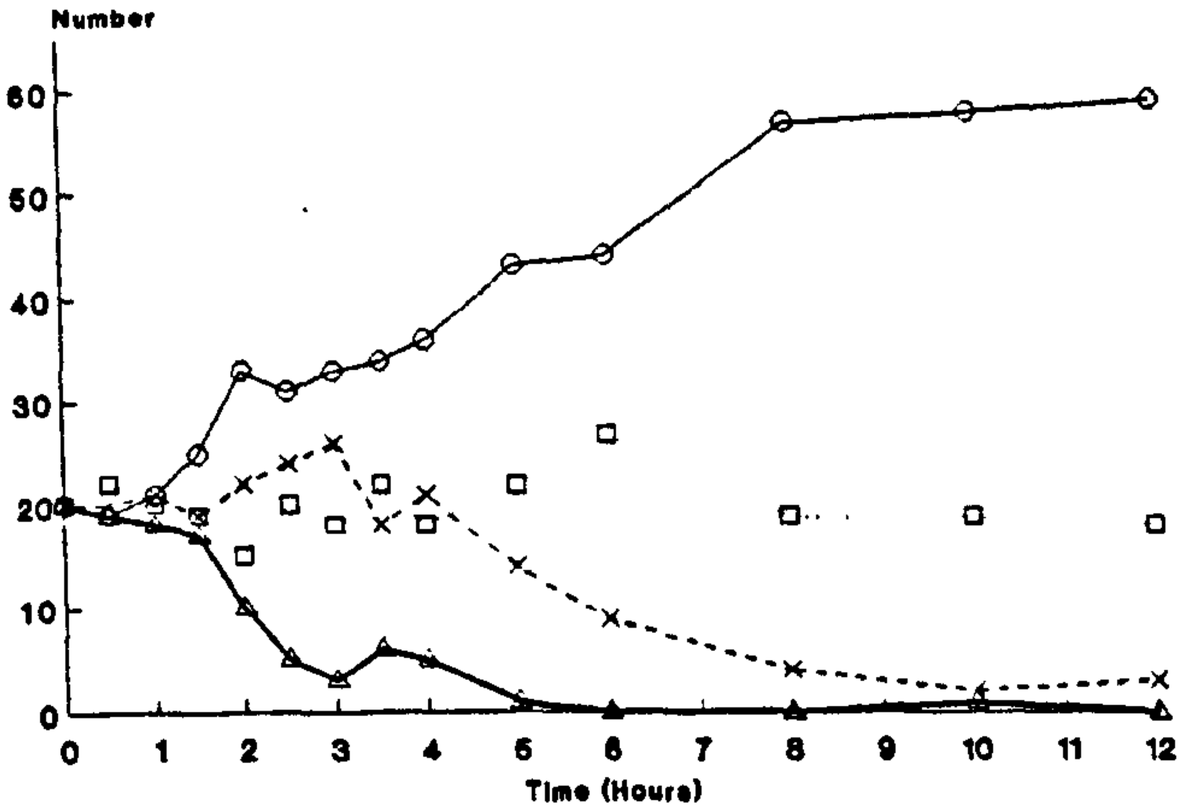
Red crabs

5ppt. - 22ppt. range

A)



B)



C)

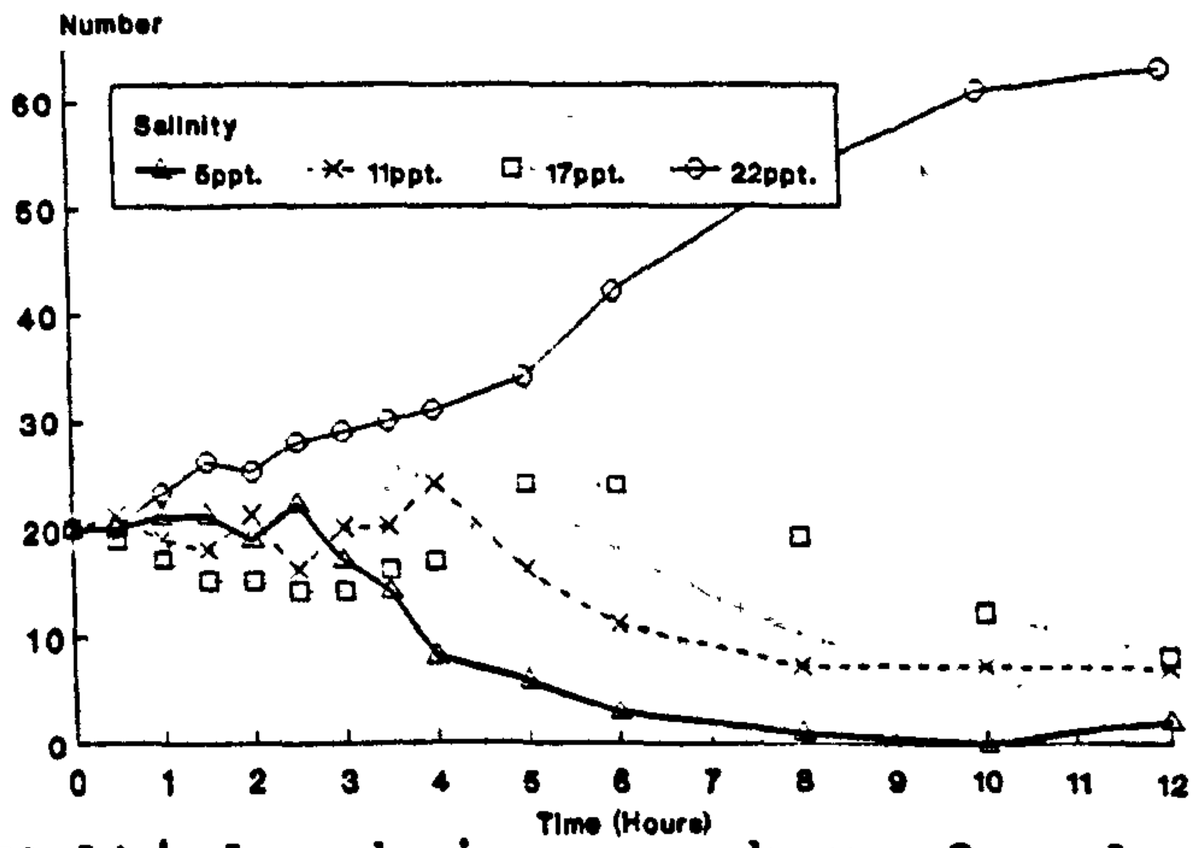


Fig. 3.2. Multiple choice, number of red crabs making a choice between salinities in the 5-22ppt. range, after acclimation to low, normal and high salinities. Graphs represent totals of 4 repetitions at each acclimation salinity, each with 5 crabs.

Fig. 3.3.

Green crabs

5ppt. - 22ppt. range

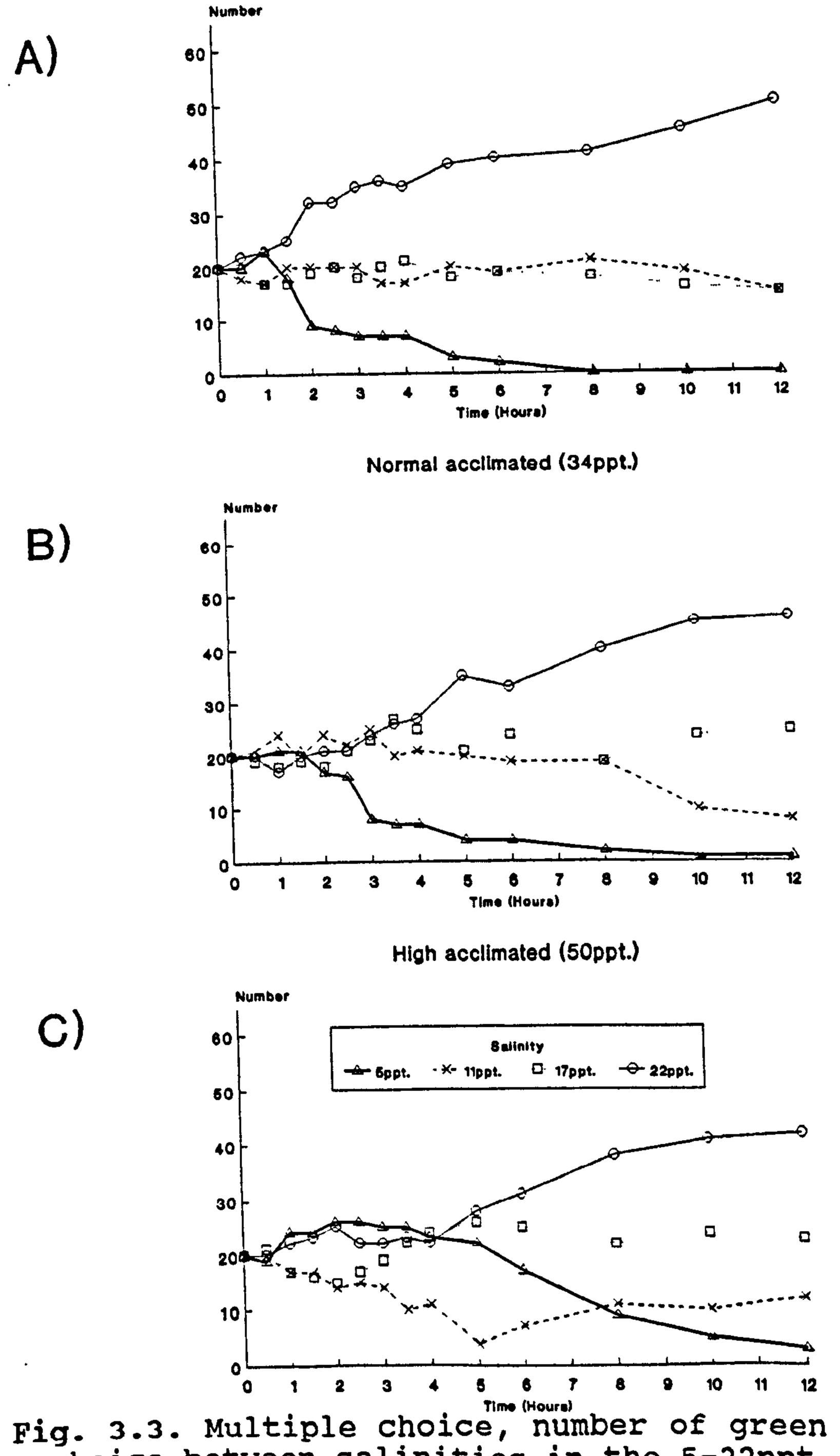


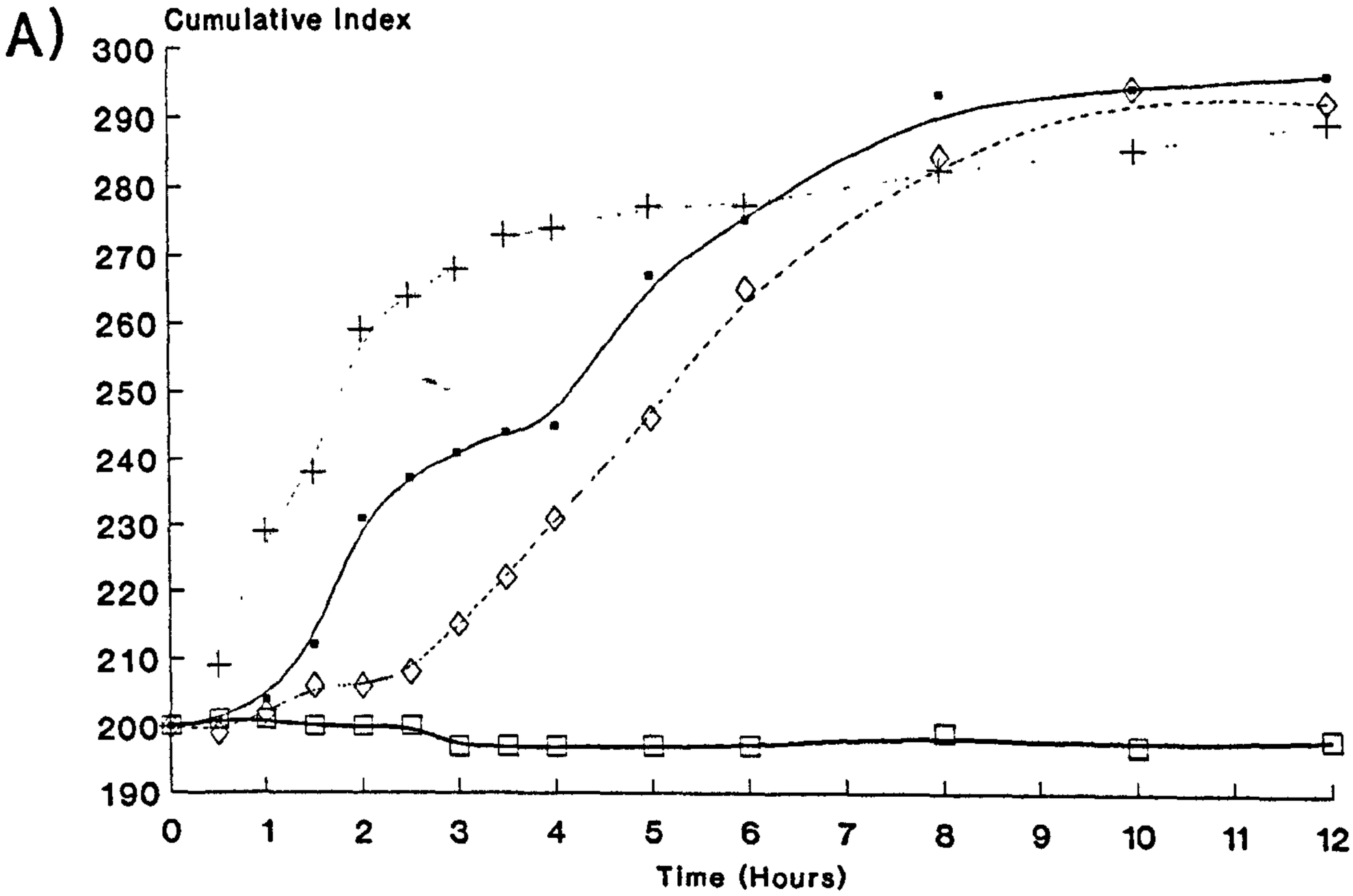
Fig. 3.3. Multiple choice, number of green crabs making a choice between salinities in the 5-22ppt. range, after acclimation to low, normal and high salinities. Graphs represent totals of 4 repetitions at each acclimation salinity, each with 5 crabs.

Fig.3.4. Cumulative Index

C.I.=(Chamber1*1)+(Chamber2*2)+(Chamber3*3)+(Chamber4*4)

This calculation is performed on the results obtained at each acclimation salinity (Fig. 3.2. and 3.3. A-C). The calculation shows movement away from lower salinities towards the higher salinities (the steeper the curve the faster the rate of directional movement; a straight line indicates no directionality).

Fig. 3.4.


Red crabs
A)
Cumulative Index
300
290
280
270
260
250
240
230
220
210
200
190
0
1
2
3
4
5
6
7
8
9
10
11
12
Time (Hours)

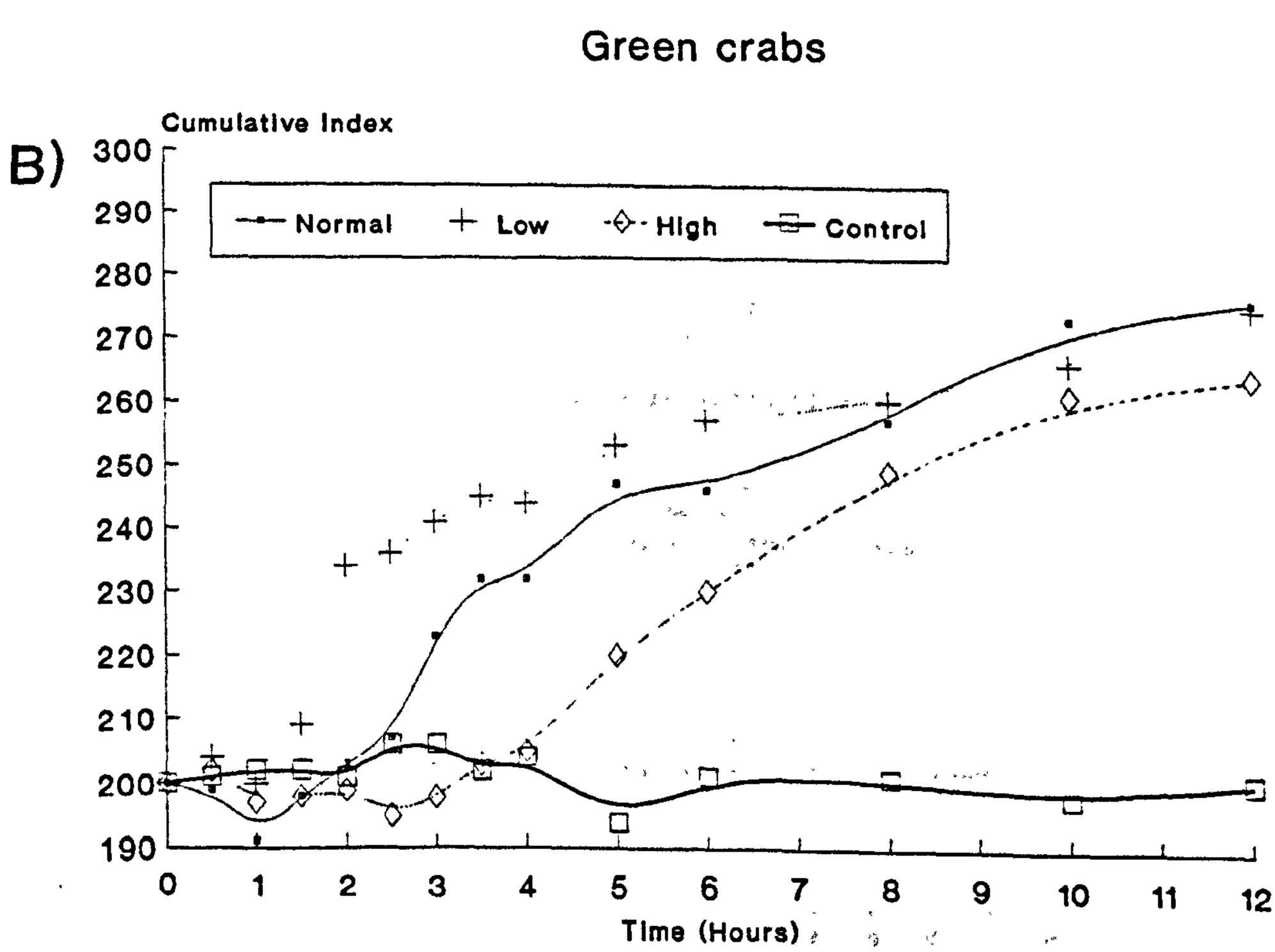

Green crabs
B)
Cumulative Index
Normal
Low
High
Control
300
290
280
270
260
250
240
230
220
210
200
190
0
1
2
3
4
5
6
7
8
9
10
11
12
Time (Hours)

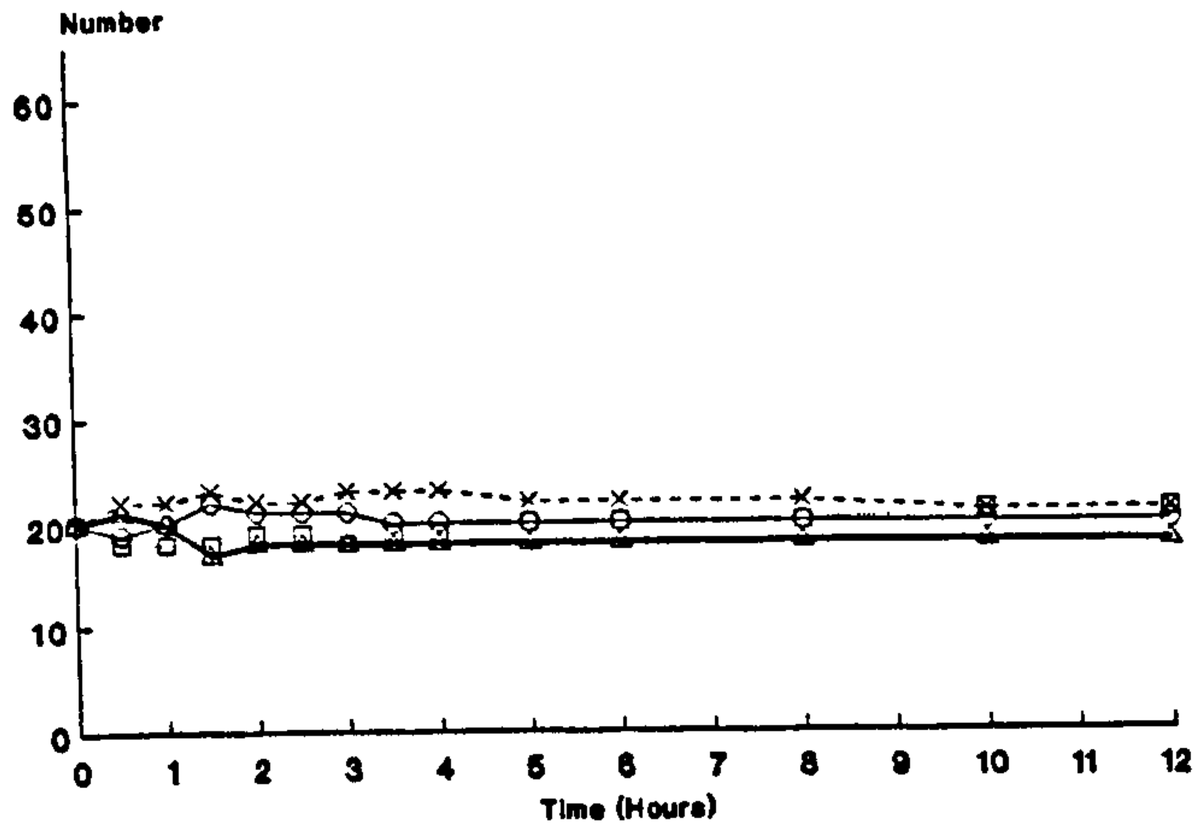
Fig. 3.5.

Red crabs

22ppt. - 40ppt. range

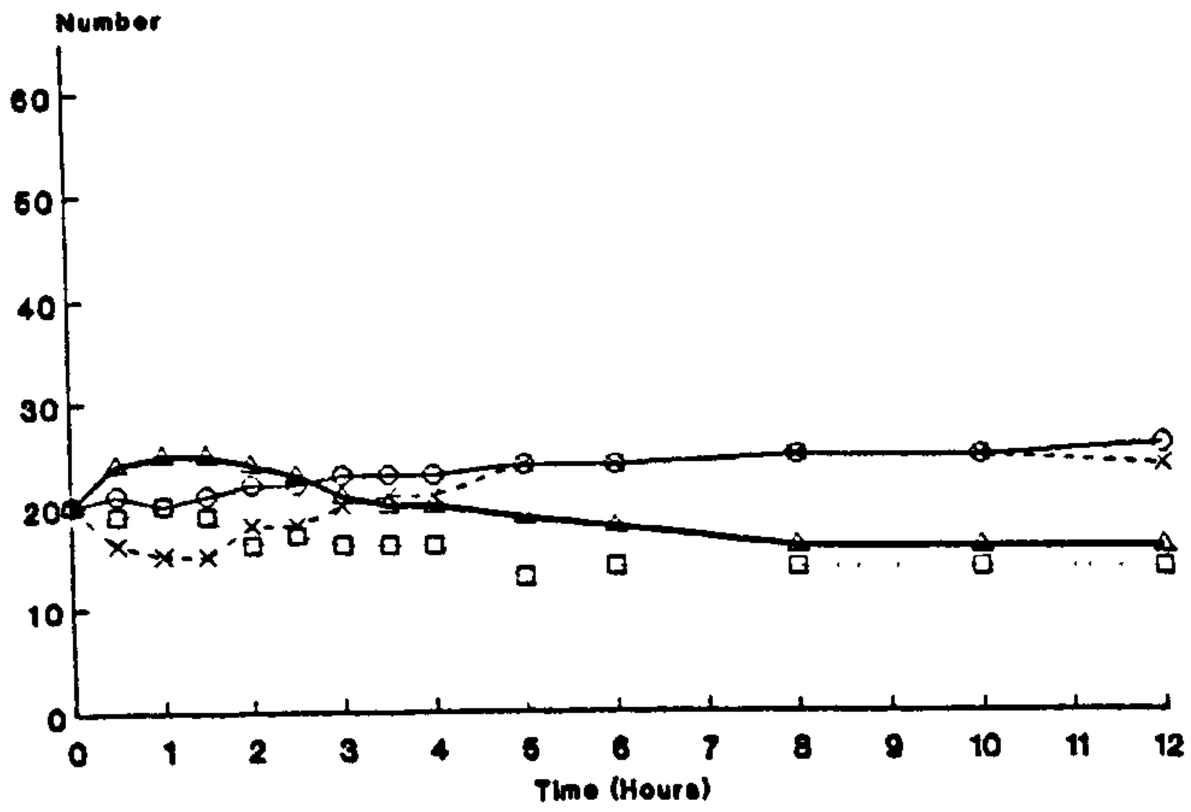
Low acclimated (17ppt.)

A)



Normal acclimated (34ppt.)

B)



High acclimated (50ppt.)

C)

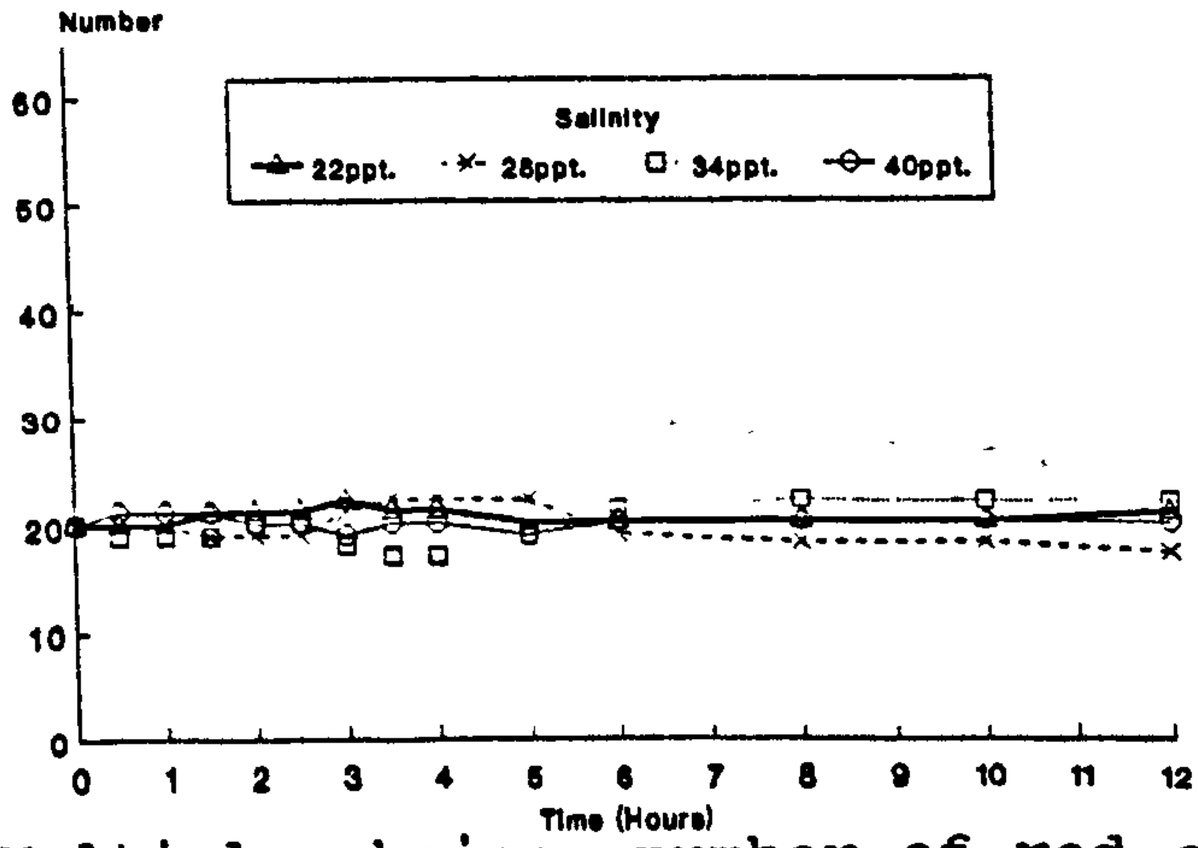


Fig. 3.5. Multiple choice, number of red crabs making a choice between salinities in the 22-40ppt. range, after acclimation to low, normal and high salinities. Graphs represent totals of 4 repetitions at each acclimation salinity, each with 5 crabs.

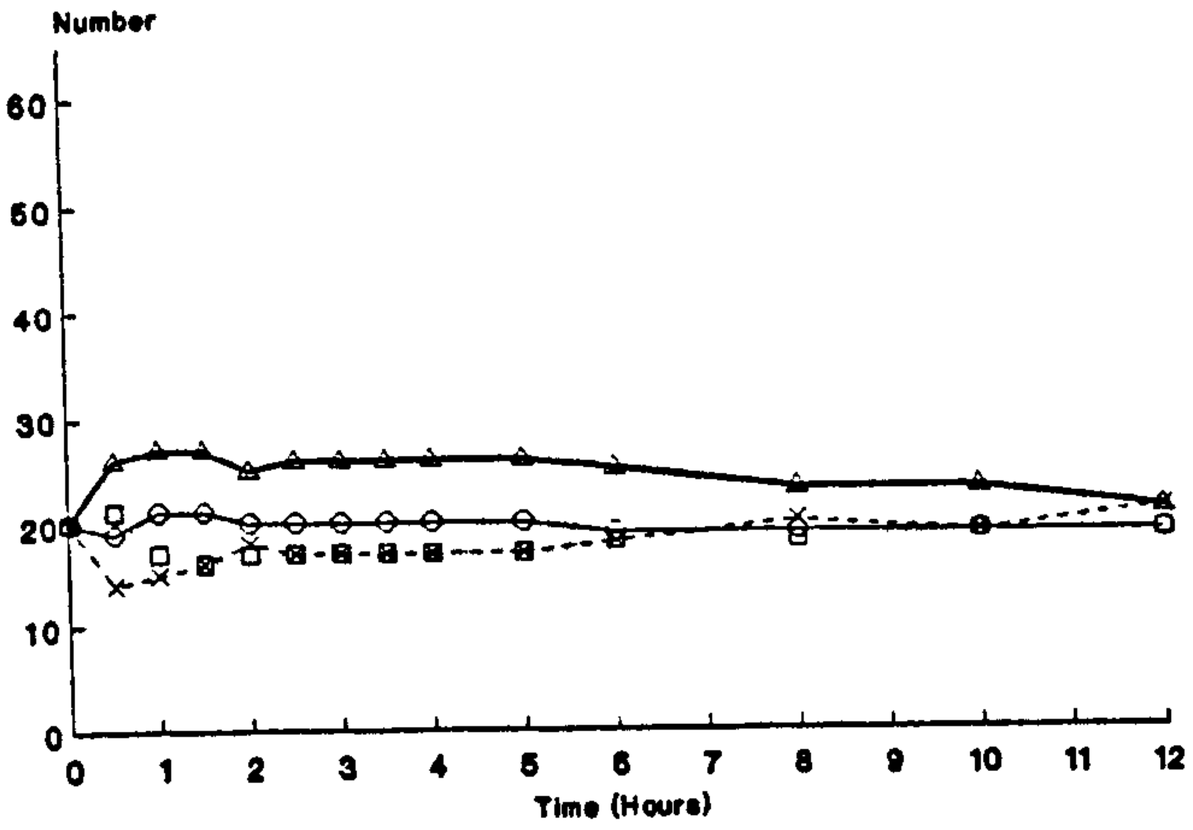
Fig. 3.6.

Green crabs

22ppt.-40ppt. range

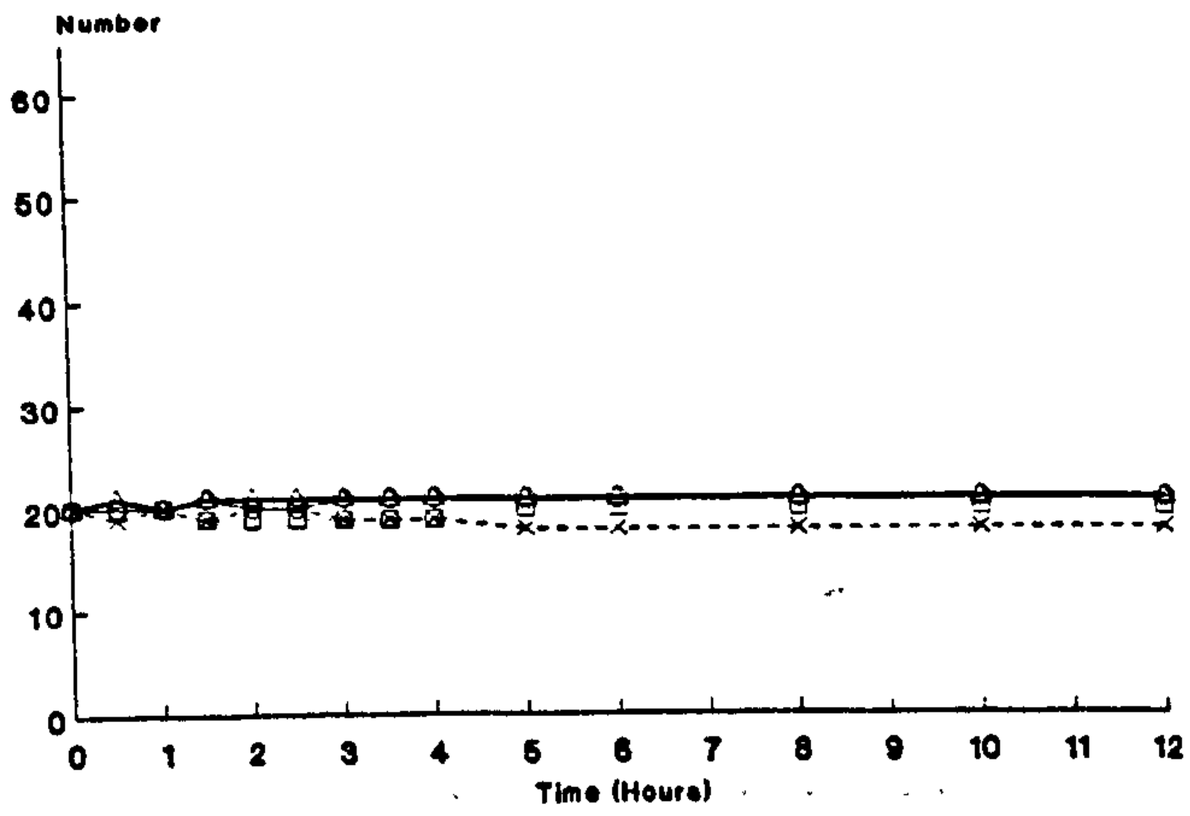
Low acclimated (17ppt.)

A)



Normal acclimated (34ppt.)

B)



High acclimated (50ppt.)

C)

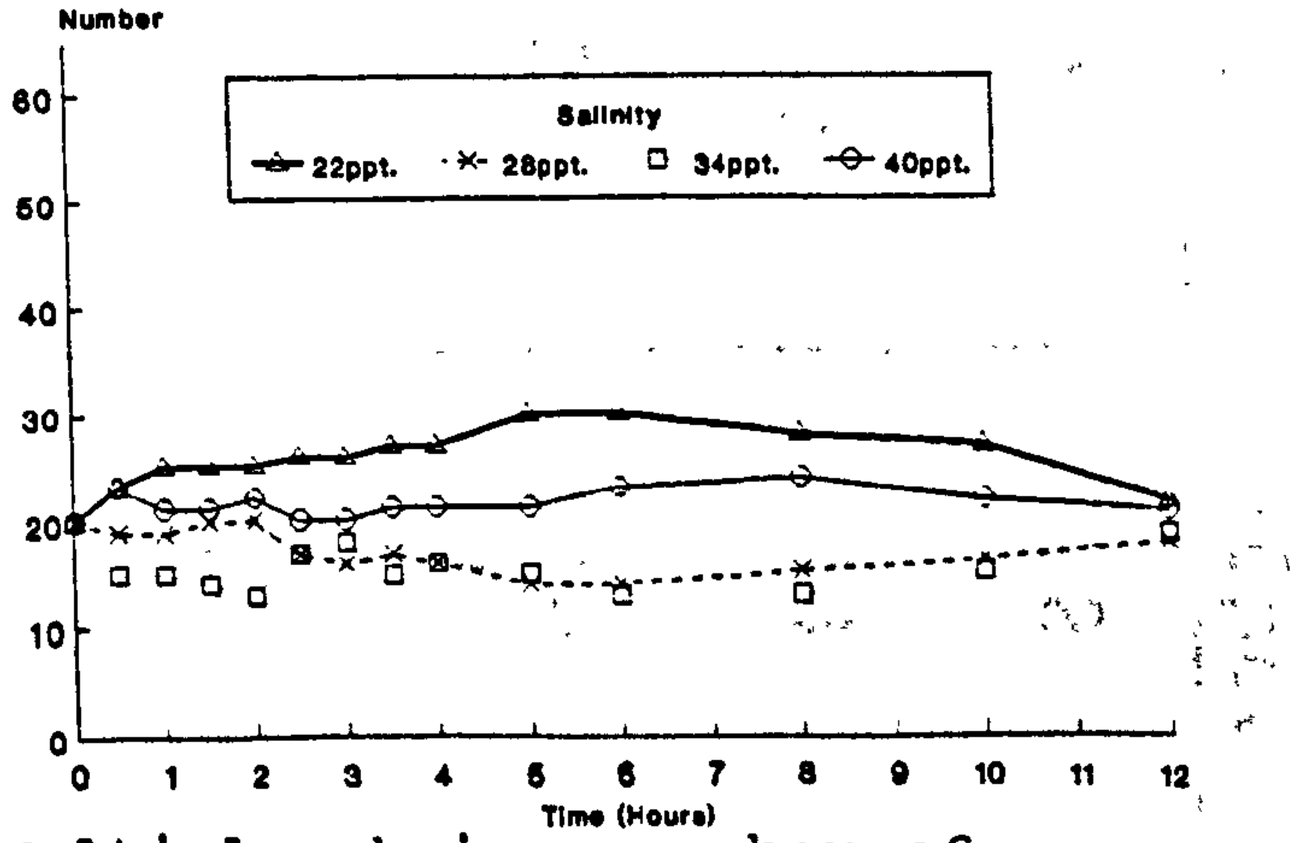
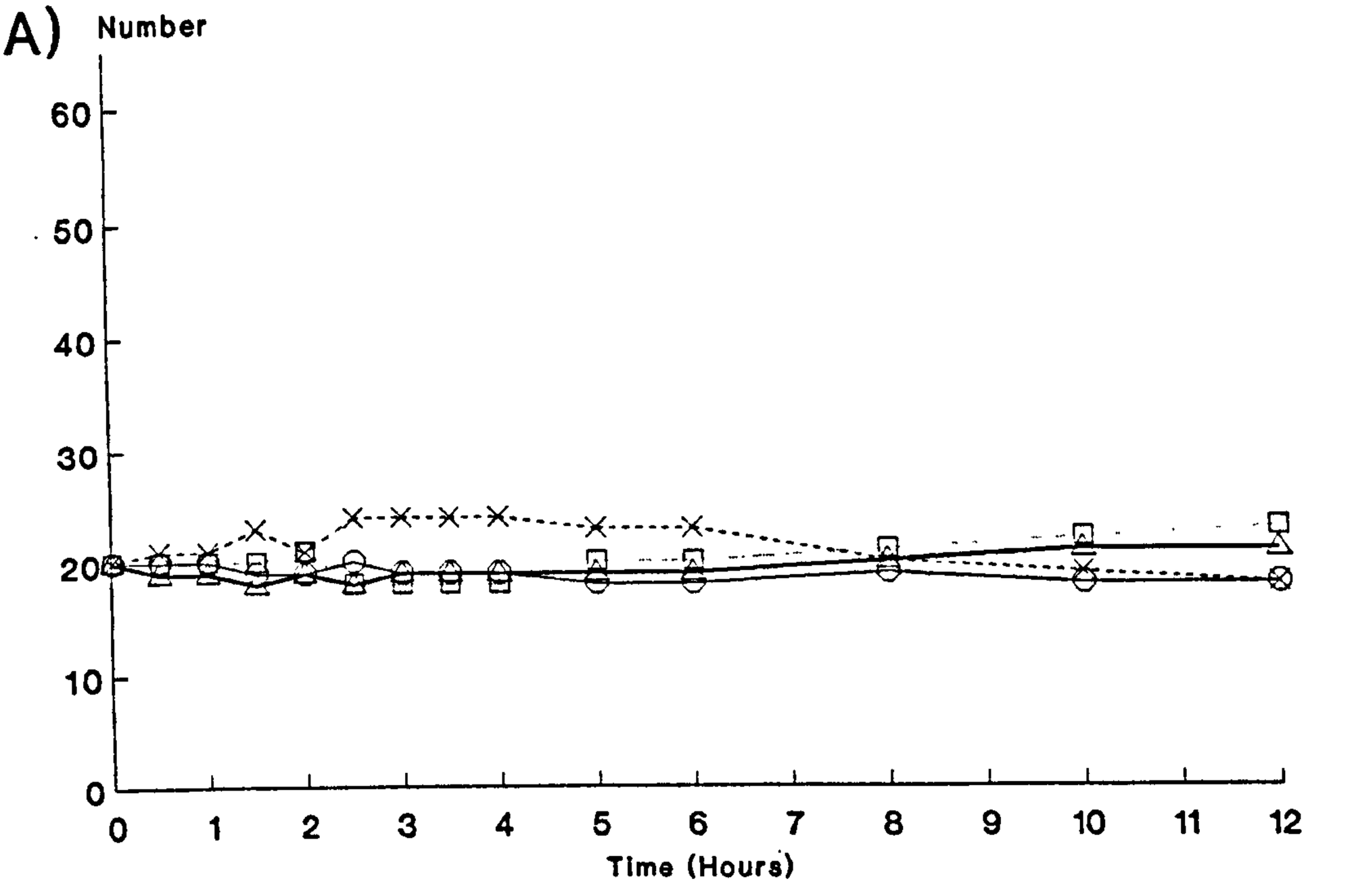


Fig. 3.6. Multiple choice, number of green crabs making a choice between salinities in the 22-40ppt. range, after acclimation to low, normal and high salinities. Graphs represent totals of 4 repetitions at each acclimation salinity, each with 5 crabs.

TABLE 3.3. Number of crabs in each chamber after 24 hours
High salinity set (salinity choice = 22ppt.- 40ppt.)

	Salinity			
Colour/Acclimation	22ppt.	28ppt.	34ppt.	40ppt.
Red Low (17ppt.)	3	18	36	22
Red Normal (34ppt.)	2	22	42	14
Red High (50ppt.)	18	20	24	18
Green Low (17ppt.)	7	23	27	23
Green Normal (34ppt.)	8	16	40	16
Green High (50ppt.)	23	18	18	21

Fig. 3.7.



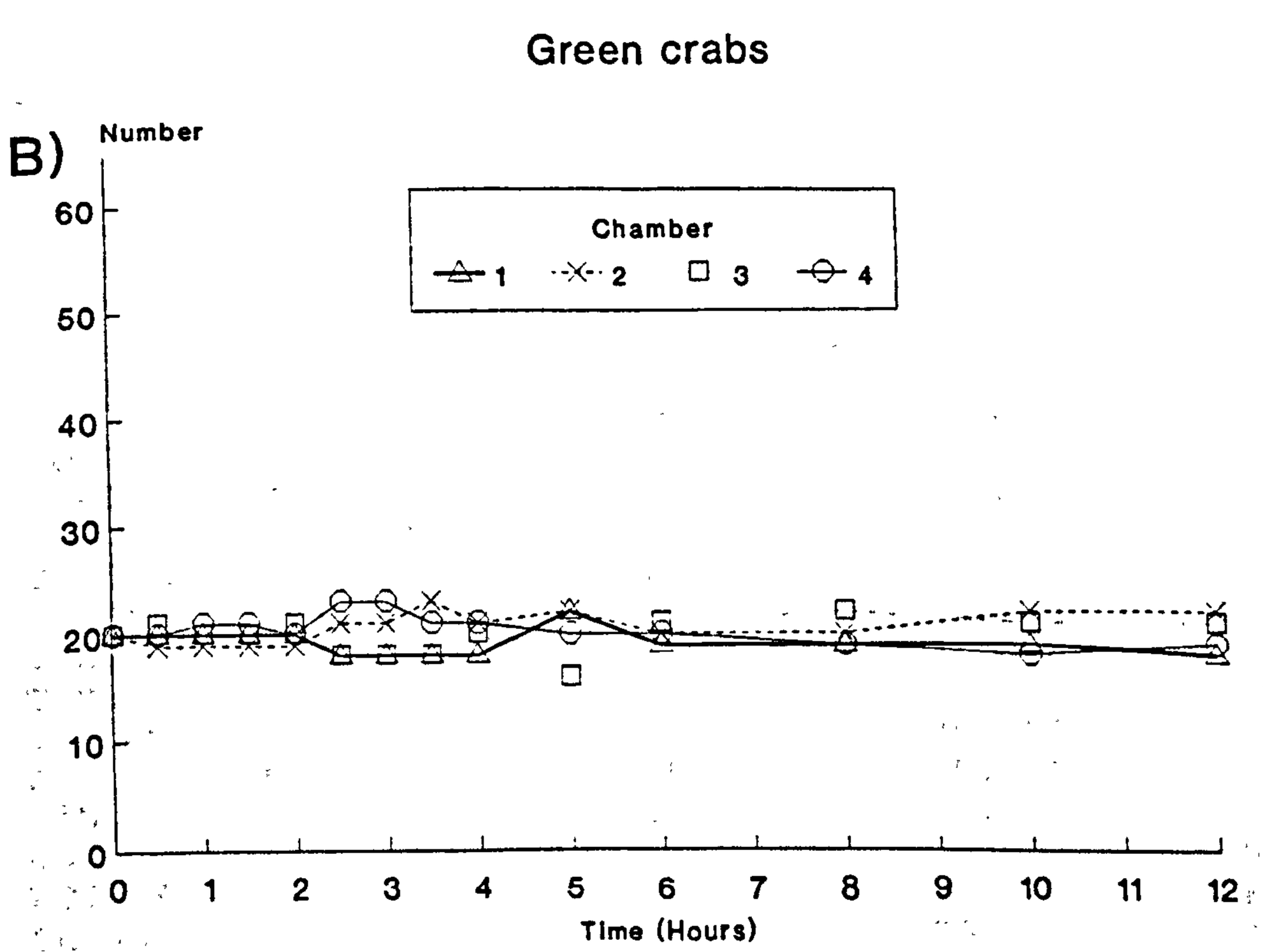


Fig. 3.7. Multiple choice, control experiment to test for any bias towards a certain chamber. Number of red and green crabs making a choice for a particular chamber when all chambers contain a salinity of 34ppt. Graphs represent totals of 4 repetitions, each with 5 crabs.

TABLE 3.1. Multi-choice experiment: Salinity choices in the range 5ppt. - 22ppt, using Hiloglinear test (see text for procedure).

Interaction	DF	Partial Chisqu	Prob
TIME*COLOUR*ACCLIM.	24	6.881	(NS)
TIME*COLOUR*CHAMBER	36	75.176	(**)
TIME*ACCLIM*CHAMBER	72	137.590	(**)
COLOUR*ACCLIM*CHAMBER	6	89.051	(**)
TIME*COLOUR	12	11.098	(NS)
TIME*ACCLIM.	24	12.943	(NS)
COLOUR*ACCLIM	2	2.355	(NS)
TIME*CHAMBER	36	716.264	(**)
COLOUR*CHAMBER	3	110.968	(**)
ACCLIM*CHAMBER	6	189.717	(**)
TIME	12	.000	(NS)
COLOUR	1	.000	(NS)
ACCLIMATION	2	.000	(NS)
CHAMBER	3	1238.443	(**)

** = P<0.01

TABLE 3.2.Multi-choice experiment: Salinity choices in the range 22ppt. - 40ppt. using Hiloglinear test (see text for procedure).

Interaction	DF	Partial Chisqu	Prob
TIME*COLOUR*ACCLIM.	24	.081	(NS)
TIME*COLOUR*CHAMBER	36	7.000	(NS)
TIME*ACCLIM*CHAMBER	72	13.606	(NS)
COLOUR*ACCLIM*CHAMBER	6	22.470	(**)
TIME*COLOUR	12	.014	(NS)
TIME*ACCLIM.	24	.014	(NS)
COLOUR*ACCLIM.	2	.016	(NS)
TIME*CHAMBER	36	5.288	(NS)
COLOUR*CHAMBER	3	26.453	(**)
ACCLIM*CHAMBER	6	8.922	(NS)
TIME	12	.010	(NS)
COLOUR	1	.001	(NS)
ACCLIMATION	2	.002	(NS)
CHAMBER	3	38.095	(**)

** = P<0.01

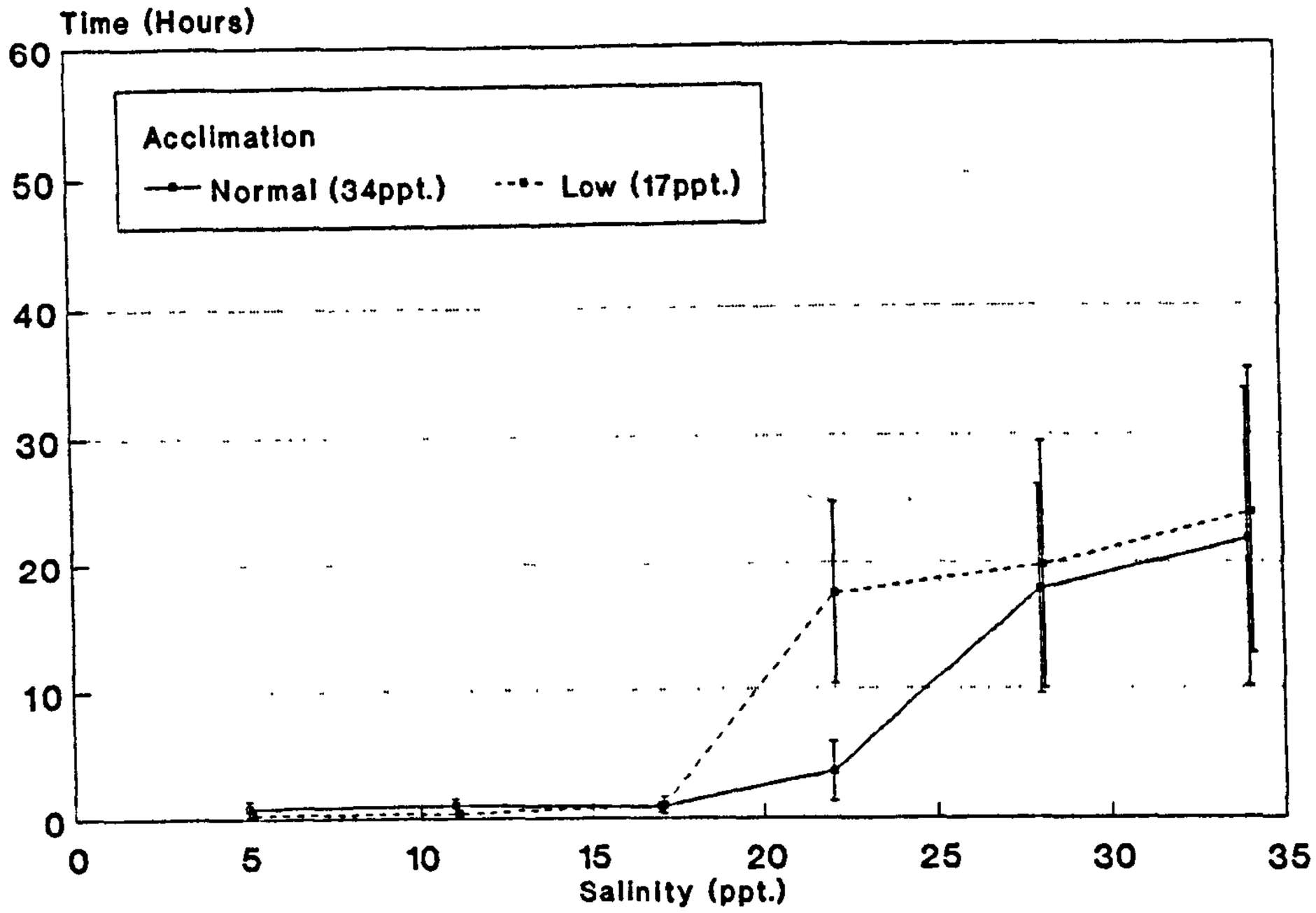
TWO CHOICE SALINITY DETERMINATION

The two choice chambers were used to determine more accurately the time course of salinity choice. Fig. 3.8. A and B shows the mean time of exit (with 95% C.L. of the mean) from the lower salinity for both the red and green colour forms. There was an increase in mean exit times with increasing salinity. At salinities of 5,11 and 17ppt. both red and green crabs acclimated to low salinity exited at a faster rate compared to those acclimated to normal salinity. At salinities of 22ppt. and above this pattern was reversed, and the crabs acclimated to normal salinity exited at faster rate. The major increase in exit times for normal acclimated crabs occurred between salinities of 22ppt. and 28ppt., whereas the principal increase in exit times for low salinity acclimated crabs occurred between salinities of 17ppt. and 22ppt. A 3 way ANOVA was applied to the Logs of time of exit (because of the large variation), and the results obtained are shown in Table 3.4.

Significant differences in time of exit are dependent upon the colour of the crabs and upon the salinity offered. In general mean exit times of the red colour crabs are lower compared to those of the green colour forms, and there is also an increase in time of exit with increasing salinity. The only significant interaction obtained was Acclimation*salinity, and the mean exit time varied depending on the salinity and

whether the crabs were acclimated to normal or low salinity. A Tukey pairwise comparison test was carried out to determine where the actual differences occurred. Both red and green crabs acclimated to normal salinity exited 22ppt. salinity at a faster rate compared to those acclimated to low salinity conditions. In all other salinities there were no significant differences in exit times dependent upon previous acclimation.

Fig. 3.8. Red crabs



Green crabs

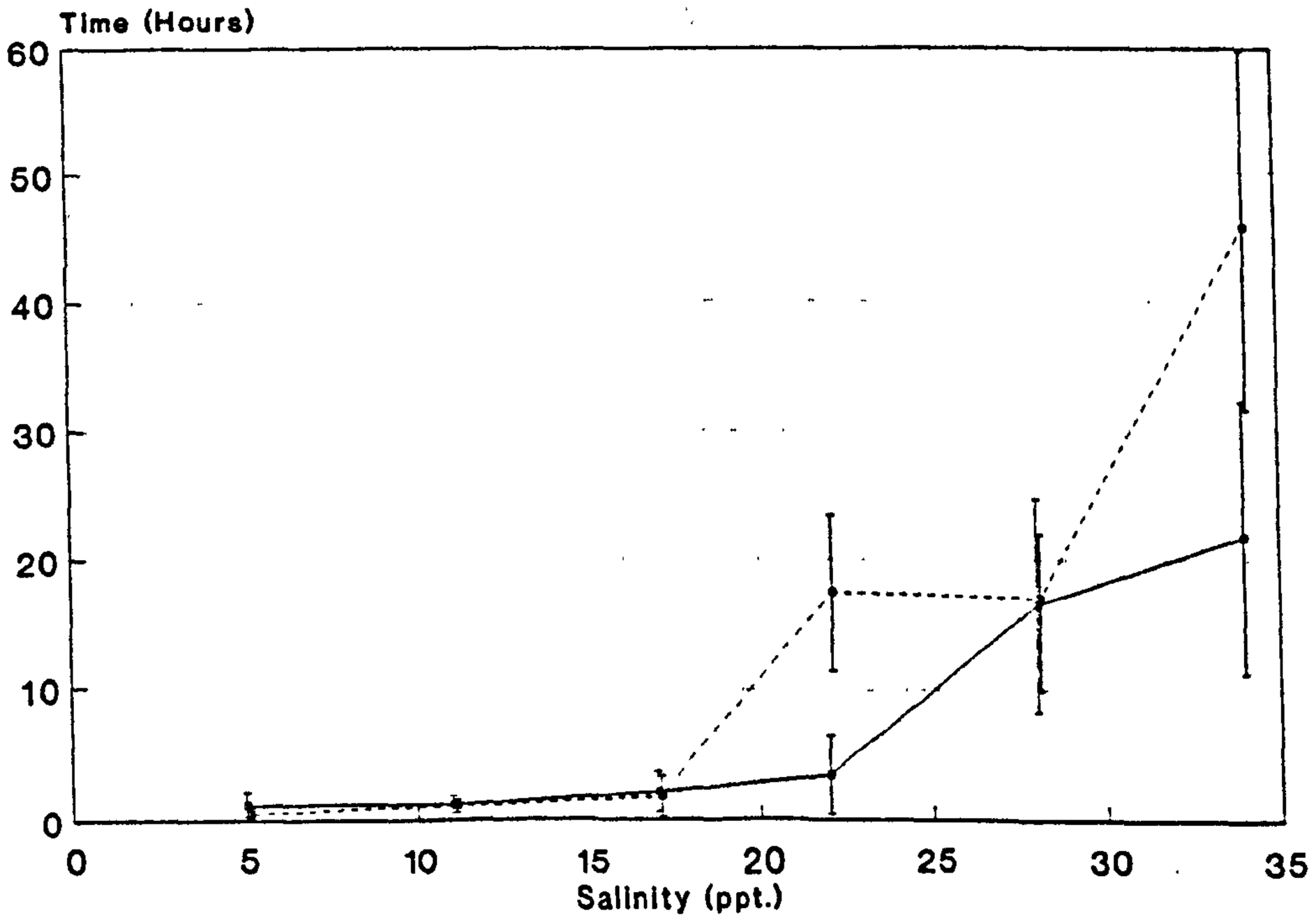


Fig. 3.8. Mean time of first exit (with 95% C.L. of the mean) from the lower salinity in the two-choice chamber. Red and green crabs were tested separately after acclimation to low and normal salinities.

TABLE 3.4. Two-choice chamber experiment: Analysis of variance performed on the time of exit from low salinity, of red and green crabs after acclimation to low (17ppt.) and normal (34ppt.) salinities (see text for procedure).

Source	DF	F	Prob.
COLOUR	1	5.80	(*)
ACCLIMATION	1	1.55	(NS)
SALINITY	5	83.15	(**)
COLOUR*ACCLIM.	1	1.34	(NS)
COLOUR*SALINITY	5	0.48	(NS)
ACCLIM.*SALINITY	5	12.09	(**)
COLOUR*ACCLIM*SALINITY	5	0.15	(NS)

* = P<0.05 ** = P<0.01

SHUTTLING BEHAVIOUR

Movement of crabs between two salinities was investigated to determine if results obtained for the multiple choice experiments (Fig. 3.4.A and B) were derived simply from increased halokinesis between chambers determined by the acclimation salinity. Fig. 3.9 shows the mean number of shuttles between the chambers. There does not appear to be a trend related to the colour of the crab or the salinity it had been previously acclimated to.

Fig. 3.9.

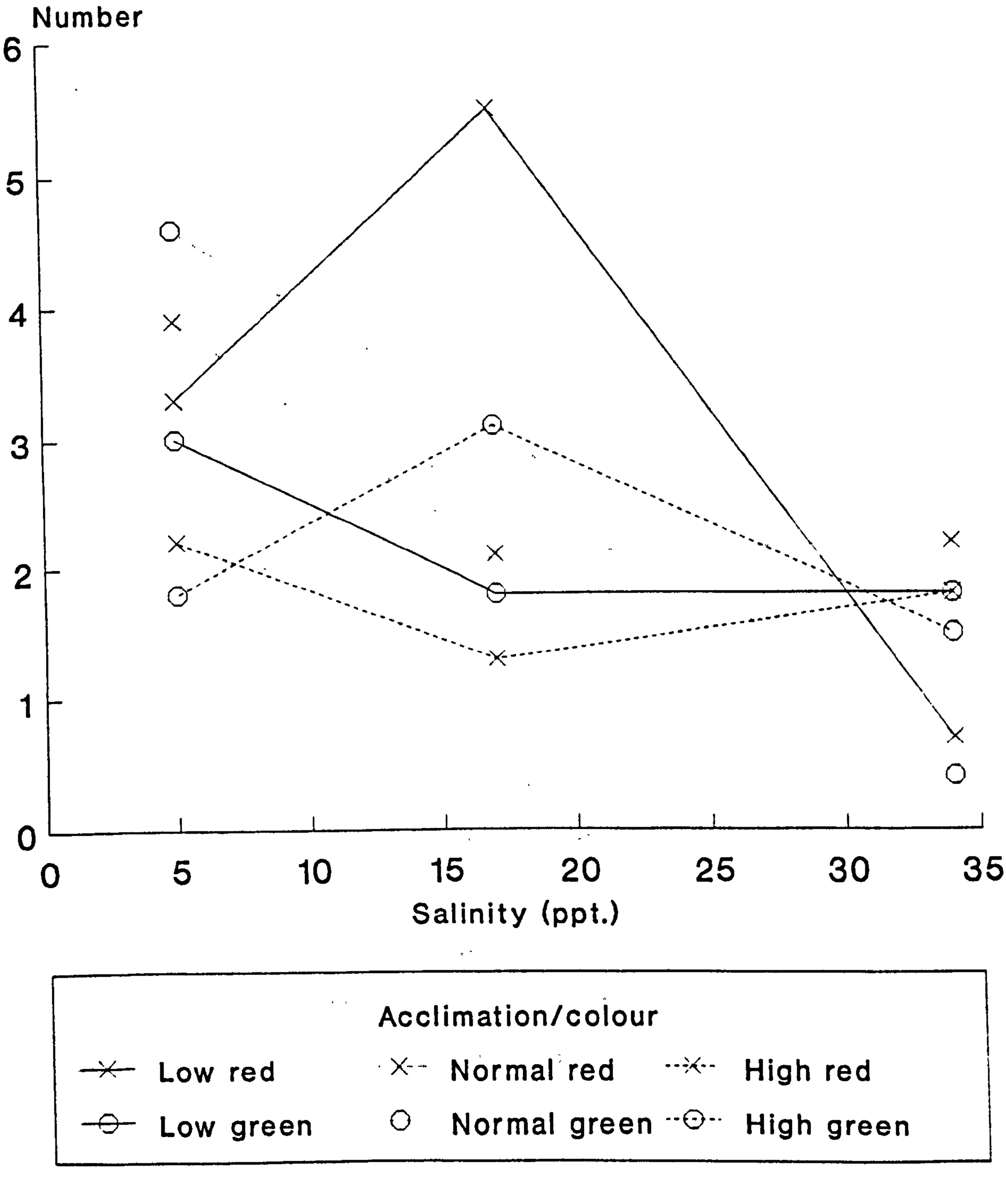


Fig. 3.9. Shuttling behaviour of crabs between two differing salinities (Salinity choice= 5,17 or 34ppt. vs. 34ppt.). Mean number of shuttles of red and green crabs in each salinity, after acclimation to low (17ppt.), normal (34ppt.) and high (50ppt.) salinities.

SALINITY CHOICE IN ACCLIMATED CRABS

The choice behaviour of acclimated red and green colour forms obtained from the results of earlier experiments (see chapter 1 Figs. 1.7. to 1.9.) was analysed further. Fig. 3.10 shows the number of green and red crabs remaining in the 5ppt. salinity, when given a choice between this and full strength seawater, after acclimation in low, normal and high salinity. In all cases red crabs exited at a faster rate compared with green crabs.

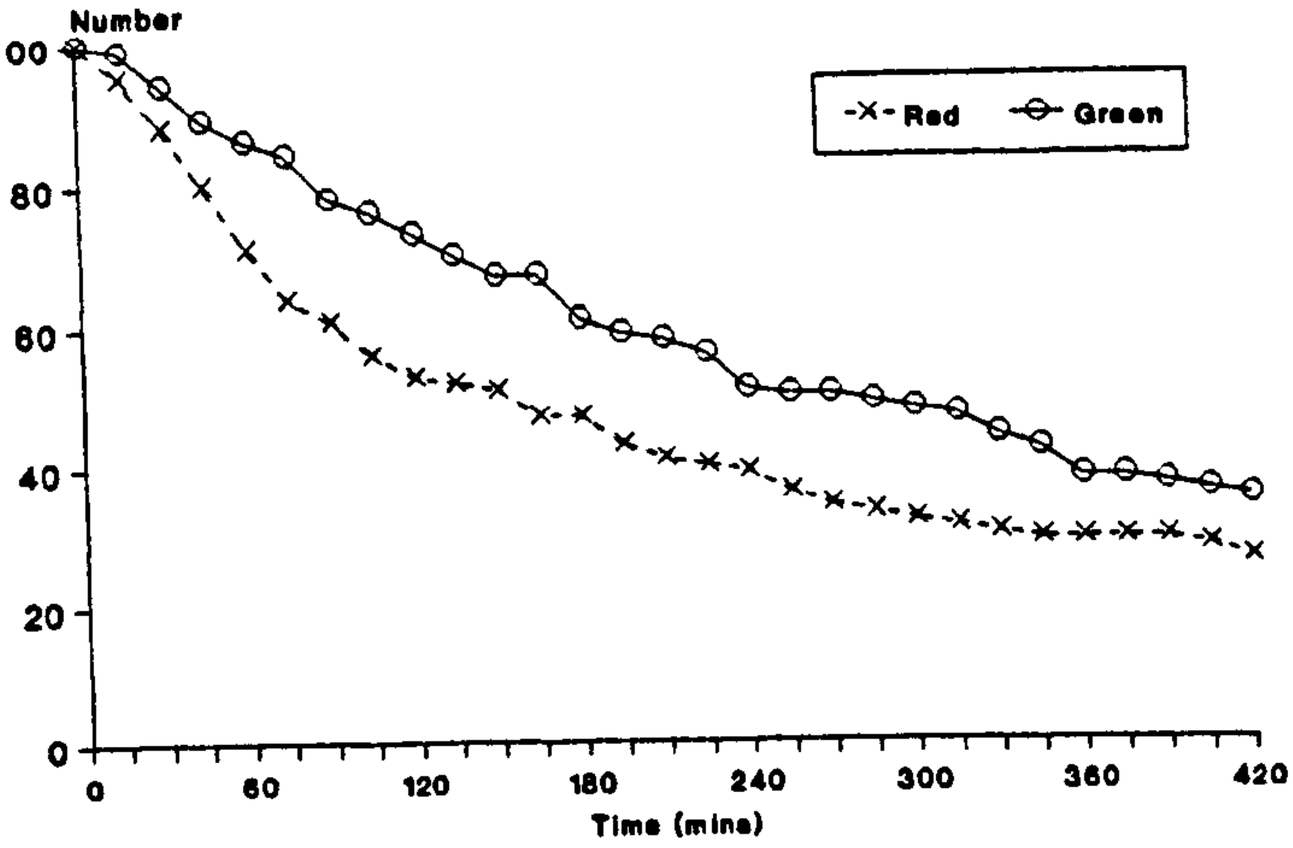
Fig. 3.11. A and B shows the number of animals remaining in the 5ppt. salinity as a function of the salinity they had been previously acclimated to. The acclimation salinity affects the choice behaviour, so that the higher the acclimation salinity the longer the crabs remain in the 5ppt. salinity. However this tendency is not so apparent in the green crabs as it is in the red crabs. A MANOVA test performed on the time of exit (Table 3.5.) confirms these findings.

The non-significant interaction confirms the results of the multi-choice experiment (Fig. 2.2 and 2.3); red and green colour morphs react in a similar fashion as a result of acclimation.

Fig. 3.10.

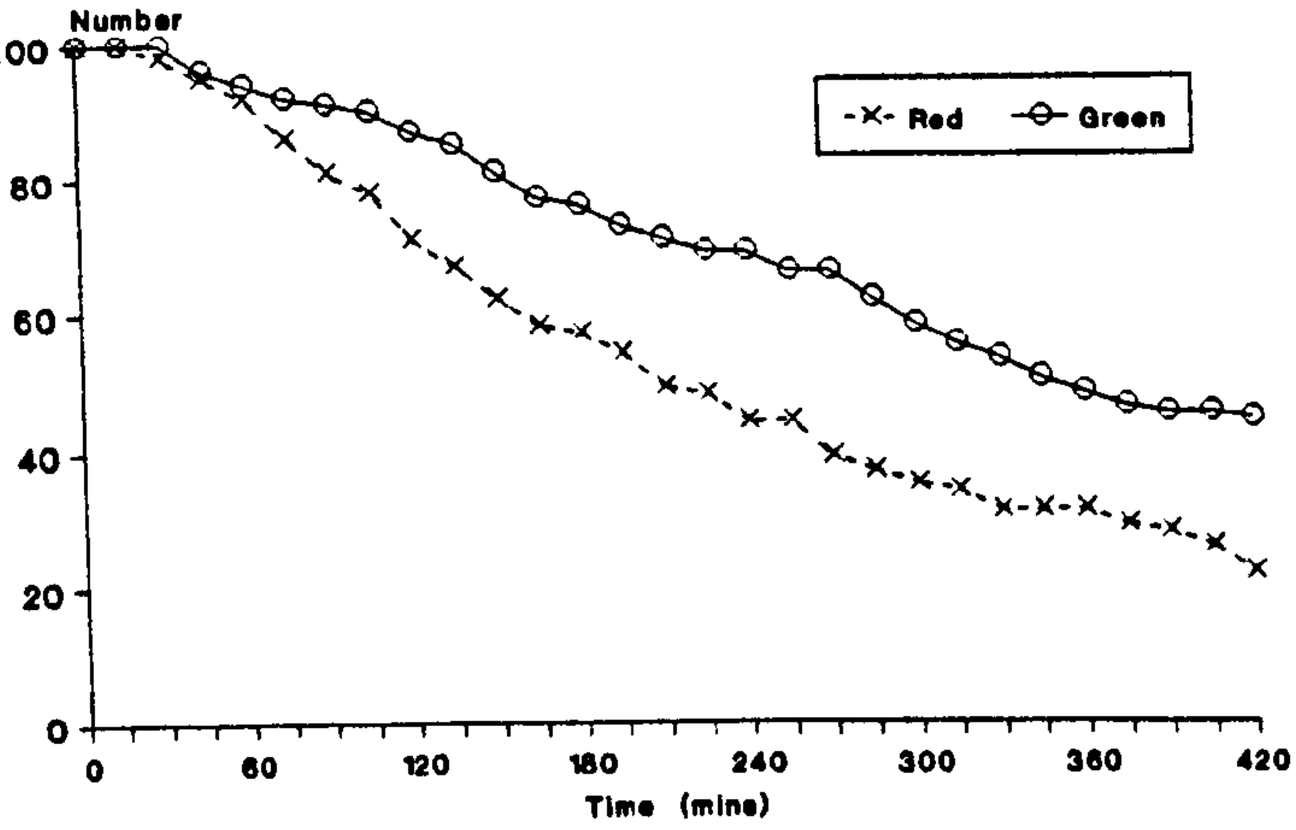
Low salinity (17ppt.) acclimated

A)



Normal salinity (34ppt.) acclimated

B)



High salinity (50ppt.) acclimated

C)

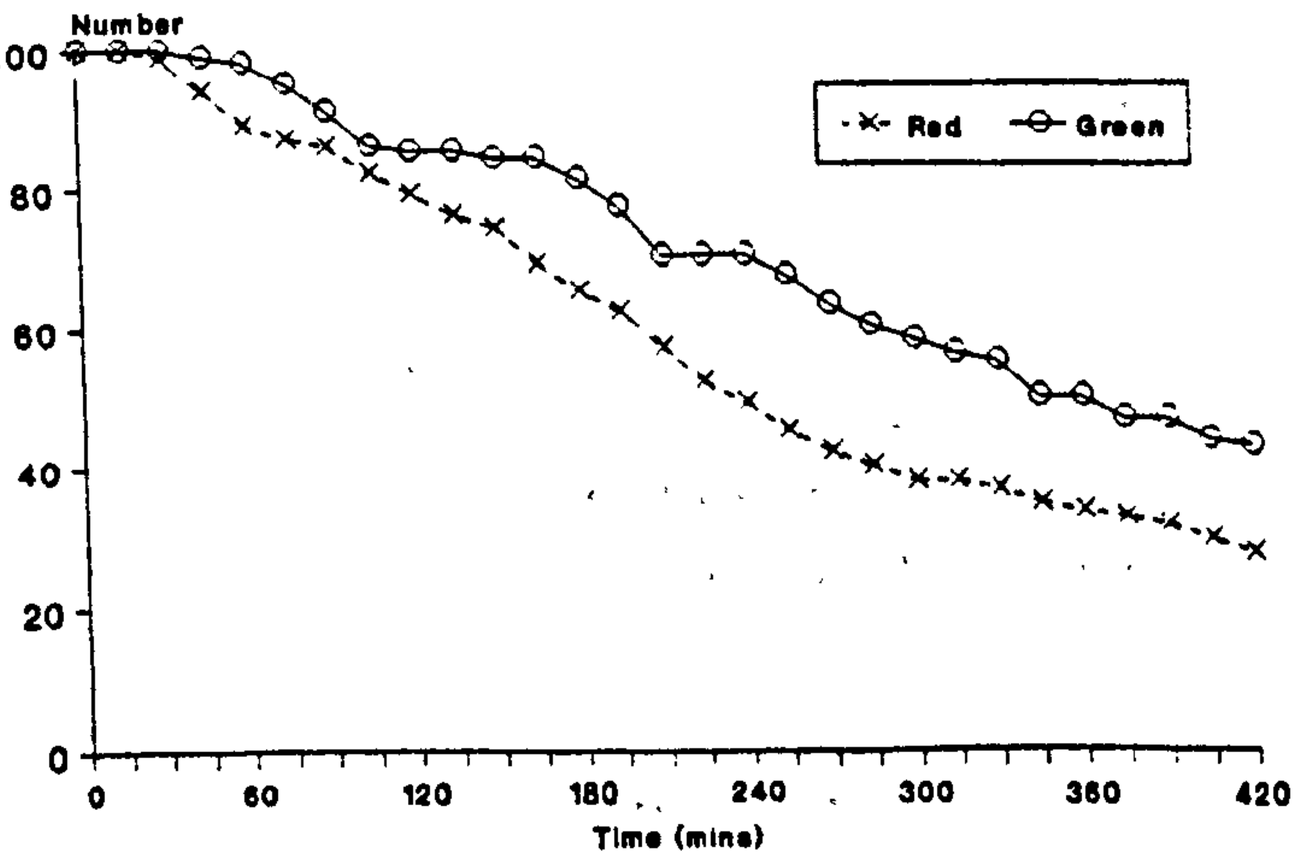
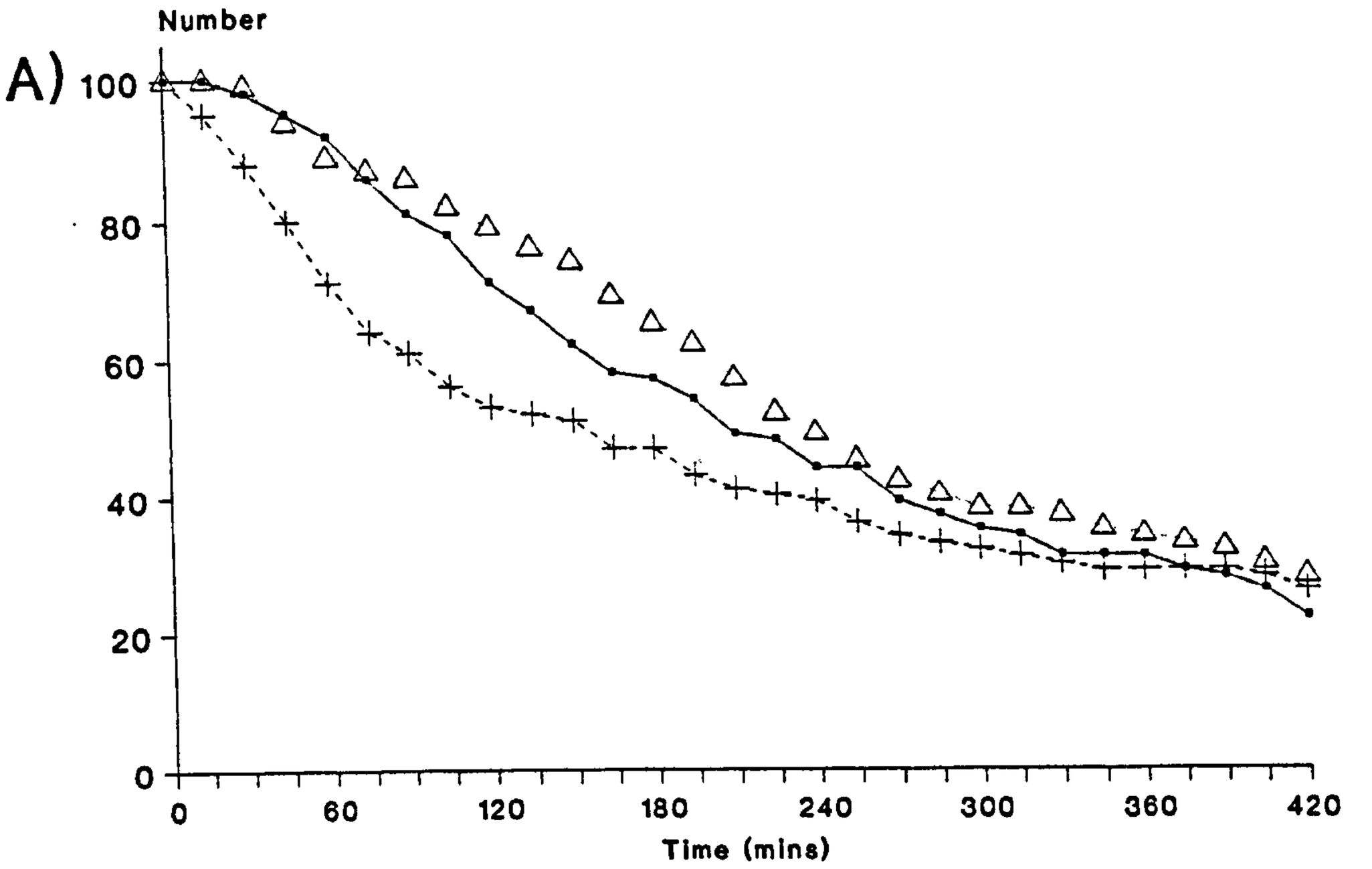


Fig. 3.10. Number of red and green crabs remaining in a salinity of 5ppt. after acclimation to low, normal and high salinities. Graphs represent totals of 4 repetitions, each with 25 crabs of each colour.

Fig. 3.11.



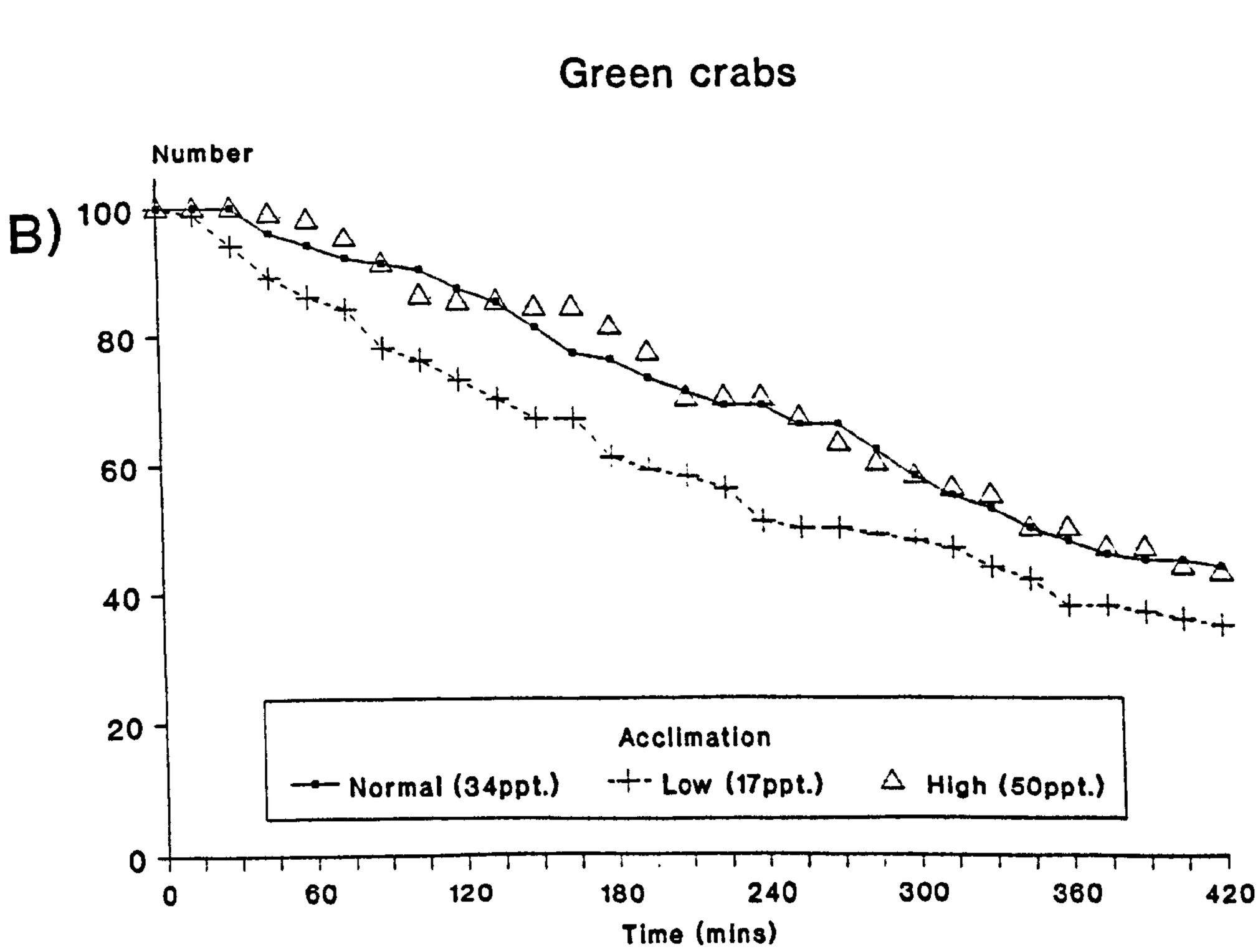


Fig. 3.11. Results of Fig. 3.10. plotted separately for the red and green crabs to show the effect of acclimation on salinity choice behaviour.

TABLE 3.5. Manova test on exit times of low (17ppt.), normal (34ppt.) and high (50ppt.) salinity acclimated red and green colour forms. Salinity choice offered = 5ppt. vs. 34ppt. Crabs initially being introduced into the lower salinity .

Source	DF	F	Prob.
COLOUR	1	10.88	(**)
ACCLIM	2	11.31	(**)
COLOUR*ACCLIM	2	.26	(NS)

** = P<0.01

EFFECT OF GROUP SIZE ON SALINITY CHOICE

Analysis of the results obtained for the previous experiment (described in detail in chapter 1) show that a number of crabs remained in the 5ppt. after 7 hours had elapsed, whereas all animals tested individually (Fig. 3.8. A and B) had left the low salinity within 4 hours.

Fig. 3.12.A shows the percentage of crabs remaining in the low salinity when varying group sizes are tested. It appears that the larger the group size the longer the crabs stay in the 5ppt. salinity. A hiloglinear test (Table 3.6.) confirms these conclusions.

The main differences occur when large groups of crabs are used. When groups sizes of 1, 5 and 10 crabs are tested, variation in exit times is not as great as when groups of 25 or 50 animals are introduced into the lower salinity (Fig. 3.12.B).

Fig. 3.12.

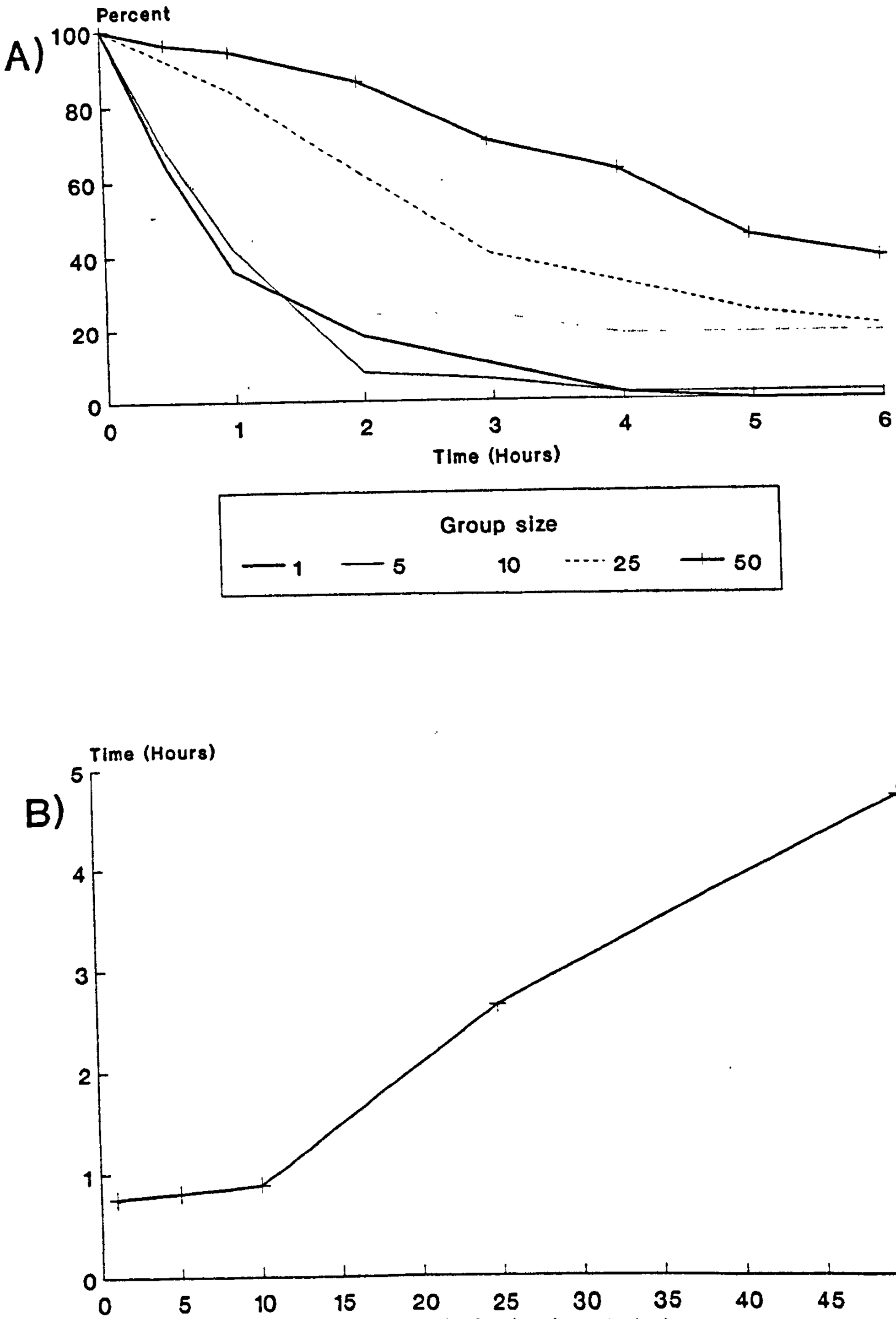


Fig. 3.12. A) Percentage of crabs remaining in a salinity of 5ppt. when the animals are introduced into the lower salinity in group sizes of 1,5,10,25 and 50 animals, with 50,10,5,2 and 4 repetitions respectively B) Time when 50% of crabs in each group size are left in the chamber containing a salinity of 5ppt.

TABLE 3.6. Effect of group size on salinity choice behaviour using Hiloglinear test. Salinity choice offered = 5ppt. vs. 34ppt. crabs initially being introduced into the lower salinity.

Interaction	DF	Partial Chisqu	Prob
TIME*GROUP SIZE	28	237.775	(**)
TIME*IN/OUT	7	1271.550	(**)
GROUP SIZE*IN/OUT	4	723.259	(**)
TIME	7	.000	(NS)
GROUP SIZE	4	1428.119	(**)
IN/OUT	1	42.414	(**)

** = P<0.01

EFFECT OF SHELTER ON SALINITY CHOICE BEHAVIOUR

The aim of this experiment was to deduce if crabs will stay longer in low salinities below their preferred range if they have a place to take refuge, as they would do beneath rocks in the estuary or intertidal zone. Fig 3.13.A illustrates that when green crabs are provided with a shelter they remain in the lower salinity for longer periods of time than they would do if no shelter was present. This also occurs in the red crabs, but it is far less pronounced. Table 3.7. shows the results obtained from a hiloglinear test on the data.

Statistical analysis shows if a shelter is available green crabs will take refuge beneath it and stay in the lower salinity for prolonged periods, unlike red crabs which will vacate the low salinity whether a shelter is present or not.

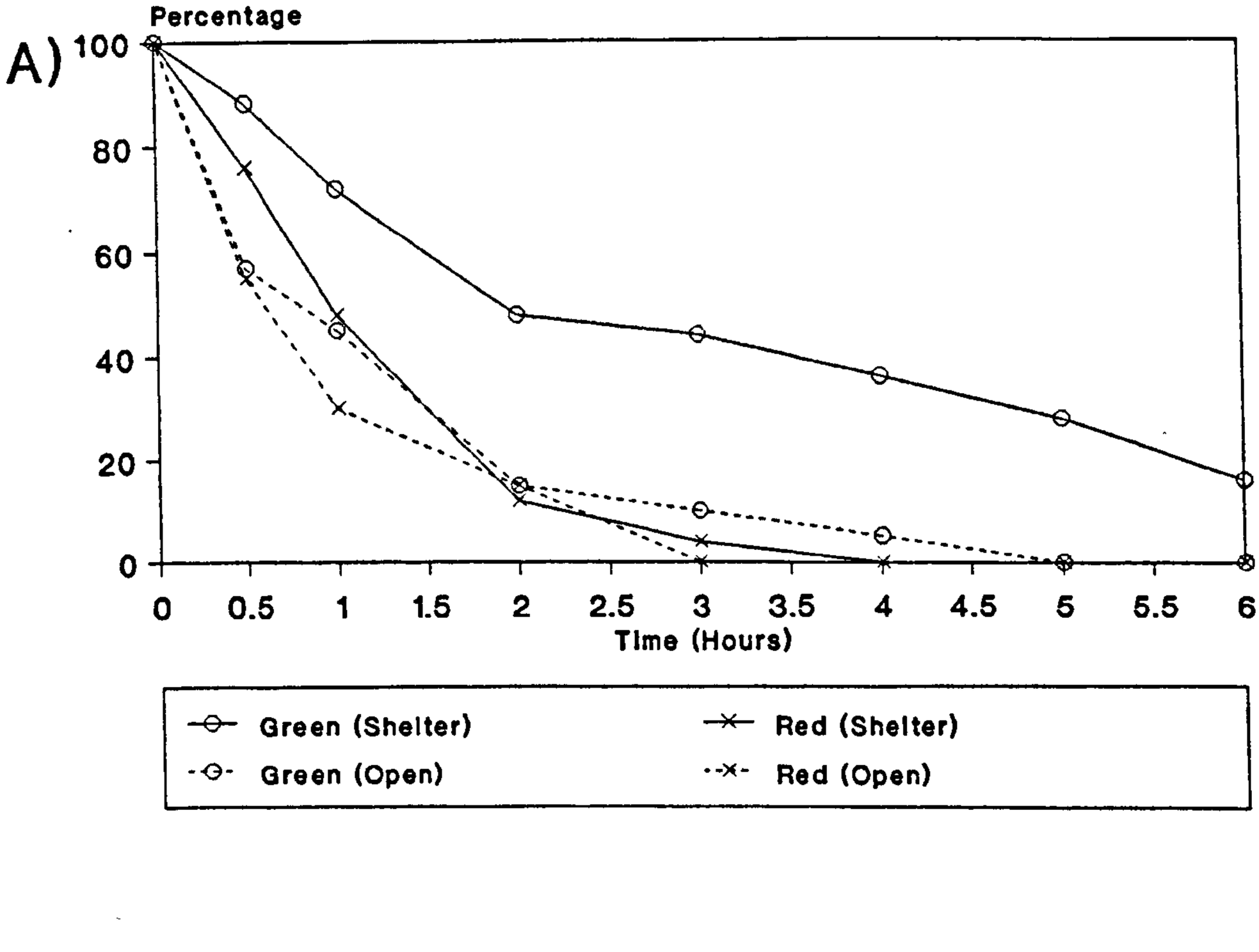
Fig. 3.13.B illustrates the preferred conditions when there is no salinity choice. Net movement of both colour forms, although more erratically so in the red crabs, is towards the chamber containing shelter.

Fig. 3.13.

A) Percentage of red and green crabs remaining in a salinity of 5ppt. when offered a place to shelter in the lower salinity. Results represent pooled data of 25 individuals of each colour at each treatment.

B) Movement of red and green crabs between two chambers each containing a salinity of 5ppt. when one chamber is provided with shelters. Results show net cumulative movement of 40 red and 40 green individual crabs.

Fig. 3.13.


A)
Percentage
100
80
60
40
20
0
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6
Time (Hours)
Green (Shelter)
Green (Open)
Red (Shelter)
Red (Open)

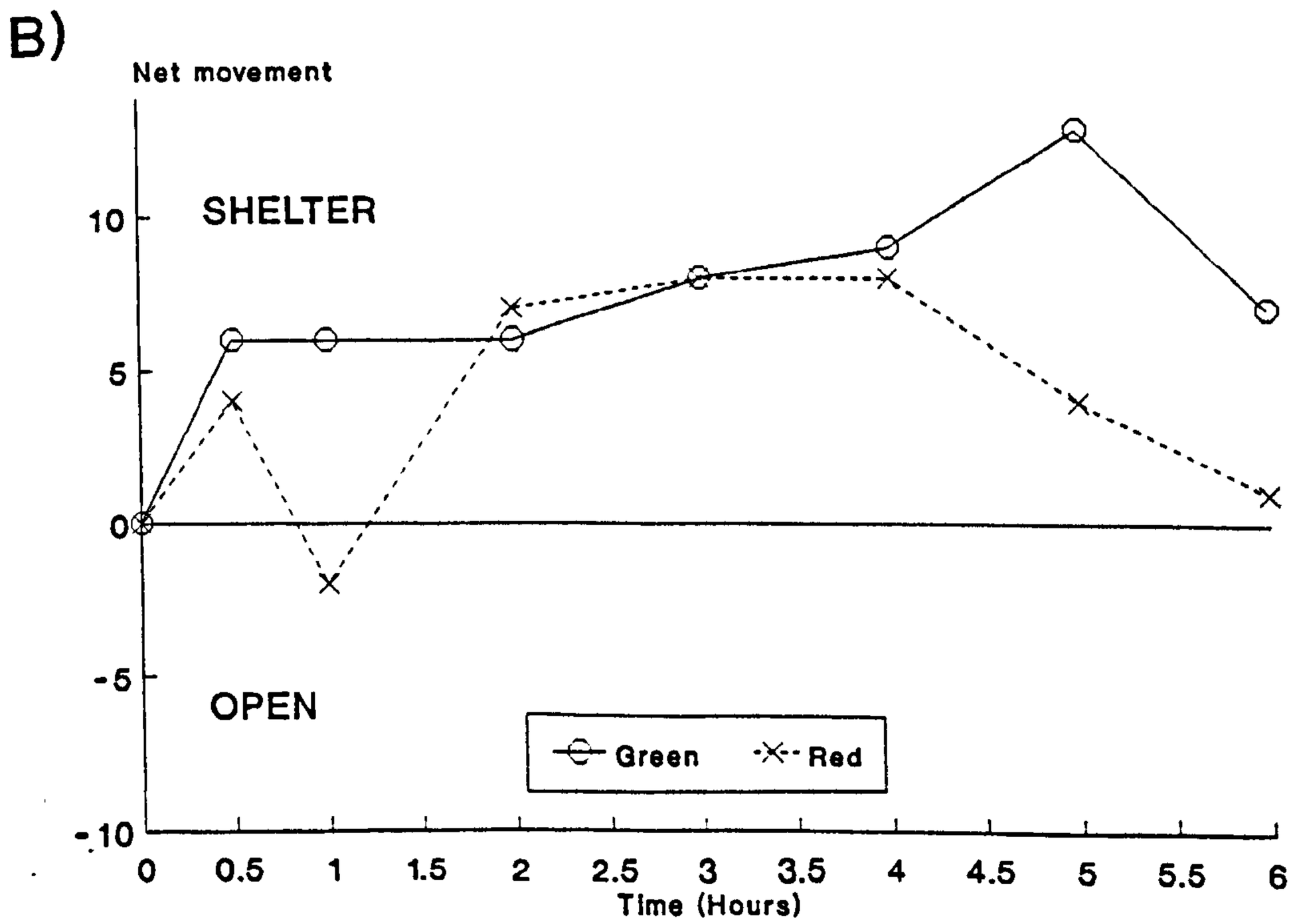

B)
Net movement
SHELTER
10
5
0
-5
OPEN
-10
Green
Red
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6
Time (Hours)

TABLE 3.7. Hiloglinear test for effect of shelter on choice behaviour of red and green crabs. Salinity choice = 5ppt. vs. 34ppt., the lower salinity being provided with and without shelters.

Interaction	DF	Red (Prob.)	Green (Prob.)
TIME	7	(**)	(**)
SHELTER	1	(NS)	(**)
TIME*SHELTER	7	(NS)	(*)

* = P<0.05 ** = P<0.01

DISCUSSION

The use of choice chambers provides a convenient method of assessing the salinity preference behaviour of *Carcinus*. When a multiple choice of salinities in the 5ppt. to 22ppt. range was offered to both red and green crabs, red crabs exited the low salinities and moved towards the higher salinities at a faster rate than the green crabs. This observed behavioural pattern is probably directly attributable to the poorer osmoregulatory abilities of red crabs (Reid *et al*, 1989, Chapter 1) and is due to increased halokinesis shown by red crabs (Chapter 4). Examination of salinity choice shows that red crabs avoid both 5ppt. and 11ppt. whereas green crabs only tend to avoid 5ppt. This appears to be related to the difference in survival times between the two colour forms (Fig. 1.1.A). It would suggest that the lower range of tolerance of red crabs is above 11ppt., whereas that of green crabs is somewhat lower.

Lockwood (1976) described two forms of salinity choice behaviour, the first of which was a crude mechanism to keep animals within a viable salinity range. The second mechanism of salinity choice was dependent on the salinity which the animal had been previously acclimated to; choices were made following which internal body fluid concentrations returned to optimum levels. In the present experiments crabs were only acclimated to salinities for 2 to 4 days, since in

the wild they are unlikely to encounter long term salinity stress. This time period allows ample time for the blood concentrations to reach a new stable value (Theede, 1969, Siebers *et al*, 1972). It also avoids the problem of analysing the effect of long term adaptation factors (such as ATP-ases) which take 2-3 weeks to reach stable values (Siebers *et al*, 1983, Winkler, 1986). The results obtained suggest that the lower the salinity of acclimation the faster the time of exit from low salinities (Figs. 3.4., 3.11.). This would tend to contradict reports that acclimation tends to increase survival times and leads to a shifting of the upper and lower salinity tolerance range (Theede, 1969, McLusky and Heard, 1971, Davenport, 1972, Mclusky, 1979, Davenport *et al*, 1980, McLusky *et al*, 1982, Delisle and Morris, 1985). These workers however acclimated animals for longer time periods and changes in ATP-ase activity may account for the observed differences (Siebers *et al*, 1983, Winkler, 1986). In the present study it is the timing of choice behaviour that is affected by previous acclimation, not the actual choice of salinities. This timing is not associated with differences in the amount of locomotor activity produced by prior acclimation (see Chapter 4, Fig. 4.11.), but may be of adaptive value in enhancing the return of the blood concentration to normal levels (Lockwood, 1976, Spaargaren, 1975b).

Similar mechanisms to that reported here for

Carcinus have been reported in the coconut crab *Birgus latro*, which appeared to control the osmolarity of its blood by selection of appropriate media (Gross, 1955). Also *Pachygrapsus crassipes* tended to choose lower salinities after acclimation to 150% seawater (Gross, 1957). In *Carcinus* regulation of fluids can be thought of as being mediated via an internal detector which registers any deviation from the standard (Spaargaren, 1975b). A crab acclimated to 50% seawater and then transferred to a lower salinity would therefore register blood concentration levels and would quickly exit such a medium. This would be advantageous if an animal already had a lowered blood concentration, since it would prevent it from penetrating any further up the salinity gradient where the blood concentration may reach a critically low level. Conversely if an animal's blood concentration became raised by desiccation on exposure to air, it could be returned to normal levels by the crab remaining in low salinity for an extended period. Spaargaren (1989) reported that if *Carcinus* is adapted to 50% seawater, then transferred to an even lower salinity, there is surprisingly an increase in permeability and efflux. This may account for the rapid exit from 5ppt. after acclimation to low salinities observed in the present study. After a short time period the loss via permeability and efflux is balanced by an increase in active uptake (Spaargaren, 1989). This

however will be limited by the amount of ions available (Spaargaren, 1975a), which will be in low concentrations in a salinity of 5ppt. Behaviour after acclimation for longer periods needs to be studied to gain a fuller understanding of the control mechanisms involved.

When offered a choice of salinities in the 22ppt. to 40ppt. range, no preference for a specific salinity was exhibited within the 12 hour time period. Nor did any trend emerge with respect to the colour of the crab or the salinity of acclimation. After 24 hours some crabs chose 34ppt. but this was by no means unanimous. It is unclear whether the crabs were unable to detect salinities within this range or if they are not osmotically stressed. Thomas *et al* (1981) reported that *Carcinus* did not discriminate between salinities in the 27ppt.- 41ppt. range, whilst Ameyaw-Akumfi and Naylor (1987) reported that the crabs did not exhibit halokinesis in salinities above 17ppt. Similar findings are reported for *Corophium volutator* (McLusky, 1970), which had a preferred salinity range of 10ppt. to 30ppt. but was unable to discriminate between pairs of salinities within this range. Likewise *Marinogammarus marinus* was shown to have a preferred range of 80% to 100% seawater, but could only discriminate between pairs of salinities outside this range (Bettison and Davenport, 1976).

The two choice chambers enabled the time course of

behavioural responsiveness to salinity to be studied in more detail. In accordance with the results of multiple choice experiments and Fig. 3.11. red crabs were found to exit lower salinities at a faster rate than green crabs. Again crabs acclimated to low salinities exited at a faster rate than those acclimated to normal salinities. However at a salinity of 22ppt. a change-over was observed. Those crabs acclimated to low salinity remained in the 22ppt. for considerably longer periods of time than those acclimated to normal conditions. This is explainable by the fact that low salinity acclimated (17ppt.) crabs were transferred to a higher salinity, whereas normal acclimated crabs were transferred to a lower salinity with obvious implications. In all cases large variations between individual exit times were observed so that, although red crabs tended to exit before green crabs and those acclimated to low salinity tended to exit before normal acclimated crabs, in most cases the preferences were not statistically significant. The cut-off point for normal acclimated crabs appeared to lie between 22ppt. and 28ppt., rather higher than found in the multiple choice chamber experiments. The upper limits of the preferred salinity range of *Carcinus* are around 40ppt., which is in accordance with the results of Thomas *et al* (1981) and Ameyaw-Akumfi and Naylor (1987). The lower discriminatory/preference limit however is approximately

22ppt., which lies between the 17ppt. and 27ppt. values reported by Ameyaw-Akumfi and Naylor (1987) and Thomas *et al* (1981) respectively. Spaargaren (1974a) reports that *Carcinus* exhibits strong regulation of its internal fluid concentration in salinities below 25ppt. This value corresponds closely with the observed cut off point in the present study. It suggests that the crabs avoid salinities where they have to expend energy regulating the internal fluids. Red crabs also avoid salinities of 17ppt. and below at a faster rate than green crabs, probably directly related to their poorer osmoregulatory abilities in such salinities (Fig. 1.3., 1.4.).

Shuttling behaviour between two salinities was analysed to determine whether it varied in extent dependent upon previous acclimation and the salinity choice offered. There was however no discernible trend. This experiment backs up those concerning the activity of crabs in each acclimation salinity (Fig. 4.11.), that the salinity choices made are not entirely dependent on changes in locomotor activity induced by prior acclimation to low or high salinities. The mean numbers of shuttles observed were lower than those recorded by Ameyaw-Akumfi and Naylor (1987). This is partly explainable by the fact that low salinities were present on both sides of the chamber, but even the control (34ppt./34ppt.) values were much lower in present

experiments. The only difference was that present experiments were carried out in constant darkness, rather than in constant illumination, which may account for some of the observed differences between present and earlier studies.

It was noticed that when crabs were introduced into low salinities in large groups, certain individuals would remain longer in those salinities, than if they had been introduced individually. There was not much difference between groups of 1, 5 and 10 crabs, and Ameyaw-Akumfi and Naylor (1987) also report no differences in choice behaviour between individuals and small groups of animals. However, when groups of 25 or 50 crabs were used, a large proportion of them remained in the low salinity after 6 hours, crowded together in the corners of the tank. Similar behaviour was observed in the holding tanks in full strength seawater, in the absence of rocks under which to shelter. Vannini (1981) also reported that crabs became quiescent when kept in large groups and suggested that release of catabolites by the animals reduced aggressive interactions. In the wild the only time they are likely to come into close contact with each other in large numbers is when they seek shelter at low water, times which are the inactive phases of their endogenous circatidal locomotor rhythms (Naylor, 1989). Thus if externally released catabolites are involved in inhibiting locomotor activity their role

as possible synchronisers of crab locomotor rhythms should be investigated. In an estuary the water column is being continually replaced and so the effects of such catabolites would be short lived. If they are important then they would presumably be most effective in enclosed bodies of water such as rockpools. This line of study was not pursued further here as it was considered, as argued below, that the effects of shelter were probably more important.

Barnes (1967) and Perkins *et al* (1969) found that crabs could penetrate into lower salinities if suitable substrate or shelter was available. In optimum conditions the animals exhibit low thigmokinesis in response to physical contact with the walls of a shelter (Cooper and Uzmann, 1977, Warman, 1990). The crowding of crabs on top of one another in the corners of a tank could presumably also induce low thigmokinesis. In the present study it was found that if a shelter was available then green crabs would remain in low salinities for longer periods of time. The red crabs, however, avoided low salinities at the same rate whether a shelter was present or not. The movements of red crabs between chambers of low salinity when no salinity choice was offered was also more erratic, and they spent considerable periods of time in the shallowest parts of the tank partially exposed to the air. This behaviour is explained if the stress imposed by low salinity overrode

the behavioural reaction to seek shelter. This response perhaps explains the absence of red crabs from the estuary (Chapter 2) even though suitable habitats were available.

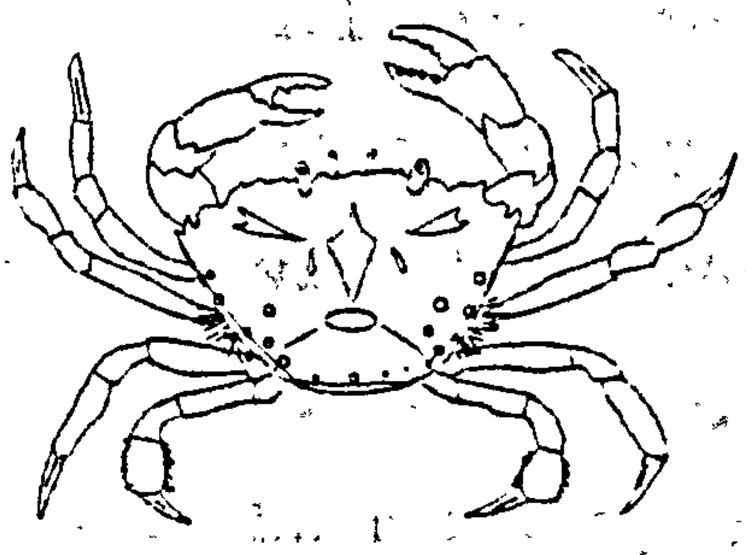
It has been shown that the salinity is not the only factor involved in determining the basis of salinity choice behaviour. The physiological state of the individual and availability of suitable habitats are also important. Future experimental procedures should therefore examine the behaviour of organisms when tested in chambers with spatial heterogeneity and fluctuating salinities, and should take account of behavioural observations in the natural environment. The observations on *Carcinus* in the Foryd estuary (Chapter 2, Fig. 2.3.) suggest that results obtained for other species would be surprisingly different from the 'classical' studies conducted hitherto in featureless choice chambers.

CHAPTER 4:

Locomotor activity of *Carcinus maenas* in response to salinity variation

SUMMARY

Estuarine green crabs exhibited endogenous locomotor activity of circatidal periodicity. They expressed less activity in response to episodes of low salinity such as would be experienced in the estuary, when compared with open shore red and green crabs. Constant low salinity initiated a rhythm of circatidal periodicity in arhythmic red and green crabs; red crabs reacted faster and were more active upon salinity change than green crabs. The amount of locomotor activity induced after prior acclimation was similar for each acclimation salinity tested.



INTRODUCTION

Many marine organisms exhibit rhythmic patterns of endogenously controlled locomotor activity of tidal or diurnal periodicity phased to specific cyclical environmental variables (for reviews see Enright, 1975, Naylor, 1976, 1985, DeCoursey, 1983). *Carcinus maenas* from intertidal areas exhibits rhythmicity, with peak periods of activity occurring at times of high tide. These rhythms were found to persist for a number of days as free running cycles of endogenous activity in constant conditions (Naylor, 1958). In subtidal populations, the tidal pattern of rhythmicity is suppressed, and crabs tend to exhibit circadian rhythmicity, with peak activity occurring during the hours of darkness (Naylor, 1960). Endogenous rhythmicity in *Carcinus* also changes annually, associated with the migration of crabs offshore during December (Naylor, 1962). Tidal rhythmicity was found to be absent in crabs collected in winter; these individuals exhibited patterns of circadian rhythmicity (Naylor and Atkinson, 1972, Atkinson and Parsons, 1973), or no discernible rhythmic behaviour at all (Bolt and Naylor, 1985). The re-appearance of endogenously controlled tidal rhythmicity coincides with the movement onshore of the animals during March and April (Naylor, 1962, Crothers, 1968), and appears to be reset by cycles of temperature and hydrostatic pressure associated with emersion cycles

on the shore (Naylor, 1963, Williams and Naylor, 1969, Naylor *et al*, 1971, Naylor and Atkinson, 1972, Naylor and Williams, 1984a).

Taylor and Naylor (1977) and Bolt and Naylor (1985) found that the tidal rhythmicity could also be reset by square and sine wave cycles of salinity of tidal periodicity. Increased locomotor activity initiated by episodes of low salinity during such treatment (Taylor and Naylor, 1977, Thomas *et al*, 1981, Ameyaw-Akumfi and Naylor, 1987) was found to be purely exogenous and the endogenous tidal rhythmicity that persisted in constant conditions after entrainment exhibited activity peaks coincident with expected times of exposure to full seawater (34ppt.). Exposure to constant low salinity also initiated rhythms of tidal periodicity in winter collected crabs, with maximum activity occurring at 12.4 hour anniversaries of the initial salinity shock (Bolt and Naylor, 1985, Reid and Naylor, 1989). This hypo-osmotic shock, however, did not modify the underlying circadian rhythmicity, suggesting a separate clock control of this system (Reid and Naylor, 1989). Freshly collected rhythmic crabs exposed to a sinusoidal cycle of salinity in tidal phase exhibited small peaks of endogenous activity at times of expected high tide (and high salinity), and larger peaks as an exogenous response to low salinity. In constant salinity afterwards, however, only the endogenous peaks of

activity entrained by exposure to full strength seawater persisted (Bolt and Naylor, 1985,1986, Reid and Naylor, 1989). When salinity was cycled in tidal antiphase clear peaks of activity occurred at times of expected high tide, which were enhanced by the exogenous effects of low salinity. These peaks however were purely exogenous and did not persist in constant salinity afterwards. After such treatment subsequent endogenous rhythmicity was phase shifted by approximately 6 hours in response to the episodes of full strength seawater (34ppt.), and therefore occurred at times of expected low water in the field (Bolt and Naylor, 1985,1986).

The aim of the present study was to use the extensive background of laboratory studies of salinity responses of rhythmicity in *Carcinus* as a basis from which to investigate and compare endogenous locomotor activity rhythms of crabs from the estuary with those from the open shore. The study also sought to examine the responses of, and the effects of acclimation on, red and green crabs when exposed to both constant and fluctuating salinity regimes.

MATERIAL AND METHODS

SALINITY SYSTEM

The salinity system shown in Fig. 4.1.A was used to supply both constant and fluctuating salinities under the control of a BBC B microcomputer and software. Freshwater and seawater were fed directly into a mixing tank where a conductivity probe was located. The conductivity meter produced a voltage between 0 and 1.8 V directly proportional to the salinity of the water. This voltage was sent to the ADC port (Analog digital converter) on the BBC where it was converted to digital code. The computer compared the salinity in the mixing tank to the pre-programmed salinity required at any one time. The I/O port of the BBC was linked directly to a pair of solid state relays which in turn controlled the solenoids which closed one valve and opened the other until the desired salinity was attained. The software checked the salinity every 15 seconds and adjusted it accordingly via the valve system. The conductivity probe was recalibrated at the beginning of each experiment. The mixing tank was supplied with an airstone which also facilitated mixing of the seawater and freshwater.

ACTIVITY DETECTION SYSTEM

Activity detection switches (Whisker switches, Fig. 4.1.C) were used to record locomotor activity of the crabs. Their use was restricted to fairly large animals

(> 40mm carapace width) since they require mechanical force to activate them, equivalent to a crab pushing past a piece of debris or algal fronds in the wild. Reed switches (6-RSR-A normally open) were connected to the I/O port of a BBC microcomputer and the voltage to/from the I/O is determined by the status of the reed switch. Once the circuit is broken by a crab pushing against the whisker and displacing the magnet from directly beneath the reed, an event is recorded. The rubber bung acts as a stabiliser, and the weight on the whisker as a counterbalance to the magnet, returning it to the original position and restoring the circuit when the crab moves away.

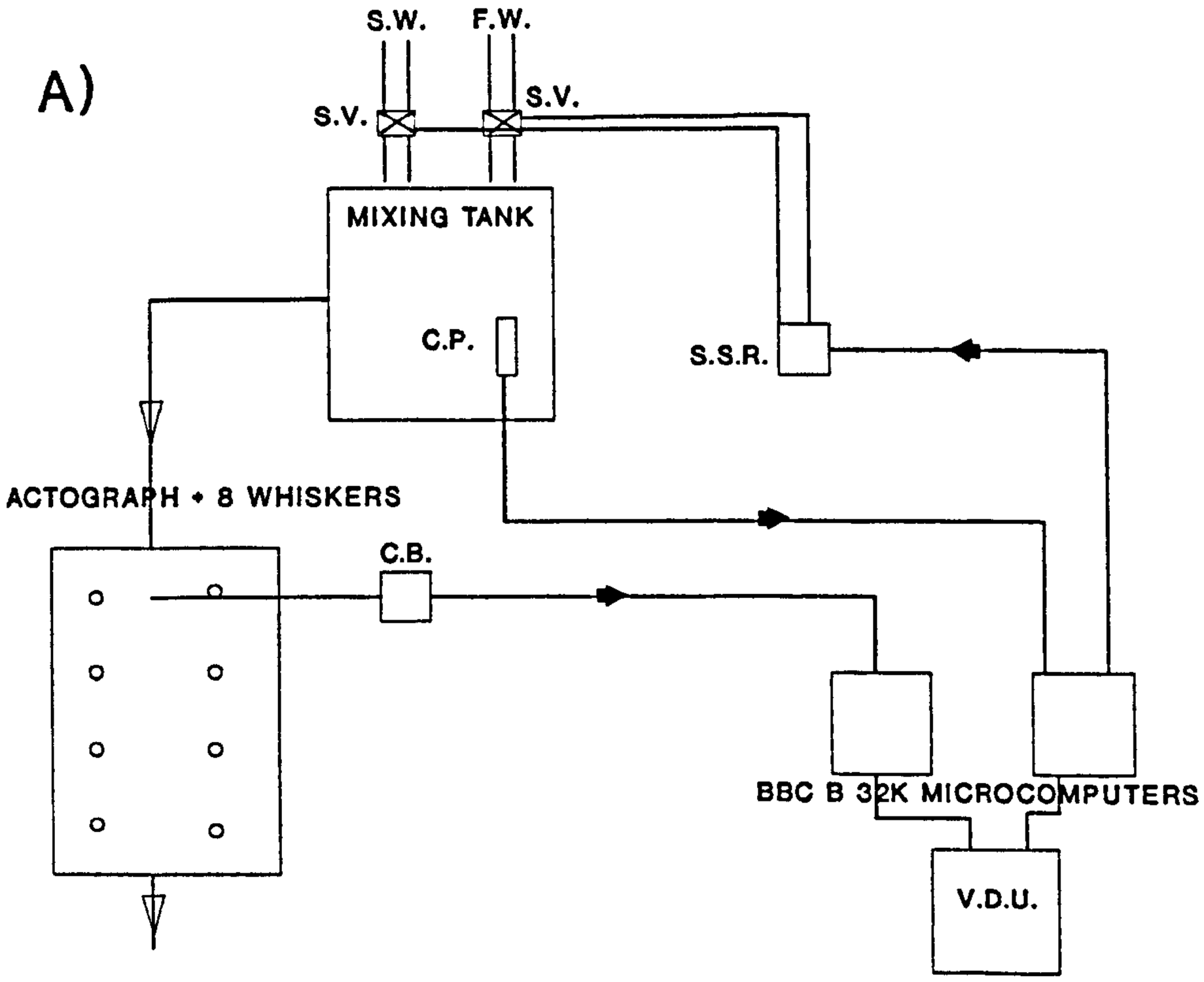
THE ACTOGRAPH

An actograph was constructed from a plastic tank 100cms x 50cms x 15cms deep. Netlon mesh was used to construct 8 equal sized chambers within the tank. An inflow pipe led directly from the mixing tank and an outflow to the waste system. The actograph was covered with a perspex lid in which holes were drilled over the centre of each chamber and one whisker was assigned per chamber. Two crabs were placed in each chamber and activity within each of the 8 chambers was individually recorded for either 15 or 30 minute intervals before being stored on cassette tape. Crabs for experiments were collected from freshly baited traps set at low

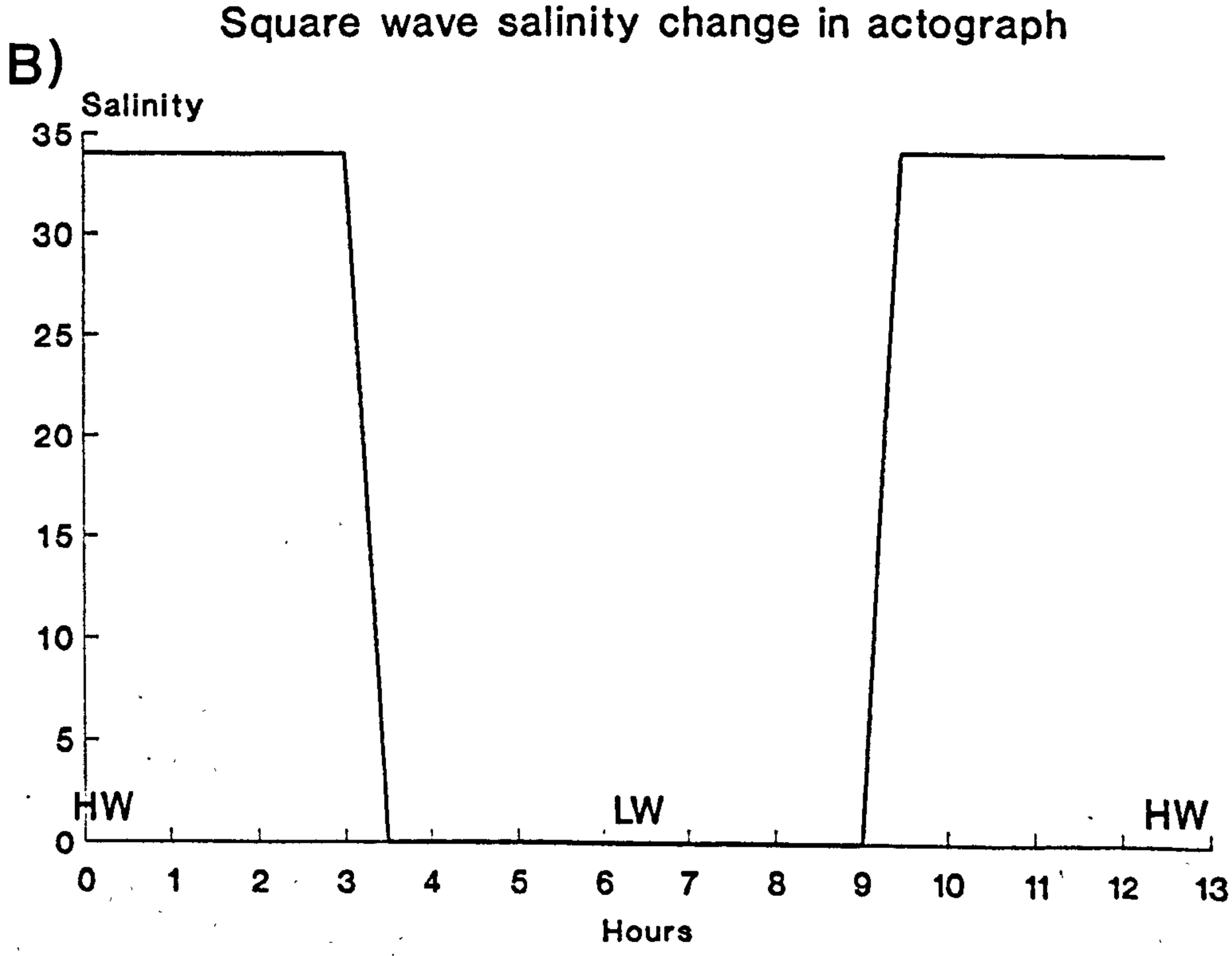
water and collected on the following low water as soon as the traps became accessible. Crabs were transferred directly to the actograph and a short time period allowed for them to settle before the experiment was started. All experiments were performed in constant darkness. All graphs shown in the results section are plotted as three point moving averages of the raw data. As mentioned in the general material and methods section only male crabs of 40-70mm carapace width were used.

A number of experiments were designed to investigate locomotor activity under a variety of conditions. These included studies of free running locomotor activity patterns of freshly collected estuarine crabs in full seawater. In other experiments crabs were kept in constant salinity until they became arhythmic. Activity was then monitored for 1 day in the salinity of acclimation, which was then lowered over a 2 -4 hour period and activity monitored for a further 3 days in this lower salinity. Salinity was varied on a 'square wave' pattern at a tidal frequency (Fig. 4.1.B) such that crabs experienced the same salinity regime as would be found at the head of the Foryd estuary. The salinity regimes used are discussed in more detail in the results section.

A)


S.W.
F.W.
S.V.
S.V.
MIXING TANK
C.P.
S.S.R.
ACTOGRAPH + 8 WHISKERS
C.B.
BBC B 32K MICROCOMPUTERS
V.D.U.

B)


Square wave salinity change in actograph
Salinity
35
30
25
20
15
10
5
0
HW
LW
HW
0
1
2
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7
8
9
10
11
12
13
Hours

C)

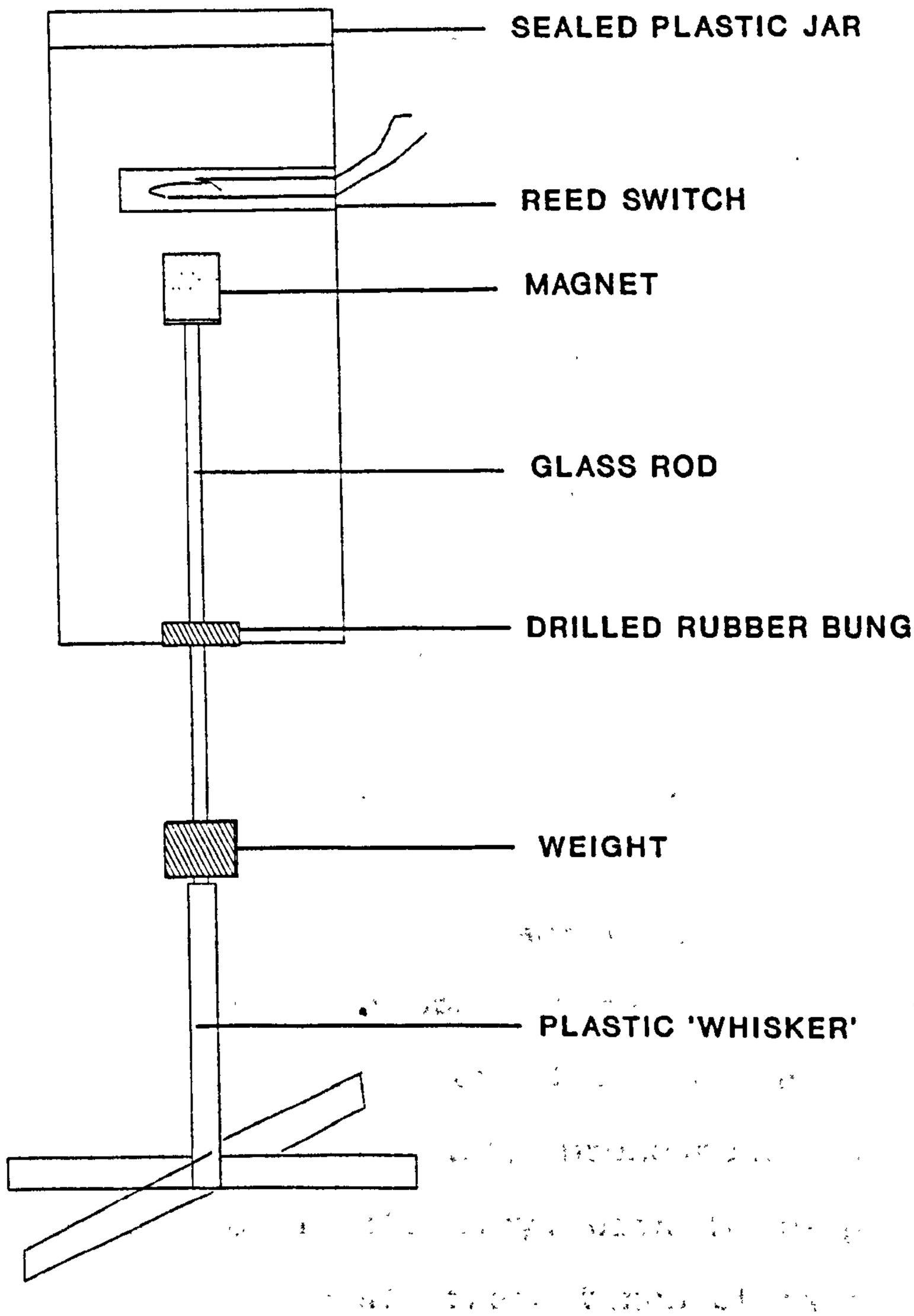


Fig. 4.1.

A) Salinity cycling system; C.B. connection board, C.P. conductivity probe, S.S.R. solid state relay box, S.V. water solenoid valve, V.D.U. visual display unit.

B) Square wave salinity cycle, showing salinity cycling on a tidal regime.

C) Activity detection switch (whisker switch).

RESULTS

ENDOGENOUS LOCOMOTOR ACTIVITY PATTERNS

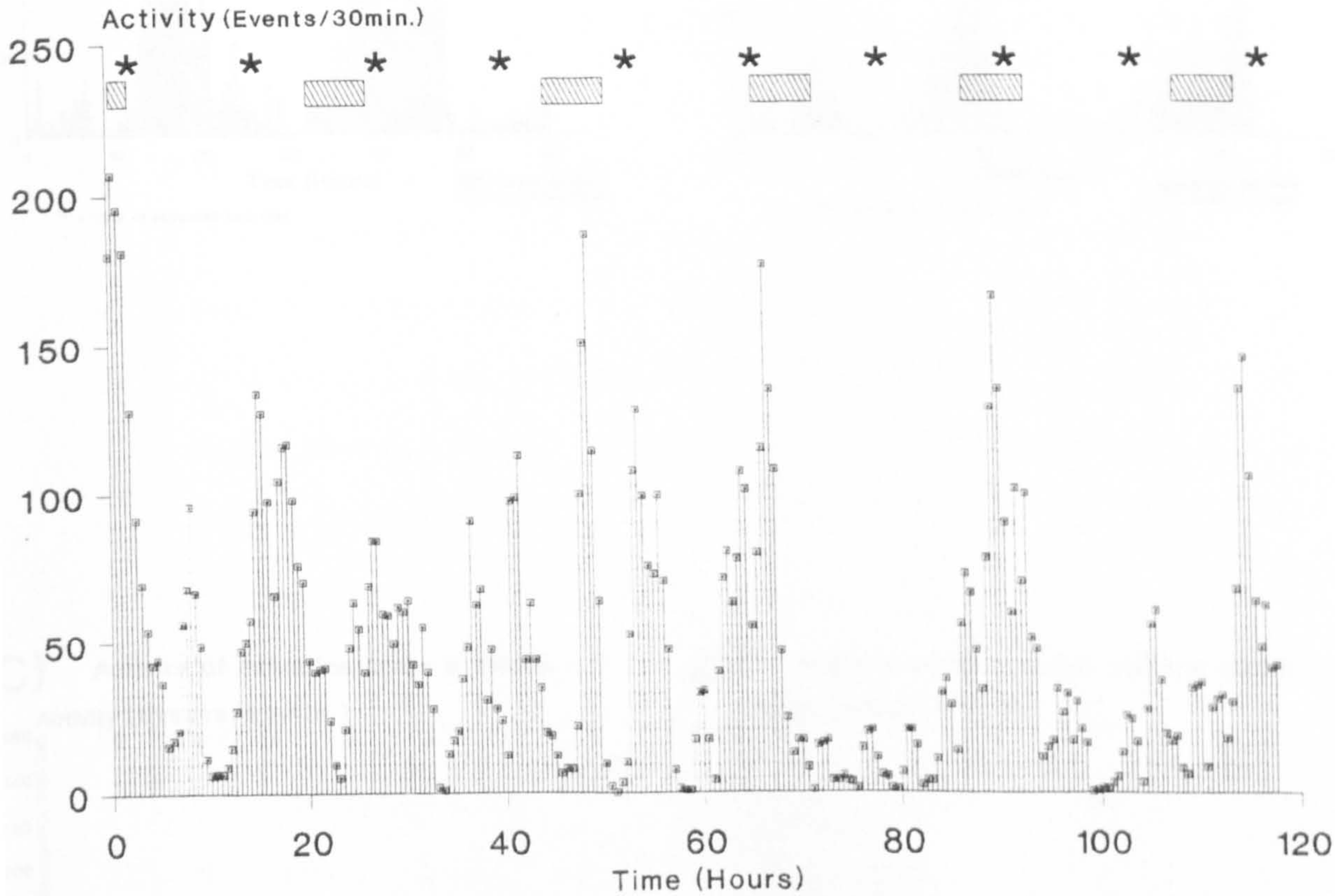
Fig. 4.2. shows endogenous locomotor activity of eight green crabs collected from the head of the Foryd estuary in early summer, held in constant salinity (34ppt.) and continuous darkness. Initially the crabs exhibited a pattern of circatidal rhythmicity, with most peaks of activity coinciding with the times of expected high tide in the estuary. After three days the circatidal rhythmicity began to break down and the crabs expressed a more circadian pattern of activity; maximum activity occurred when high tides occurred at expected dusk, although a weaker tidal component still exists. This pattern resembles that of crabs collected in summer on the open shore (Naylor, 1958).

The endogenous locomotor activity of green crabs from the estuary and the open shore (Ynys Faelog) was compared during December (Fig. 4.3.A,B). Both sets of crabs exhibited a particularly pronounced, apparently circadian pattern of rhythmicity, with large peaks more or less coincident with alternate times of expected high tide, but with little evidence of correlation with the expected light/dark cycle. During March both estuarine and open shore crabs showed some qualitative evidence of circatidal rhythmicity, several peaks coinciding with the time of expected high tide. The pattern of endogenous locomotor activity in December and in March

was therefore roughly similar for crabs collected from the estuary and from the open shore.

Fig. 4.2.

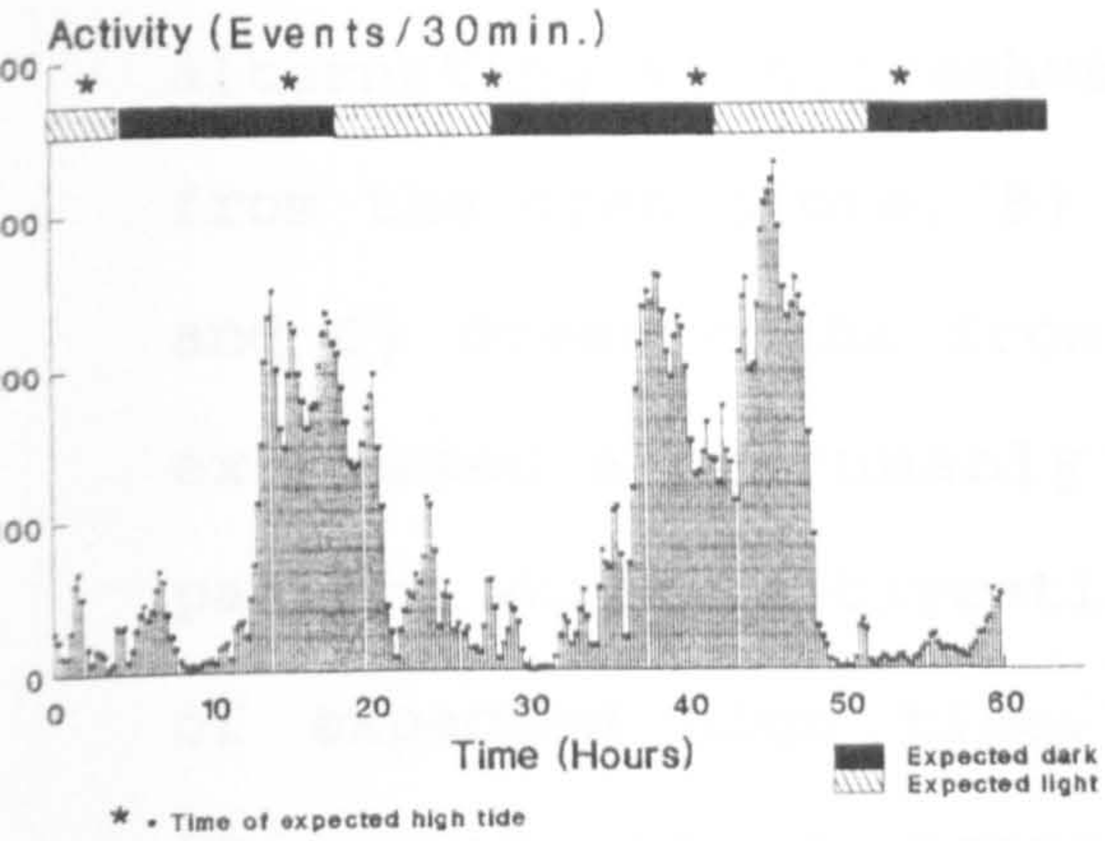
Activity of estuarine crabs in June



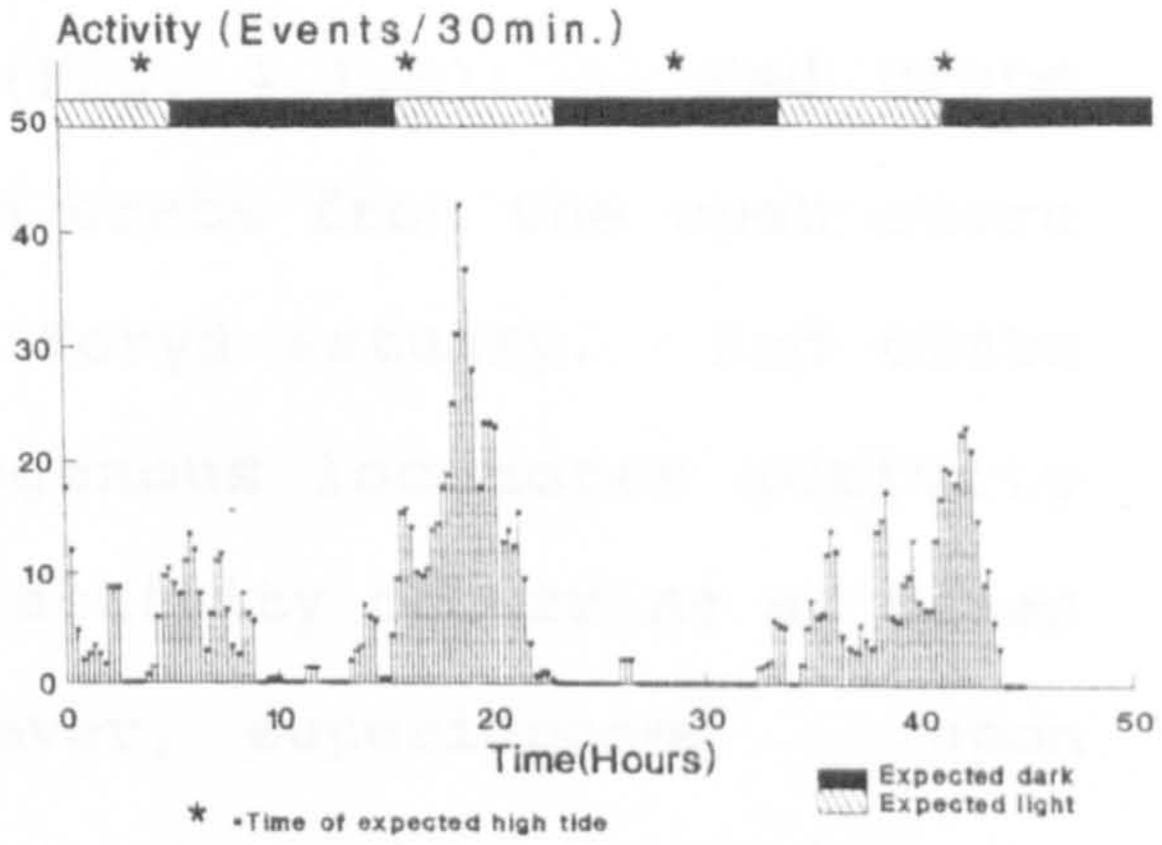
Endogenous locomotor activity of 8 green crabs from the Foryd estuary, in constant salinity (34ppt.) and constant darkness

g. 4.3.

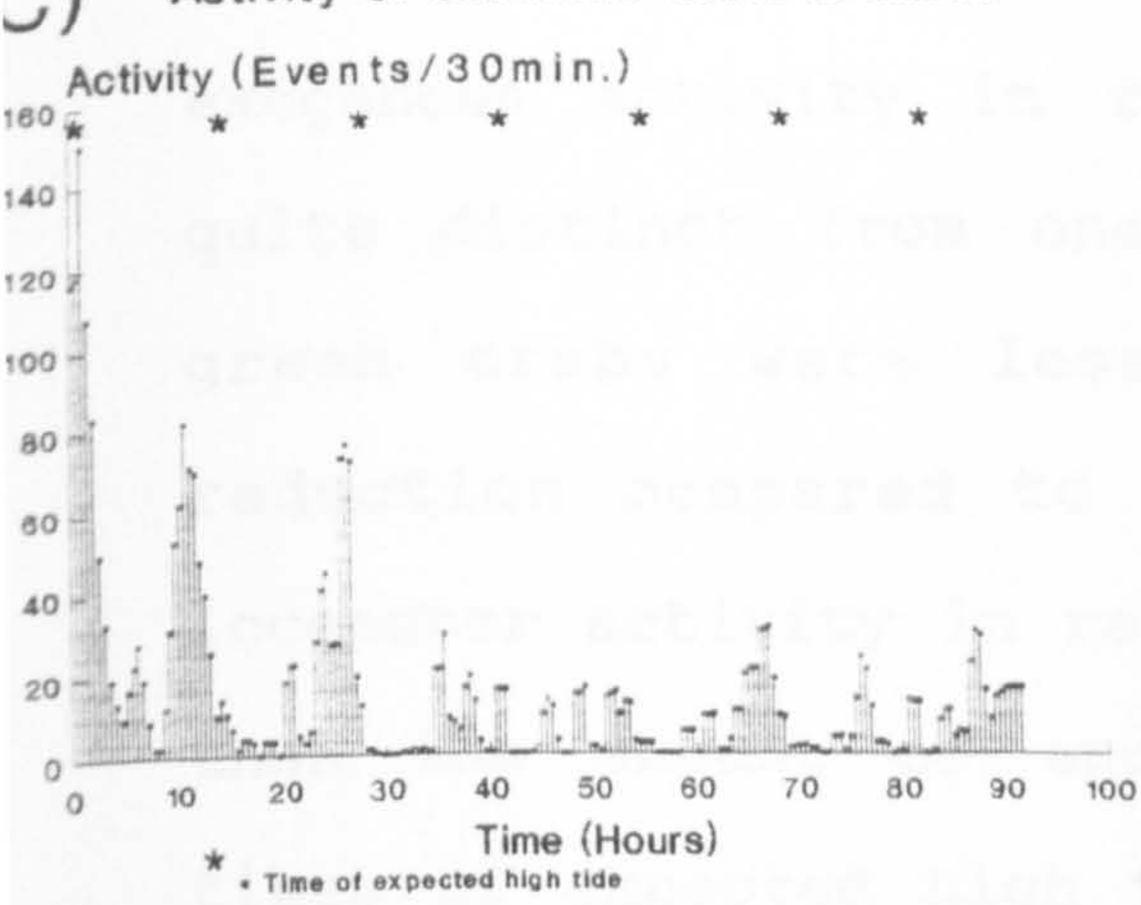
A) Activity of estuarine crabs in December



B) Activity of open shore crabs in December



C) Activity of estuarine crabs in March



D) Activity of open shore crabs in March

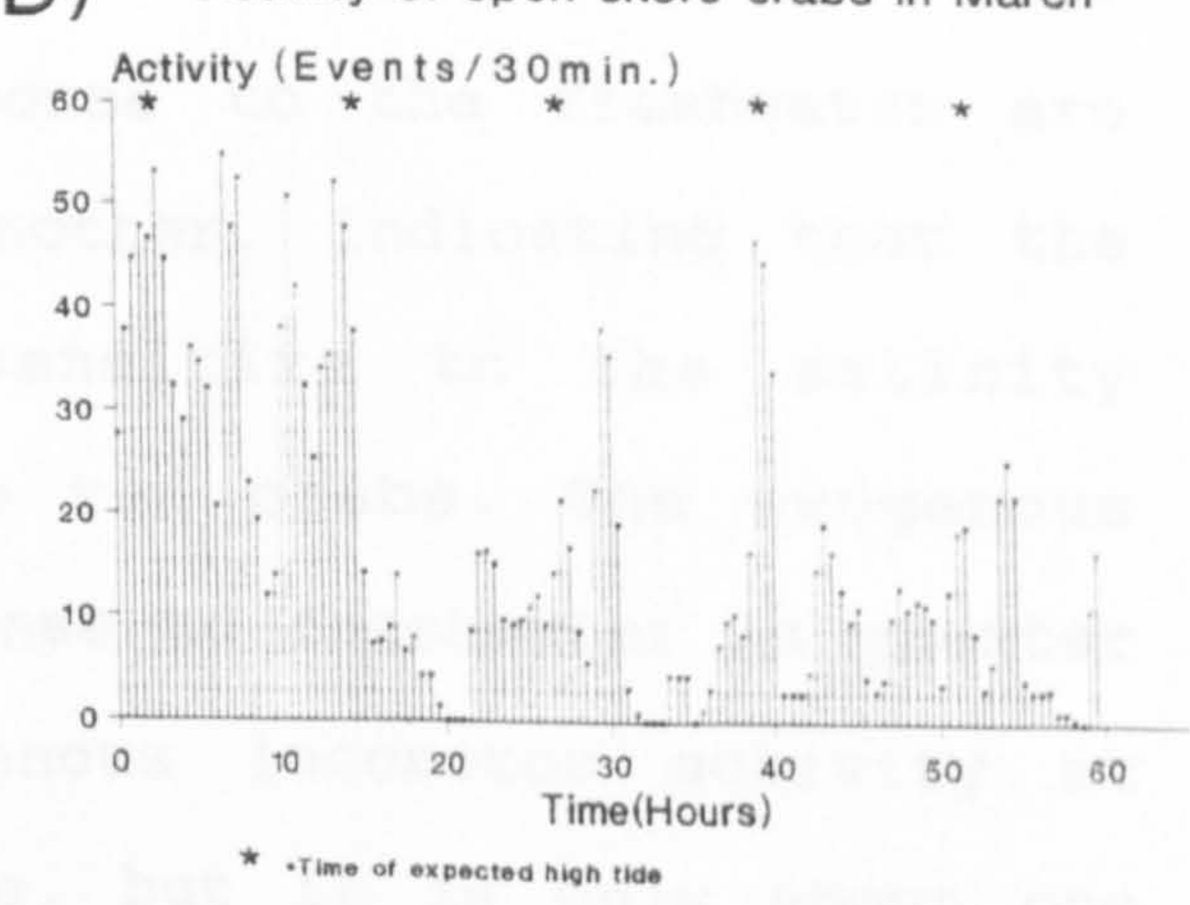


Fig. 4.3. Activity of between 5 and 8 green crabs freshly collected from the Foryd estuary and the open shore (Ynys Faelog) in December (A,B), and in March (C,D). Activity recorded in constant salinity (34ppt.) and constant darkness.

SALINITY CYCLING

Fig. 4.4. illustrates the activity of groups of eight crabs freshly collected in summer, and exposed to square wave artificial tidal cycles of seawater alternating with freshwater (Fig. 4.1.B): A) Red crabs from the open shore, B) Green crabs from the open shore and C) Green crabs from the Foryd estuary. Red crabs expressed a presumably endogenous locomotor activity pattern which is circatidal, activity occurring at times of expected high tide. However, superimposed upon this endogenous response were peaks generated by exogenous responses to freshwater, which were of particularly large amplitude over the first 48 hours of the experiment. Green crabs from the open shore show a different pattern of activity when exposed to cycles of salinity. The peaks of endogenous locomotor activity in response to times of expected high tide and the exogenous activity in response to the freshwater are quite distinct from one another, indicating that the green crabs were less sensitive to the salinity reduction compared to the red crabs. The exogenous locomotor activity in response to freshwater is greater than the amount of endogenous locomotor activity at times of expected high tide, but it is only about one third the magnitude of that of the red crabs. Crabs collected from the estuary also show a peak of exogenous locomotor activity as a result of exposure to

freshwater. However, this exogenous effect is smaller in magnitude than the endogenous locomotor activity which occurs at times of expected high tide. There is evidence of this type of pattern emerging towards the end of the recording period for both red and green crabs from the open shore, the exogenous response starts to diminish in magnitude compared to the endogenous locomotor activity. Throughout all these experiments the peaks of locomotor activity are presumably entrained by repeated exposure to full seawater coincident with the times of expected high tide.

Fig. 4.4.

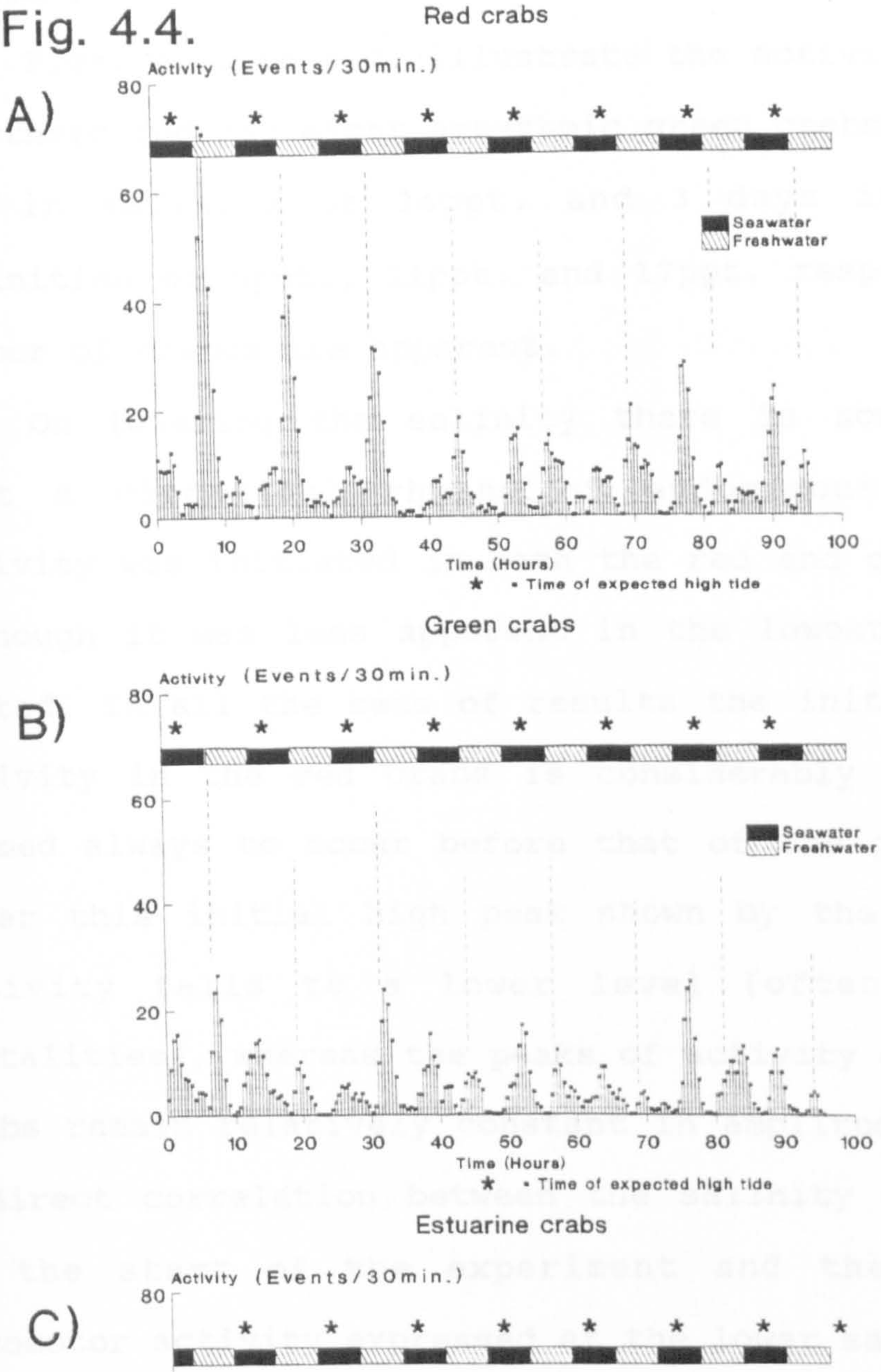


Fig. 4.4. Activity of eight freshly collected A) red crabs, B) green crabs C) green crabs from the Foryd estuary, in late summer exposed to a 6.25 hour, 6.25 hour cycle of seawater and freshwater (see Fig. 4.1.B).

CONSTANT SALINITY REGIMES

Figs. 4.5. to 4.7. illustrate the activity of eight arhythmic red and eight arhythmic green crabs held for 1 day in salinity of 34ppt. and 3 days in constant salinities of 5ppt., 11ppt. and 17ppt. respectively. A number of trends are apparent.

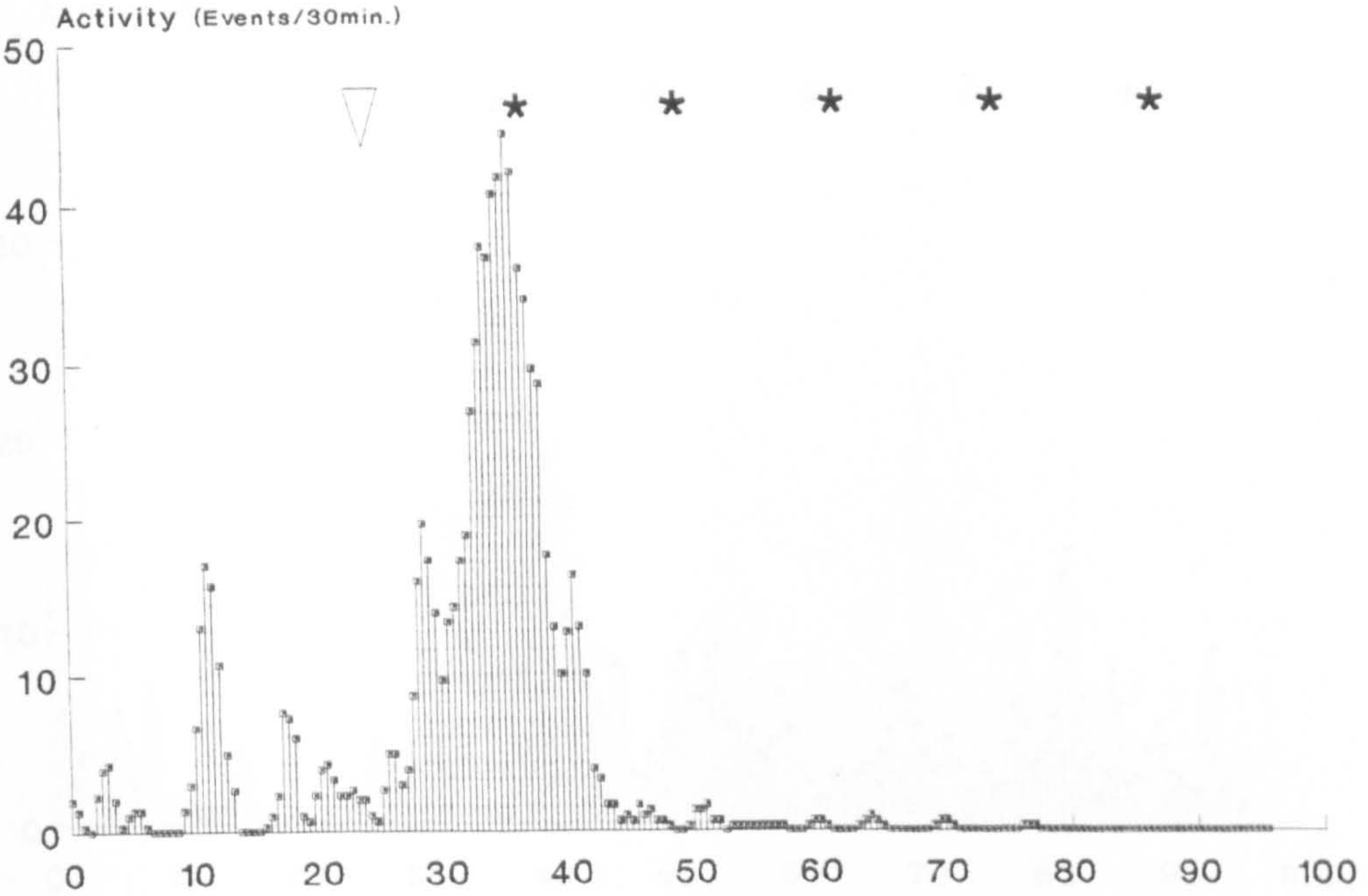
On lowering the salinity there is some evidence that a circatidal rhythm of endogenous locomotor activity was initiated in both the red and green crabs, although it was less apparent in the lowest salinities tested. In all the sets of results the initial peak of activity in the red crabs is considerably greater and seemed always to occur before that of the green crabs. After this initial high peak shown by the red crabs, activity falls to a lower level (often caused by mortalities), whereas the peaks of activity of the green crabs remain relatively constant in amplitude. There is a direct correlation between the salinity differential at the start of the experiment and the amount of locomotor activity expressed at the lower salinity.

Crabs were also acclimated to either low (Fig. 4.8.) or high salinities (Fig. 4.9.) for two days, with locomotor activity then monitored for a further day in the salinity of acclimation and for 3 days in a salinity of 5ppt. Broadly similar findings to those for normal acclimated crabs (Fig. 4.5.) were recorded. Both red and green crabs that had been previously acclimated to low

salinity showed very pronounced bursts of locomotor activity immediately after transfer to 5ppt. compared to those previously acclimated to normal salinity. Red and green crabs acclimated to high salinity however, also reacted to the salinity change before those acclimated to normal salinity, the degree of activity being very similar.

Fig. 4.5.

Red crabs



Green crabs

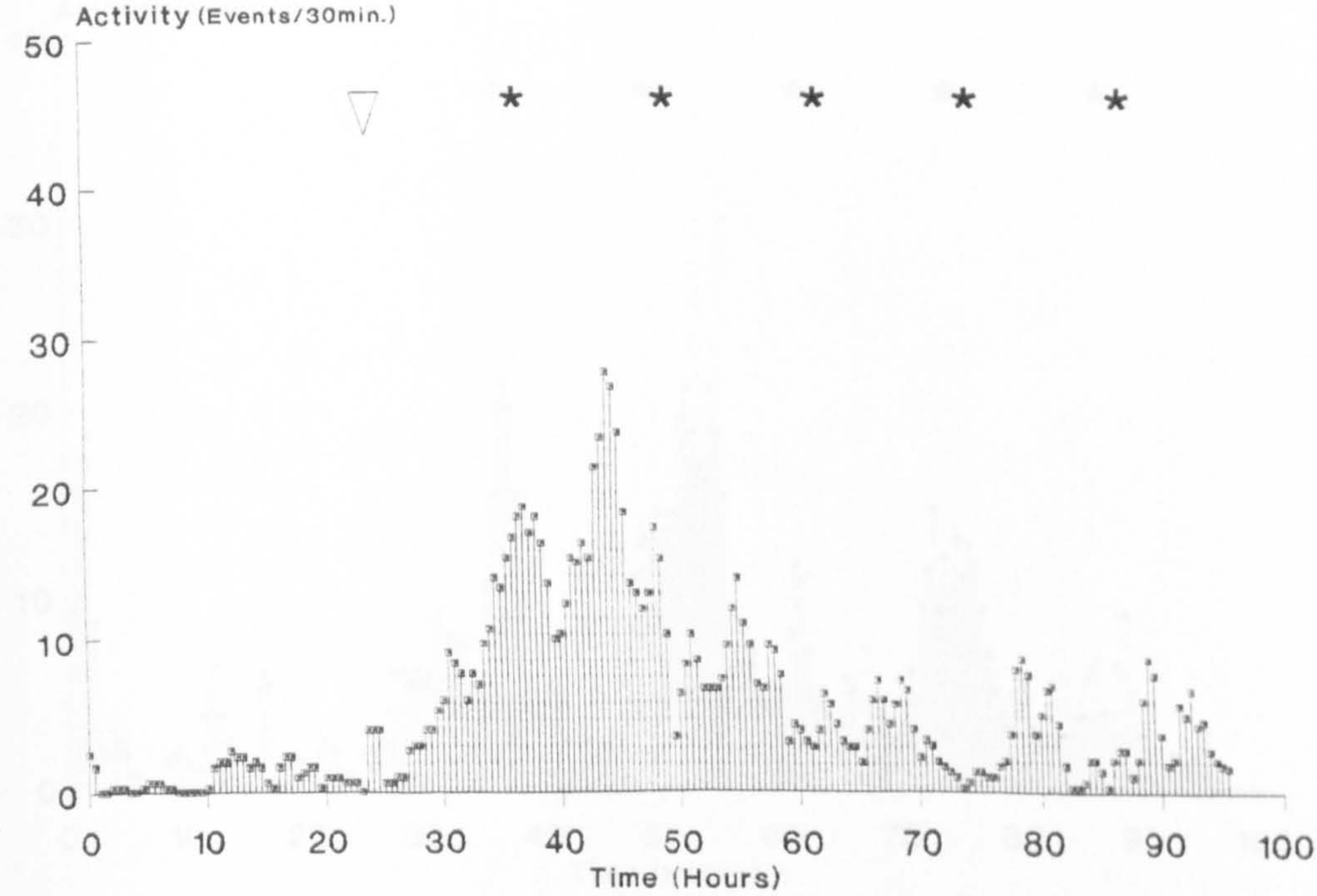
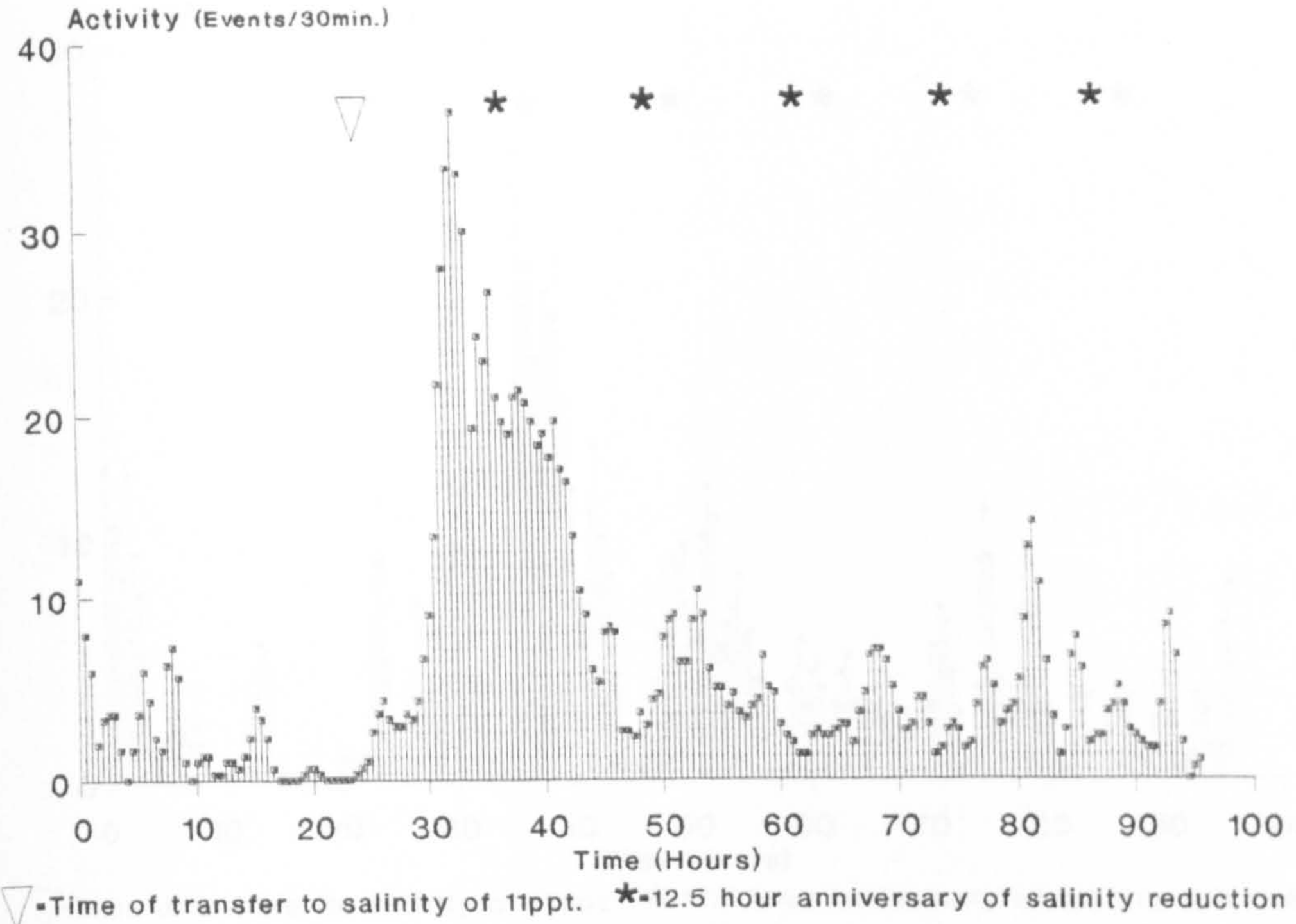


Fig. 4.5. Activity of eight arhythmic red and eight arhythmic green crabs, during 1 day in salinity of 34ppt. and 3 days in constant salinity of 5ppt. 6 red crabs and 2 green crabs were dead at the end of the experiment.

Fig. 4.6.



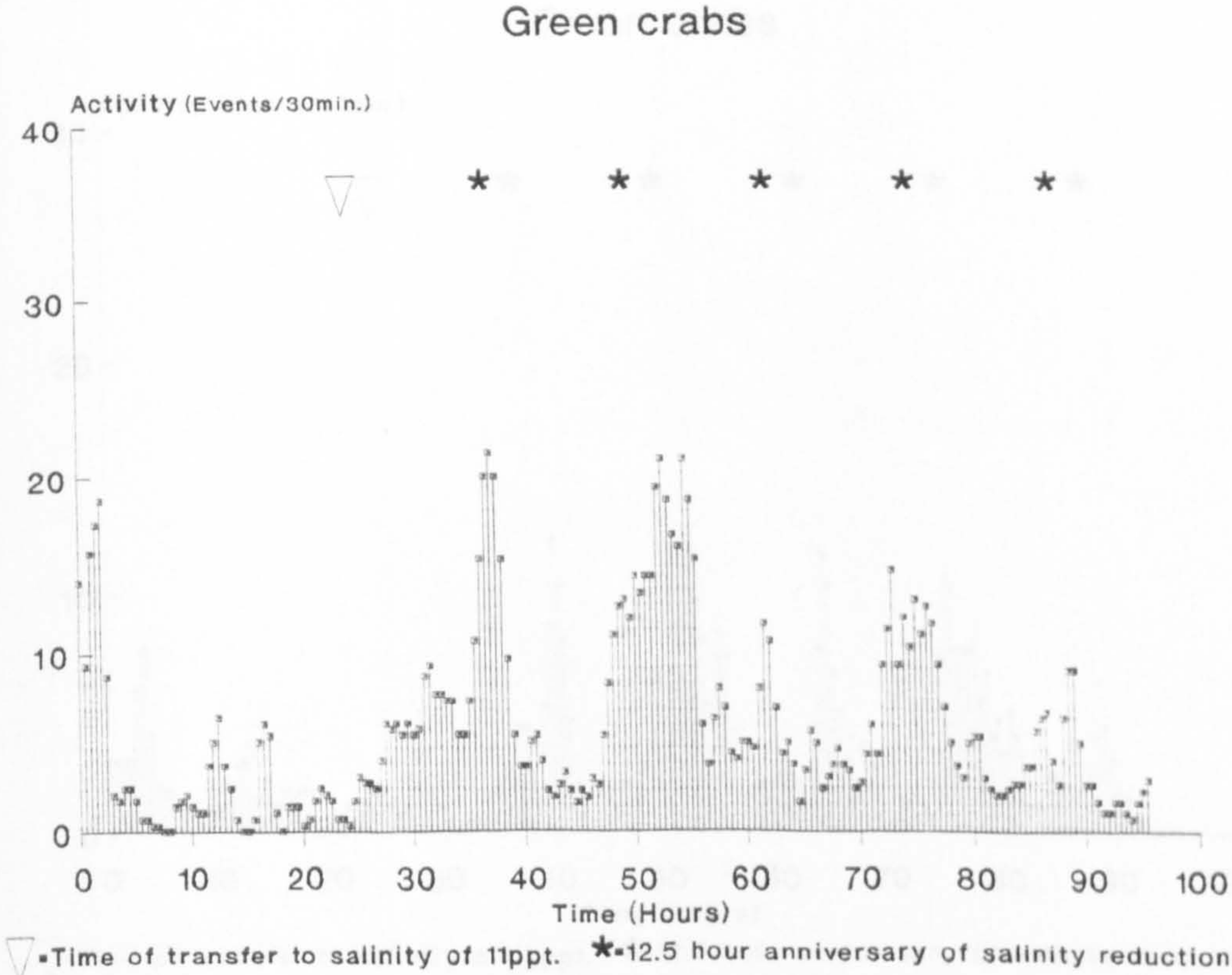


Fig. 4.6. Activity of eight arhythmic red and eight arhythmic green crabs, during 1 day in salinity of 34ppt. and 3 days in a constant salinity of 11ppt. 2 red crabs were dead at the end of the experiment.

Fig. 4.7.

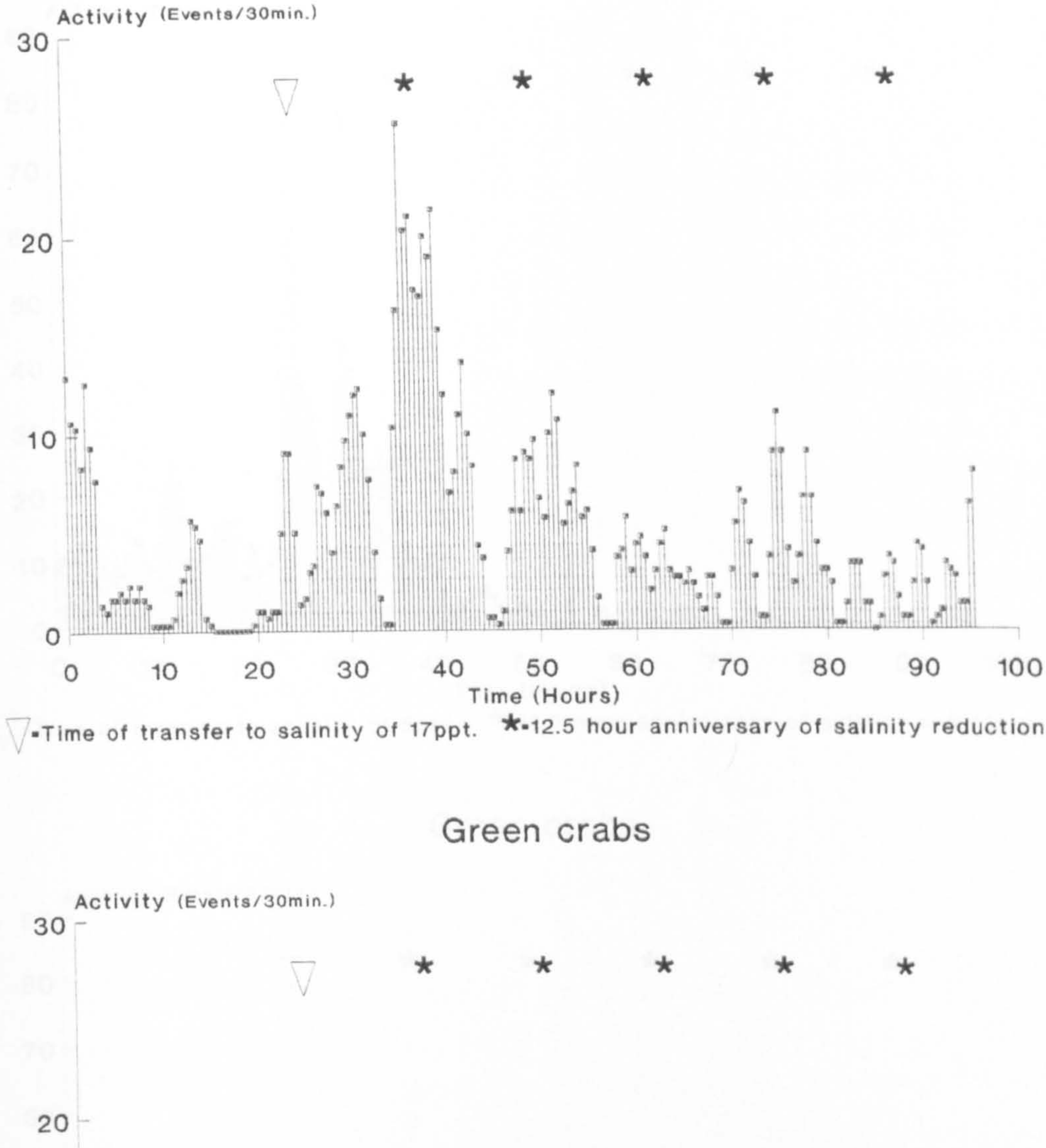


Fig. 4.7. Activity of eight arhythmic red and eight arhythmic green crabs, during 1 day in salinity of 34ppt. and 3 days in a constant salinity of 17ppt.

Fig. 4.8.

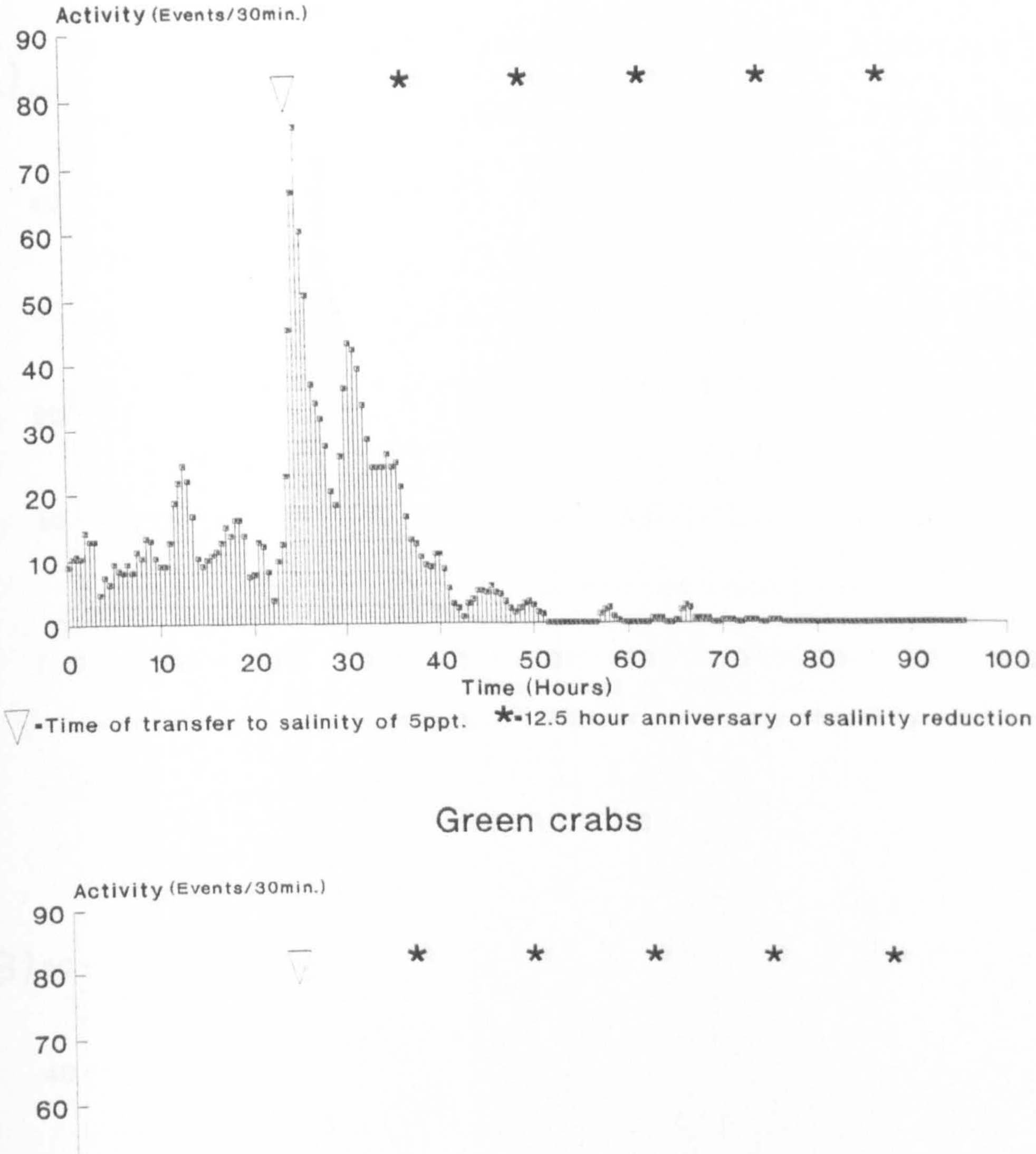
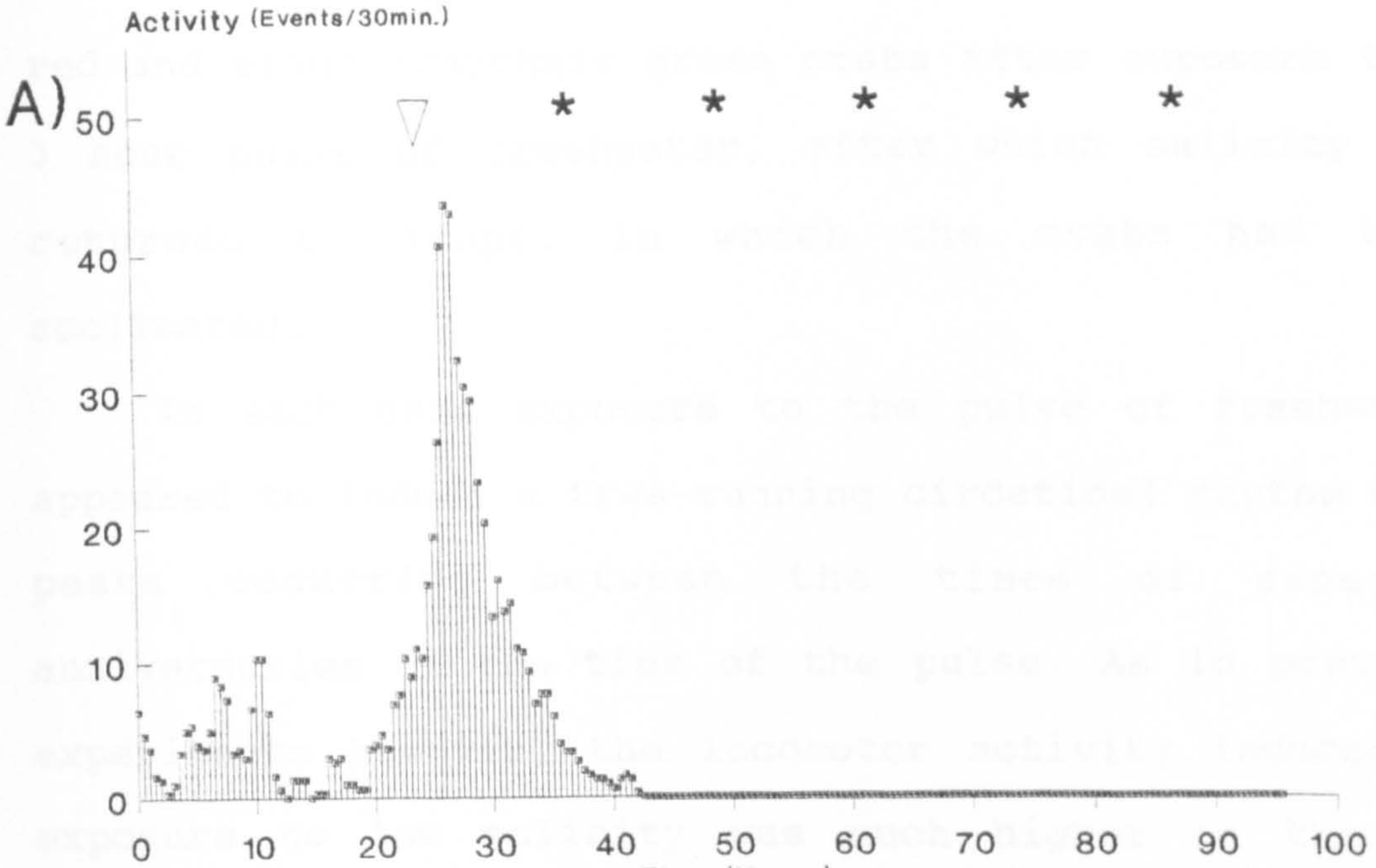


Fig. 4.8. Activity of eight red and eight green crabs previously acclimated to low salinity (17ppt.). Activity recorded for 1 day in salinity of 17ppt. and for 3 days in a constant salinity of 5ppt. 5 red crabs and 2 green crabs were dead at the end of the experiment.

Fig. 4.9.



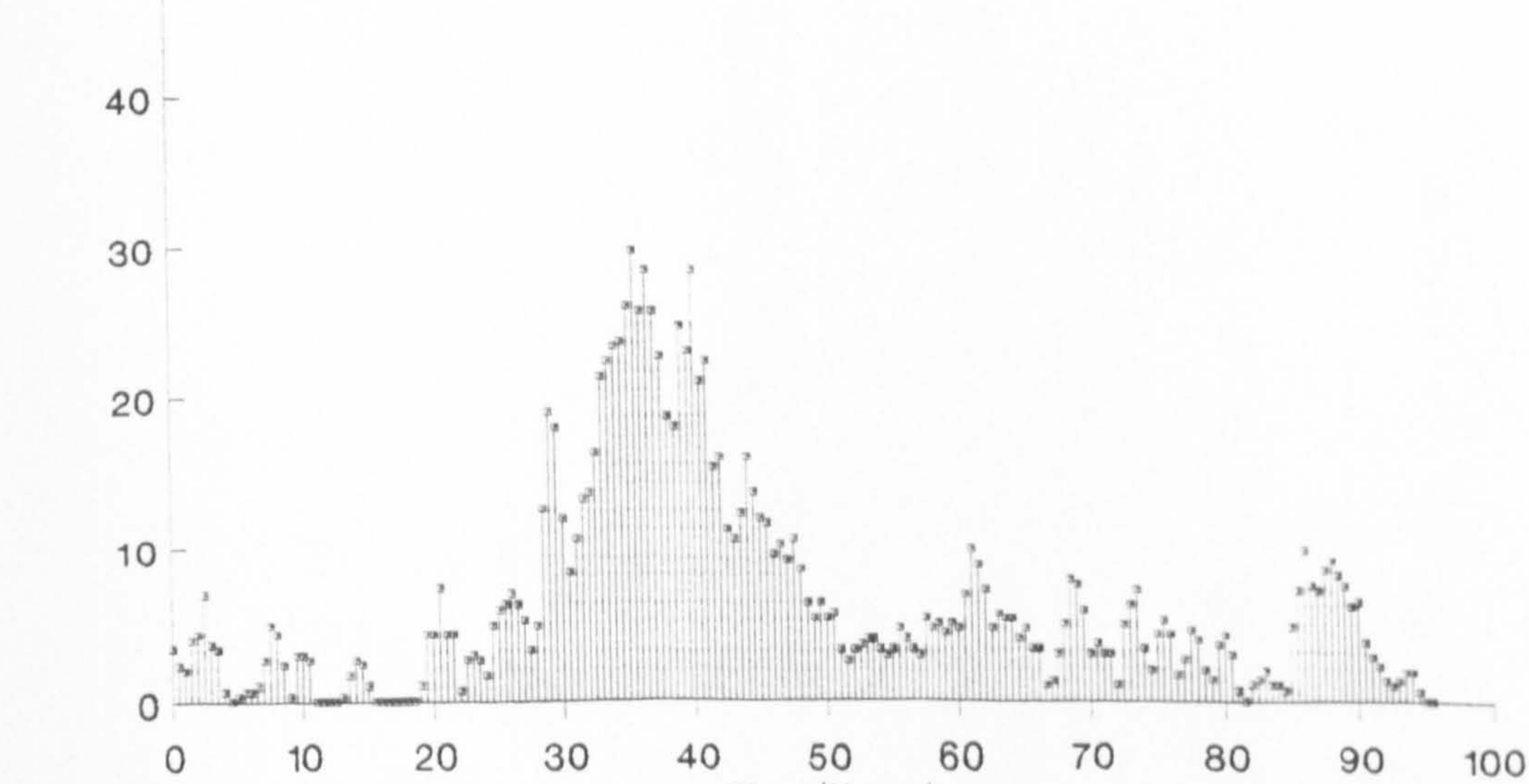


Fig. 4.9. Activity of eight red and eight green crabs acclimated to high salinity (50ppt.) for 2 days. Activity recorded for 1 day in salinity of 50ppt. and 3 days in a constant salinity of 5ppt. 8 red crabs and 4 green crabs were dead at the end of the experiment.

PULSES OF FRESHWATER

Fig. 4.10. shows the activity of eight arhythmic red and eight arhythmic green crabs after exposure to a 3 hour pulse of freshwater, after which salinity was returned to 34ppt. in which the crabs had been acclimated.

In each case exposure to the pulse of freshwater appeared to induce a free-running circatidal rhythm with peaks occurring between the times of expected anniversaries of the time of the pulse. As in previous experiments however, the locomotor activity induced by exposure to low salinity was much higher in the red crabs than in green crabs.

Fig. 4.10.

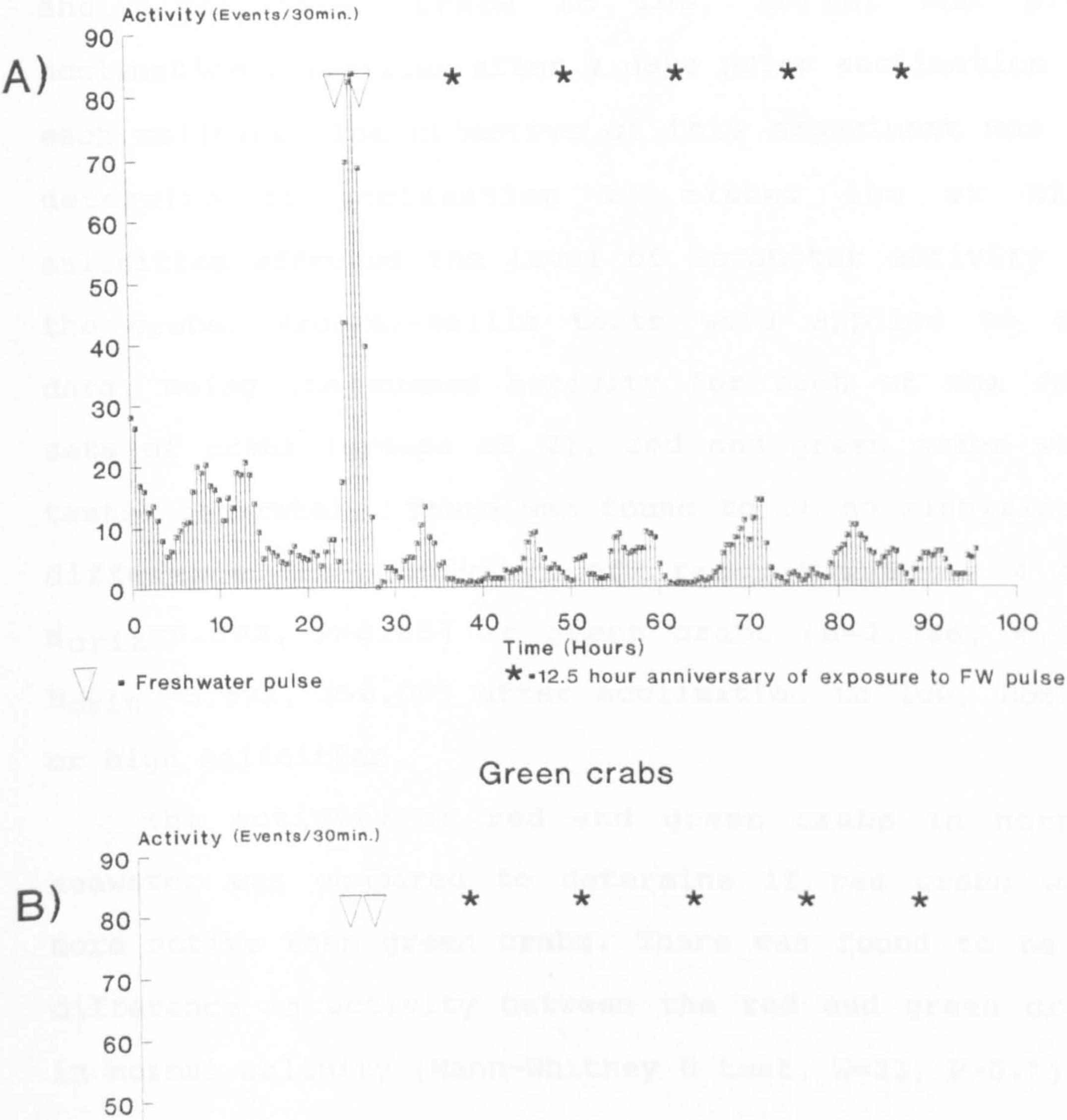


Fig. 4.10. Activity of eight arhythmic red and eight arhythmic green crabs, during 1 day in salinity of 34ppt., and subsequently at 34ppt. for a further 3 days after exposure to a 3 hour pulse of freshwater.

EFFECT OF SALINITY OF ACCLIMATION ON LOCOMOTOR ACTIVITY

Fig. 4.11. illustrates the activity of eight red and eight green crabs in low, normal and high acclimation salinities after 2 days prior acclimation in each salinity. The objective of this experiment was to determine if acclimation to either low or high salinities affected the level of locomotor activity of the crabs. Kruskal-Wallis tests were applied to the data, using the summed activity for each of the four sets of crabs (groups of 2), red and green crabs were tested separately. There was found to be no significant difference in activity of red ($H=1.846$, $4,4,4$ $H_{crit}=5.692$, $P>0.05$) or green crabs ($H=2.346$, $4,4,4$ $H_{crit}=5.692$, $P>0.05$) after acclimation to low, normal or high salinities.

The activity of red and green crabs in normal seawater was compared to determine if red crabs were more active than green crabs. There was found to be no difference in activity between the red and green crabs in normal salinity (Mann-Whitney U test, $W=23$, $P>0.1$).

Fig. 4.11.

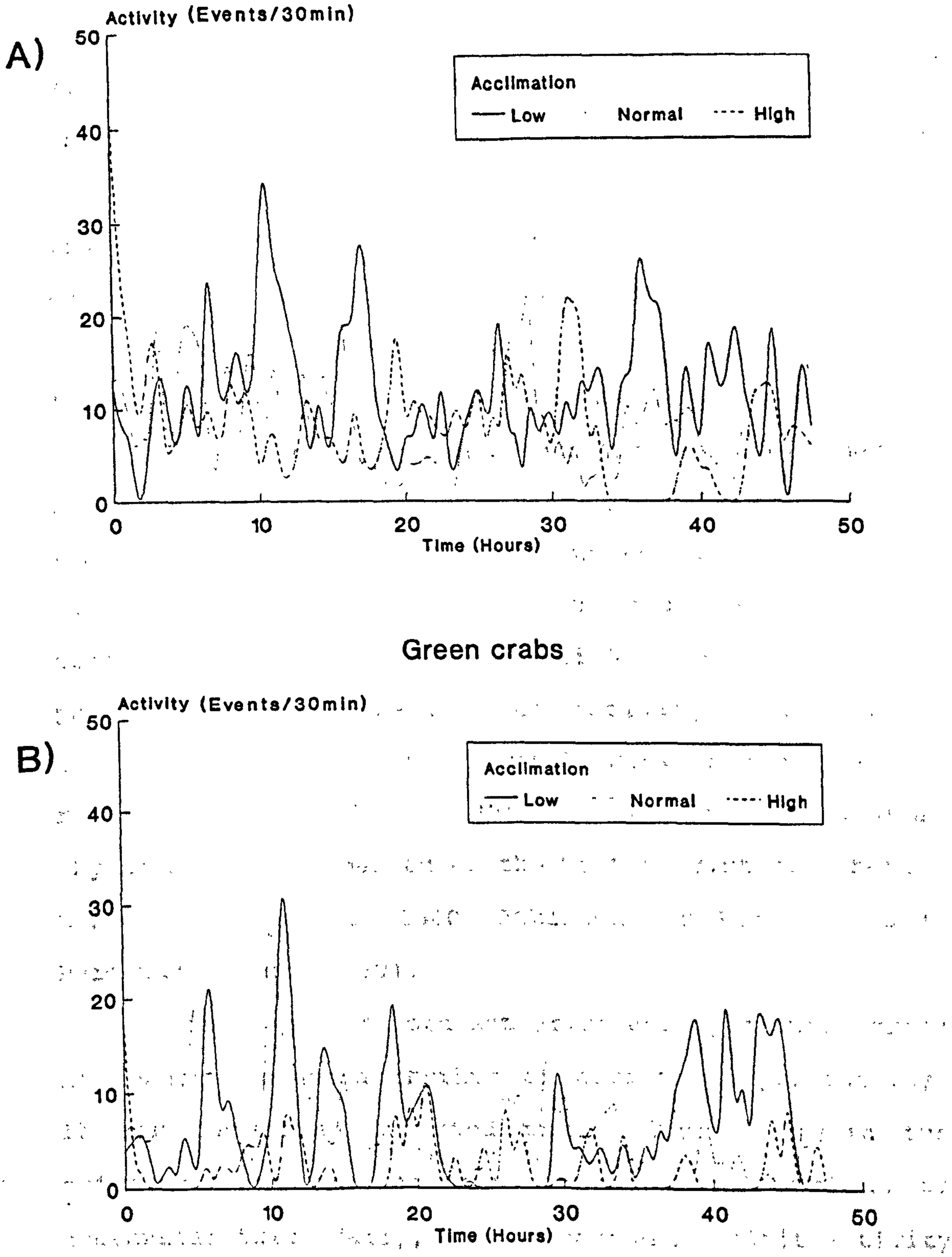


Fig. 4.11. Activity of eight red and eight green crabs in low (17ppt.), normal (34ppt.) and high (50ppt.) acclimation salinities after 2 days prior acclimation in each given salinity.

DISCUSSION

The endogenous locomotor activity expressed by crabs from the Foryd estuary in constant conditions is similar to that of animals collected from the open shore. The crabs showed periods of peak locomotor activity coincident with the time of expected high tide (Naylor, 1958). In simulated tidal cycles of salinity change estuarine crabs also show bursts of activity coincident with falling salinity but, as with open shore forms, this effect is purely exogenous. The free-running circatidal pattern persists, entrained to the times of expected high salinity and high tide in the estuary (Taylor and Naylor, 1977, Bolt and Naylor, 1985, 1986). This circatidal rhythm only persisted for 3 days in constant conditions, after which time a circadian pattern with peak periods of activity occurring on evening high tides was observed. Similar findings are reported by Naylor (1958, 1960) and circadian rhythmicity is suggested as the most persistent inherent periodicity (Naylor, 1960, Atkinson and Parsons, 1973, Reid and Naylor, 1989).

During December both estuarine and open shore crabs no longer expressed rhythms of circatidal periodicity. It may have been expected that, since crabs in the estuarine environment were experiencing periods of freshwater twice daily, that they would exhibit activity of circatidal periodicity, which would persist

throughout the winter months. Exposure to low salinity has been shown to re-induce circatidal rhythmicity in apparently arhythmic crabs in winter (Bolt and Naylor, 1985). Instead the peak periods of activity observed occurred on alternate high tides, suggesting strong circadian modulation of the rhythm. This change of pattern of rhythmicity from circatidal to predominately circadian is characteristic of crabs in winter (Atkinson and Parsons, 1973). Interestingly too the phasing of the largest activity peaks in December (Fig. 4.3.) is not consistently at the times of expected night time, probably related to the coincidence of some of the expected tide times with expected dawn and dusk. This shows that crabs in the estuary in winter exhibit a similar pattern of activity to those occurring in the sublittoral zone of the open shore in the absence of zeitgebers such as temperature and pressure. This suggests that salinity may not be effective in modulation of the circatidal clock during the winter. Evidence of endogenous circatidal rhythmicity beginning to be re-instated was apparent in March in both open shore and estuarine forms. This is consistent with movement of crabs into the intertidal zone of fully marine shores as the temperature increases (Naylor, 1962, 1963, Naylor and Atkinson, 1972, Atkinson and Parsons, 1973, Naylor and Williams, 1984a).

During exposure to square wave salinity cycles,

similar to those experienced in the Foryd estuary (Fig. 2.2.A), different patterns of endogenous rhythmicity were observed between red and green crabs from the open shore and estuarine green crabs. Red crabs exhibited a large exogenous response almost immediately upon lowering of the salinity, which almost totally obliterated the underlying endogenous circatidal rhythmicity. The exogenous response has been proposed as escape behaviour in *Carcinus* (Taylor and Naylor, 1977, Thomas *et al*, 1981, Bolt and Naylor, 1985, Ameyaw-Akumfi and Naylor, 1987). Present results indicate that this response is most pronounced in red crabs, which are rare in estuaries, suggesting that salinity avoidance behaviour may be of particular importance in this form. Green crabs also react to the hypo-osmotic shock with increased halokinesis, but being more tolerant of low salinity (Reid *et al*, 1989, Chapter 1, Chapter 3) they exhibit this exogenous response later into the freshwater cycle and it is much less marked than that of the red crabs. This would explain the observed behavioural reactions mentioned in Chapter 3, and would account for the selective retention of green crabs within the estuary (Chapter 2).

The responses exhibited by estuarine crabs to cycles of salinity are different from those of open shore forms (Fig. 4.4. and Taylor and Naylor, 1977, Bolt and Naylor, 1985). Estuarine crabs are much less

responsive than open coast forms which exhibit marked exogenous locomotor on exposure to freshwater. On falling salinities estuarine crabs show activity peaks which are much smaller in amplitude than the endogenous peaks at times of expected high tide. Thus green crabs in an estuary appear to become habituated to episodes of falling salinity, crabs retained in the estuary clearly exhibiting tidal and daily rhythmic locomotor patterns as though they were on the open shore, with no apparent mechanism to induce escape. The results suggest that for estuarine crabs the episodes of high salinity are the major zeitgeber entraining the behavioural rhythm, since they do not seek to avoid the low salinity that occurs twice daily in the estuary. The endogenous rhythm of open shore forms is also entrained by high salinity episodes (Taylor and Naylor, 1977, Bolt and Naylor, 1985), but in such crabs other factors such as pressure and temperature might be expected to be more important among the known zeitgebers (Bolt *et al*, 1989, Naylor, 1989). Present results suggest that the halokinesis response to reduced salinity reported for *Carcinus* (Taylor and Naylor, 1977, Bolt and Naylor, 1985) is most marked in open shore forms and virtually absent in estuarine forms, indicating some behavioural basis for the ecological separation of estuarine and open shore crabs.

Exposure to constant low salinity in winter has

been shown in *Carcinus* to induce a locomotor rhythm of circatidal periodicity (Bolt and Naylor, 1985, Reid and Naylor, 1989). Once again, however, present results show that the pattern exhibited by red crabs was quite distinct from that of green individuals. In all of the salinities tested the initial peak of activity of red crabs on salinity reduction was much larger and occurred before that of green crabs. This initial high halokinesis (Taylor and Naylor, 1977) enabled the crabs to avoid adverse salinities (Thomas *et al*, 1981, Ameyaw-Akumfi and Naylor, 1987), and would account for differences in choice behaviour between the red and green crabs reported here (Chapter 3).

The subsequent peaks of activity expressed by red crabs in low salinities rapidly decreased in amplitude, whereas those of the green crabs were comparatively uniform in height. This difference appeared to be due more to osmotic stress and eventual mortality of red crabs rather than a true behavioural difference. This was confirmed by giving both sets of crabs a pulse of freshwater (Fig. 4.10), after which the salinity was returned to 34ppt. The crabs entrain to this, exhibiting a rhythm of circatidal periodicity, the amplitude of which is similar for both colours of crab. Moreover the activity peaks appeared consistently to alternate with the 'tidal' anniversaries of the freshwater pulse, suggesting that such treatment generates rhythms with

peak times of 'expected' high salinity in a tidal cycle. There was a decrease in the amount of locomotor activity within the increasing salinity range tested. This appears to be directly related to the escape response; the lower the salinity the faster the time of exit (Fig. 3.8.).

After acclimation to either low or high salinities a similar rhythm of circatidal periodicity was initiated upon lowering of the salinity (Figs. 4.8. and 4.9.). For both red and green crabs acclimated to low salinity the initial peak of activity was greater and occurred before that of normal acclimated crabs. This increased locomotor activity would account for the faster exit rates from low salinities exhibited by both red and green crabs (Figs. 3.8., 3.11.). However, after acclimation to high salinity the amount of activity exhibited is similar to, and the first peak occurs before that of normal acclimated crabs. This presents something of an anomaly, since previous experiments (Figs. 3.2., 3.3., 3.11.) have shown that high salinity acclimated crabs tend to exit low salinities after those acclimated to normal conditions. It may be that the increased halokinesis caused by lowering of the salinity is not solely responsible for the pattern of choice behaviour exhibited by acclimated crabs.

Activity was therefore monitored for two days in each acclimation salinity to determine if the time of

exit from low salinities (see Figs. 3.4., 3.11.) was dependent on the activity beforehand, since magnesium salts in seawater tend to have a depressing effect on neuromuscular transmission (Katz, 1936, Waterman, 1941, Boardman and Collier, 1946). Robertson (1949, 1953) working with *Maia* and other spider crabs found that the amount of magnesium in the blood was inversely related to the active movement and an excitatory effect of magnesium free seawater has been found to occur in *Cancer magister* (Holliday, 1980). However, the activity of both red and green crabs within each of the acclimation salinities was found to be similar. The altered magnesium concentrations would appear therefore not to have an appreciable affect on the locomotor activity of *Carcinus*. Lockwood and Riegel (1969) found that despite the fact that blood magnesium concentrations of *Carcinus* fell to 1/4 of their original level there was no change in activity. In high magnesium seawater *Carcinus* was found to be generally hyperactive and in good condition; concentrations in the blood increased up to 5 times the normal level without obvious narcotic effects (Zanders, 1981), but satisfactory explanations for this phenomenon are lacking. Comparison of activity levels of red and green crabs in 34ppt. seawater showed no differences. This would suggest that escape responses shown are as a result of the salinity encountered, rather than red crabs being initially more

active than their green counterparts.

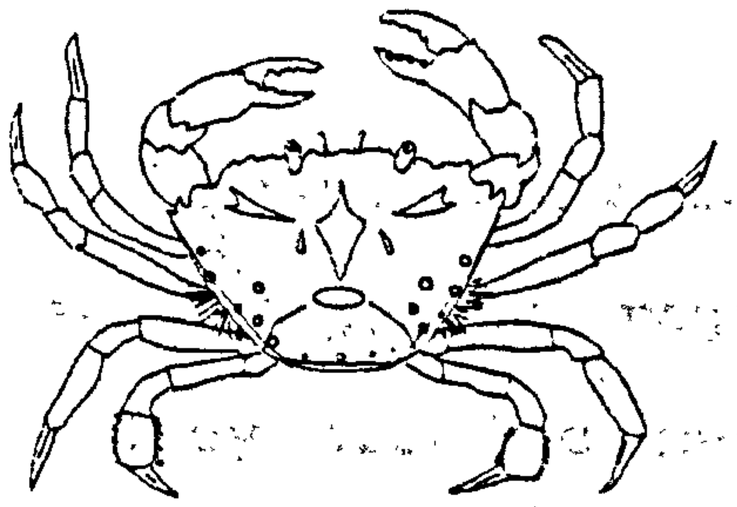
This study has shown that the previous environment encountered by the organism can affect the endogenous locomotor activity. Future investigations should now consider differences between the two colour forms of *Carcinus* to cycling environmental variables. Investigations of the effects of a particular zeitgeiber and its importance should be related to the general behaviour of the animal under study and to the environment from which it was collected.

CHAPTER 5:

Detection of the medium and thresholds of salinity discrimination by *Carcinus maenas*

SUMMARY

Carcinus detected salinity variation by responding to the concentrations of Na and Cl in seawater, and was able to differentiate between salinities separated by as little as 0.5ppt.



INTRODUCTION

The intertidal zone and brackish water environments have been invaded a number of times during evolutionary history by different classes of organisms (Rankin and Davenport, 1981). Similarly mechanisms of detection of the medium, permitting animals to exhibit behavioural traits to maintain themselves within preferred salinity regimes, have evolved several times

Two main methods of salinity detection have been described. Animals respond either to the total osmotic pressure as in *Jasus lalandii* (Krijgsman and Krijgsman, 1954), *Porcellana platycheles* (Davenport, 1972a), *Panulirus japonicus* (Tazaki, 1975), *Marinogammarus marinus* (Bettison and Davenport, 1976) and *Lasaea rubra* (Davenport and Beard, 1988). Alternatively they may react to a specific ion or group of ions found in seawater. Barnes (1939) showed that *Ligia baudiniana* reacted negatively to Na^+ and K^+ but not to Ca^{2+} ions. *Gammarus pulex* detects Ca^{2+} (Vincent, 1973), and *Mytilus edulis* responds to Na^+ and Mg^{2+} (Davenport, 1981), whilst *Scrobicularia plana* responds to all three of these ions in seawater (Akberali and Davenport, 1982).

Chemoreceptors responsible for these detection abilities are located on various regions of the body. In many crustaceans they are located on the antennae, antennules or antennular flagella, as in *Jasus lalandii*

(Krijgsman and Krijgsman, 1954), *Cambarus bartonii* (Hodgson, 1958), *Gammarus oceanicus* (Lagerspetz and Matilla, 1961), *Panulirus japonicus* (Tazaki, 1975) and *Porcellana platycheles* (Davenport, 1972a). In addition the last species also employed the second and third pairs of walking legs when making a choice between salinities (Davenport and Wankowski, 1973). In *Carcinus* the dactyls of the walking legs are covered with hair peg organs which link to ion sensitive chemosensory neurons, which are reported to detect salinity changes (Schmidt, 1989).

The thresholds of these detection abilities vary between different species of crustaceans. *Asellus aquaticus* could detect differences between freshwater and NaCl solutions of 1ppt., but was unable to discriminate between NaCl solutions separated by a difference of less than 5ppt. (Lagerspetz and Matilla, 1961), whereas *Marinogammarus marinus* makes a significant choice for 2.5% seawater when this solution is tested against freshwater (Bettison and Davenport, 1976). The discrimination abilities of *Corophium volutator* were dependent upon the salinity of acclimation, and the range of pairs of salinity choices offered, but the amphipod could detect differences between salinities as small as 2.5ppt. (McLusky, 1970). *Porcellana platycheles* differentiated between seawater strengths separated by 10% seawater, making a choice

towards the highest salinity offered (Davenport, 1972a). Thomas *et al* (1981) found that *Carcinus* was able to discriminate between pairs of salinities separated by a 25% seawater difference over a two hour period, making a choice for the salinity closest to full seawater. Over a period of 4 to 5 hours *Carcinus* can detect between pairs of salinities separated by a 4ppt. to 6ppt. difference (Ameyaw-Akumfi and Naylor, 1987).

The aim of this chapter was to determine whether *Carcinus* was responsive to the osmotic pressure of the medium or to a certain ion or group of ions. The ability of *Carcinus* to discriminate between sets of salinities was investigated to find the minimal detection threshold, and to determine if this was sufficient for them to perceive and move along a salinity gradient.

MATERIAL AND METHODS

Detection of the medium by *Carcinus* was studied using the multiple choice chamber tank described in Chapter 3. Two alternate chambers were filled with the solution under test, and the other two with freshwater. Initial experiments were designed to test whether the crabs responded to ionic or osmotic pressure of the medium. It was assumed that any detectable ionic/osmotic pressure in the test solution would make this more 'favourable' to the crabs than freshwater.

Five crabs were introduced into each individual chamber after being rinsed in test solution to prevent contamination of the solutions with seawater. The number of animals per chamber was then recorded after 1, 3 and 6 hours in constant darkness. Approximately equal numbers of both red and green male crabs between 40-70mm carapace width were used. Results for each of three repetitions were pooled, for each of the two alternate chambers containing a given solution. Data were analysed with standard chi-square contingency tables and tested at the $p<0.01$ level since a relatively large number of trials were carried out.

If a significant difference was obtained between the choice of test solution and freshwater, then the test solution was compared against seawater to ascertain whether *Carcinus* could discriminate between a single ion solution and full seawater. Ionic solutions were made up

using GPR grade compounds, and unless otherwise stated were isotonic with that ion in seawater (Rankin and Davenport, 1981). An optimum pH of 6-8,(Davenport pers. comm.) was achieved by addition of Sodium Hydroxide, Potassium Hydroxide or Hydrochloric acid, temperature being maintained between 12-14^{o}C.

Experiments involving detection of concentrations of various media were conducted in a similar fashion, five crabs being introduced into each test solution. The results for three repetitions for each individual chamber were pooled and are shown graphically in the Results section. Salinities separated by a difference of 1ppt. or less were determined using a Braystoke Series 600 CDTS probe and read-out unit. Salinities were monitored at regular intervals and maintained at a constant level for the duration of each experiment.

RESULTS

DETECTION OF IONS

When given a choice between a 1M solution of non electrolyte mannitol which has an osmotic pressure close to that of full seawater (1000 mOsm/Kg), and freshwater (Fig.5.1.) *Carcinus* showed no significant preference for the mannitol solution. *A priori* therefore, this suggests that *Carcinus* does not respond to the osmotic pressure of its environment but possibly to a single ion or group of ions present in seawater. The crabs responses to all the major cations in normal seawater i.e. Na^+, Mg^{2+}, Ca^{2+} and K^+ plus the major anion Cl^- were therefore tested.

Initial experiments were performed using an isotonic solution of sodium chloride (isotonic for sodium) against freshwater to deduce whether *Carcinus* responds to these ions in seawater (Fig. 5.2. A). The crabs showed a statistically significant preference for the sodium chloride solution. The isotonic sodium chloride solution was then tested against full strength seawater and crabs showed no statistically significant preference for either solution. (Fig. 5.2.B).

An iso-osmotic solution of sodium chloride (Weast, 1979) was then tested against full strength seawater, this solution being isotonic for chloride (Akberali and Davenport, 1982). Crabs appeared to show a preference for seawater over iso-osmotic sodium chloride solution

but this preference was not significant at the 1% level specified (Fig 5.3.A).

If *Carcinus* detects the sodium concentration of the medium then this has to be considered in relation to the fact that an iso-osmotic solution of sodium chloride has a sodium content equivalent to that of 43ppt. seawater. A 43ppt. seawater solution was therefore tested against normal strength seawater (34ppt.) (Fig. 5.3.B). Crabs appeared to show a preference for the normal seawater over 43ppt. seawater but this was not statistically significant at the 1% level required. However a temporal pattern of choice similar to that of iso-osmotic sodium chloride vs. seawater was recorded (Fig. 5.3. A,B).

Additional experiments were performed to determine whether *Carcinus* responds to sodium, to chloride or to concentrations of both these ions in seawater. An isotonic solution of sodium sulphate was first tested against freshwater (Fig 5.4.A), in which *Carcinus* showed a significant preference for the sodium solution. When the sodium solution was tested against seawater (Fig. 5.4.B) *Carcinus* appeared to show a preference for the seawater, but this preference was not statistically significant at the 1% (or 5%) level.

The ability of *Carcinus* to detect chloride was tested against freshwater using an isotonic solution of magnesium chloride (Fig. 5.5.A), in which the crabs

showed a significant preference for the chloride solution. When the crabs were given a choice between magnesium chloride solution and seawater the crabs appeared to show a preference for the seawater but this was significant only at the 5% level and not at the required 1% level (Fig. 5.5.B).

The experiments so far suggest that *Carcinus* responds to both sodium and chloride ions individually in the seawater. Further experiments were then conducted to investigate whether they respond to any of the other cations present in normal seawater. Detection of magnesium ions by *Carcinus* was tested by giving crabs a choice between an isotonic solution of magnesium sulphate and freshwater. Although crabs appeared to show a preference for the magnesium solution there was no statistically significant choice between solutions at the 1% (or 5%) level (Fig. 5.6.).

Calcium and potassium were tested against freshwater using calcium chloride and potassium chloride solutions, the amount of chloride in isotonic solutions of these ions being assumed to be negligible. In both cases it is found that *Carcinus* did not respond to calcium or potassium ions in similar concentrations to those found in normal seawater (Fig. 5.7.A and B).

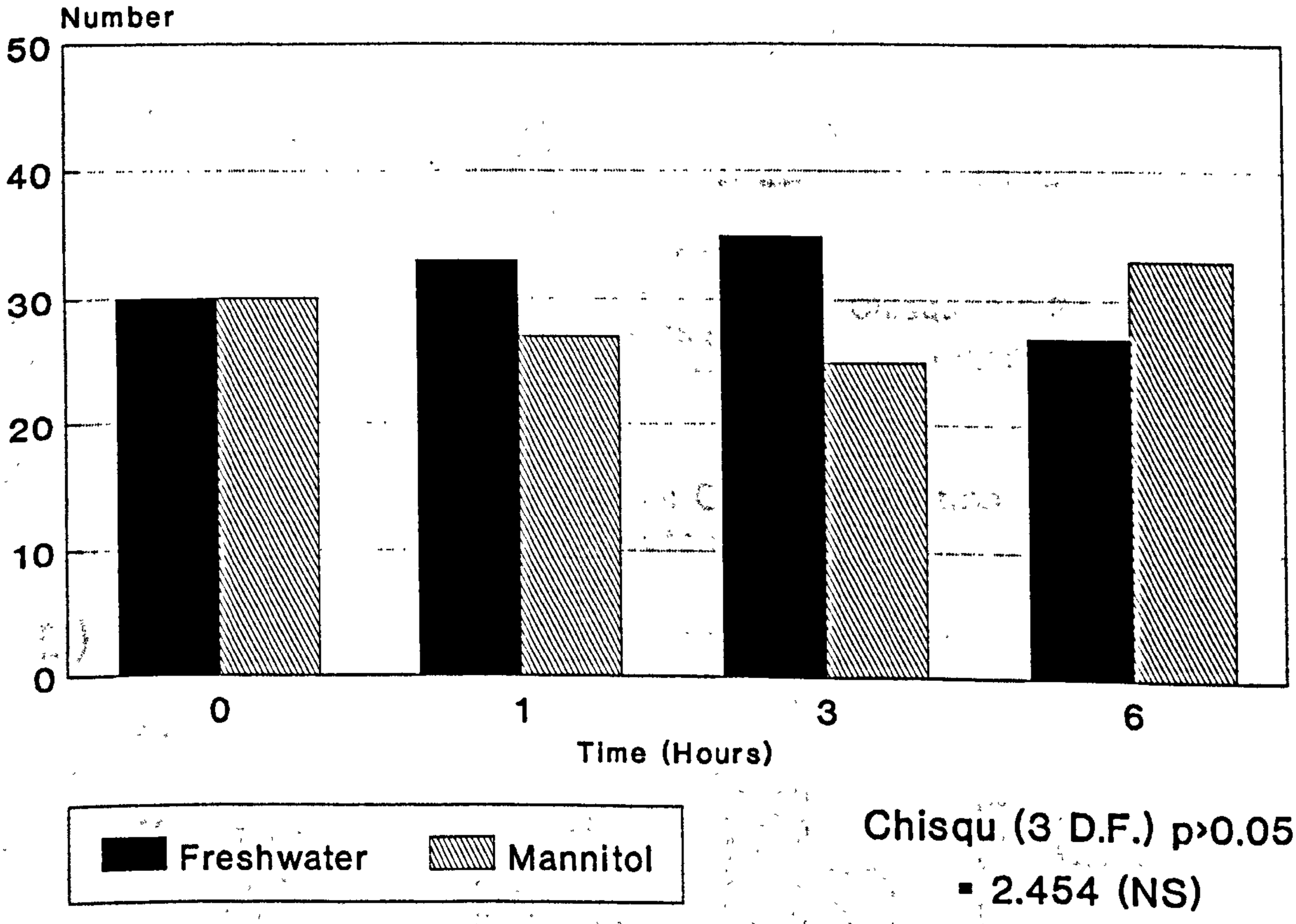
Finally since *Carcinus* appeared to show a preference for a solution containing magnesium ions over freshwater (Fig. 5.6.), experiments were designed

to check whether *Carcinus* was responding to the chloride or to the magnesium in magnesium chloride solution. A solution was prepared using calcium chloride and tested against freshwater (Fig. 5.8.A). The crabs showed a strong preference for the chloride solution. This choice was statistically significant at the 1% (or 5%) level. However when calcium chloride solution was tested against seawater the crabs showed a significant preference for the seawater at the 1% level. (Fig. 5.8.B).

Figs. 5.1. to 5.8.

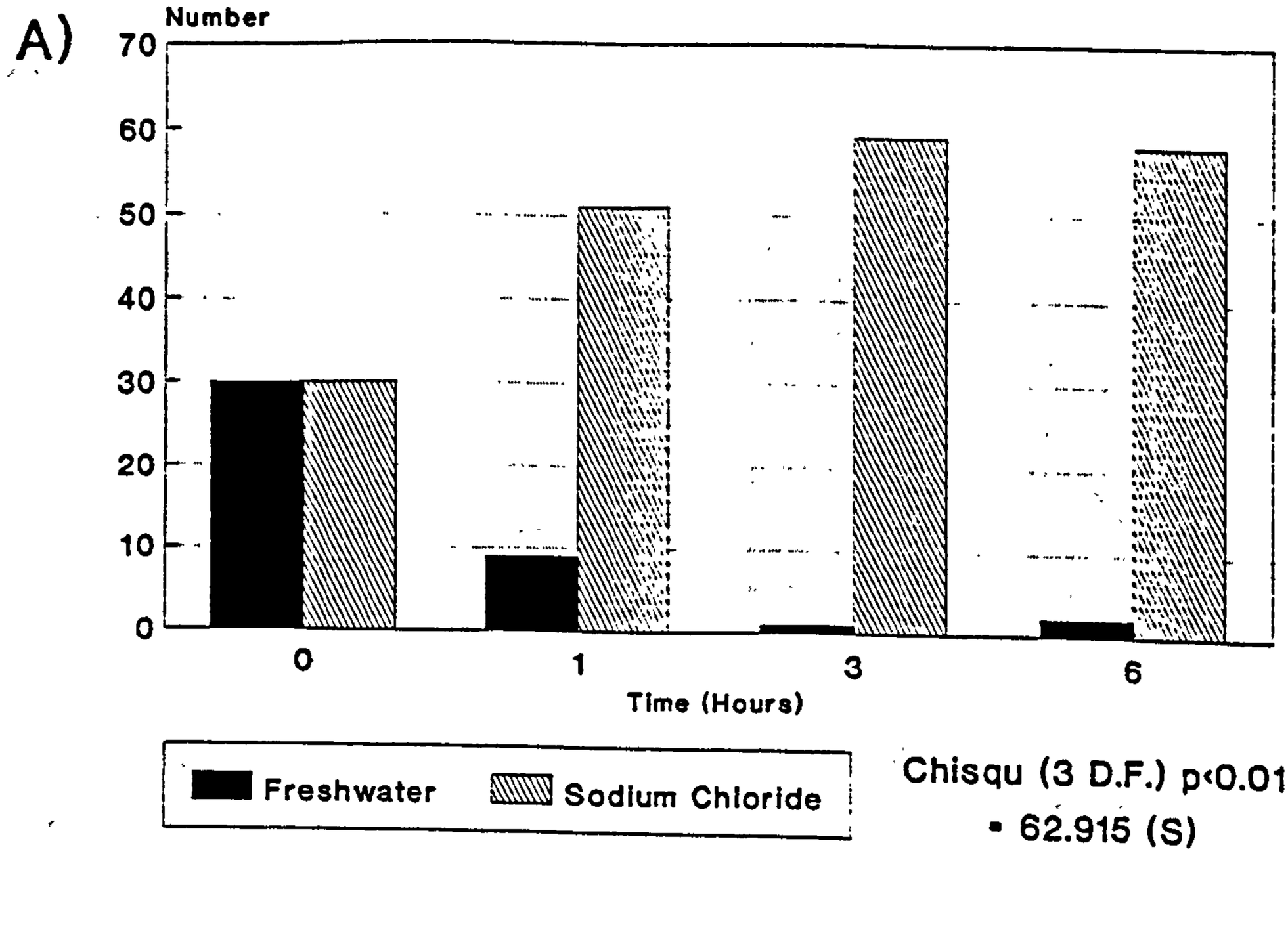
Histograms (totals of 3 repetitions) showing the choice made by 60 crabs between either freshwater or seawater and the solution under test after intervals of 0, 1, 3 and 6 hours. The particular solution under test is specified at the top of each graph.

Fig. 5.1. Freshwater/Mannitol



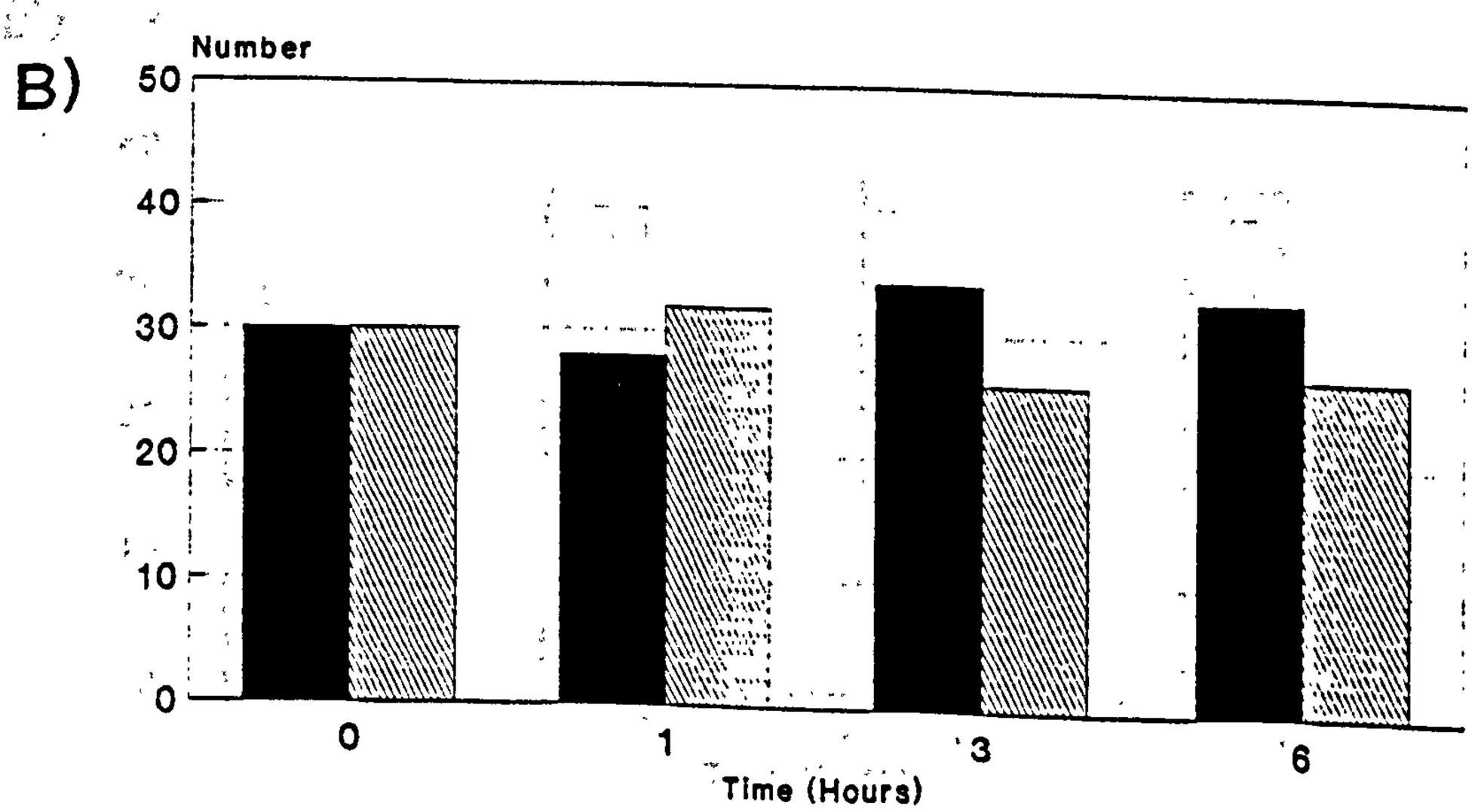
Chisqu (3 D.F.) p>0.05
= 2.454 (NS)

Fig. 5.2. Freshwater/Sodium Chloride solution



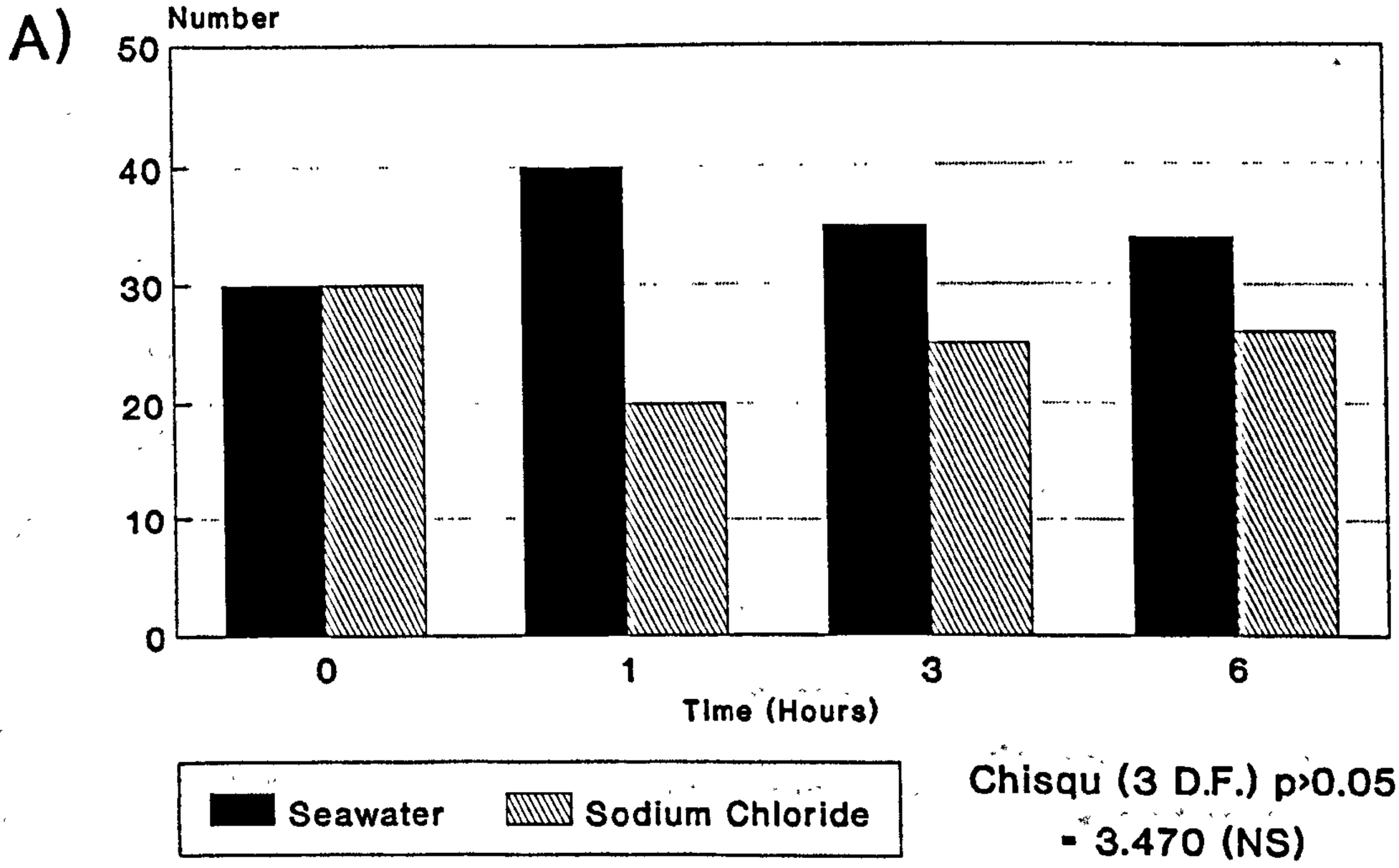
Chisqu (3 D.F.) p<0.01
▪ 62.915 (S)

Seawater/Sodium Chloride solution
(Isotonic)



Chisqu (3 D.F.) p>0.05
▪ 1.519 (NS)

Fig. 5.3. Seawater/Sodium Chloride solution (Iso-osmotic)



Seawater/43ppt. seawater

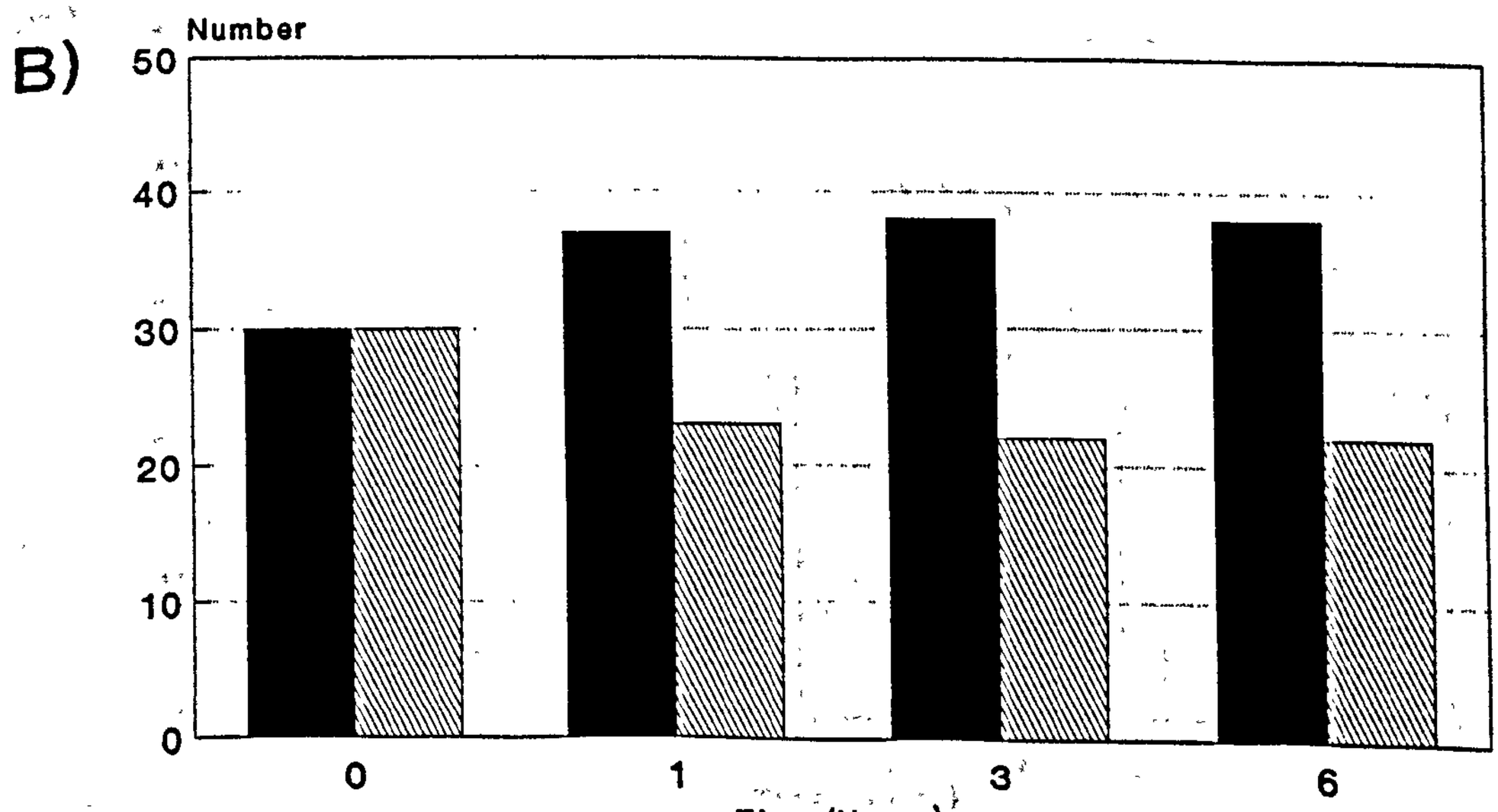
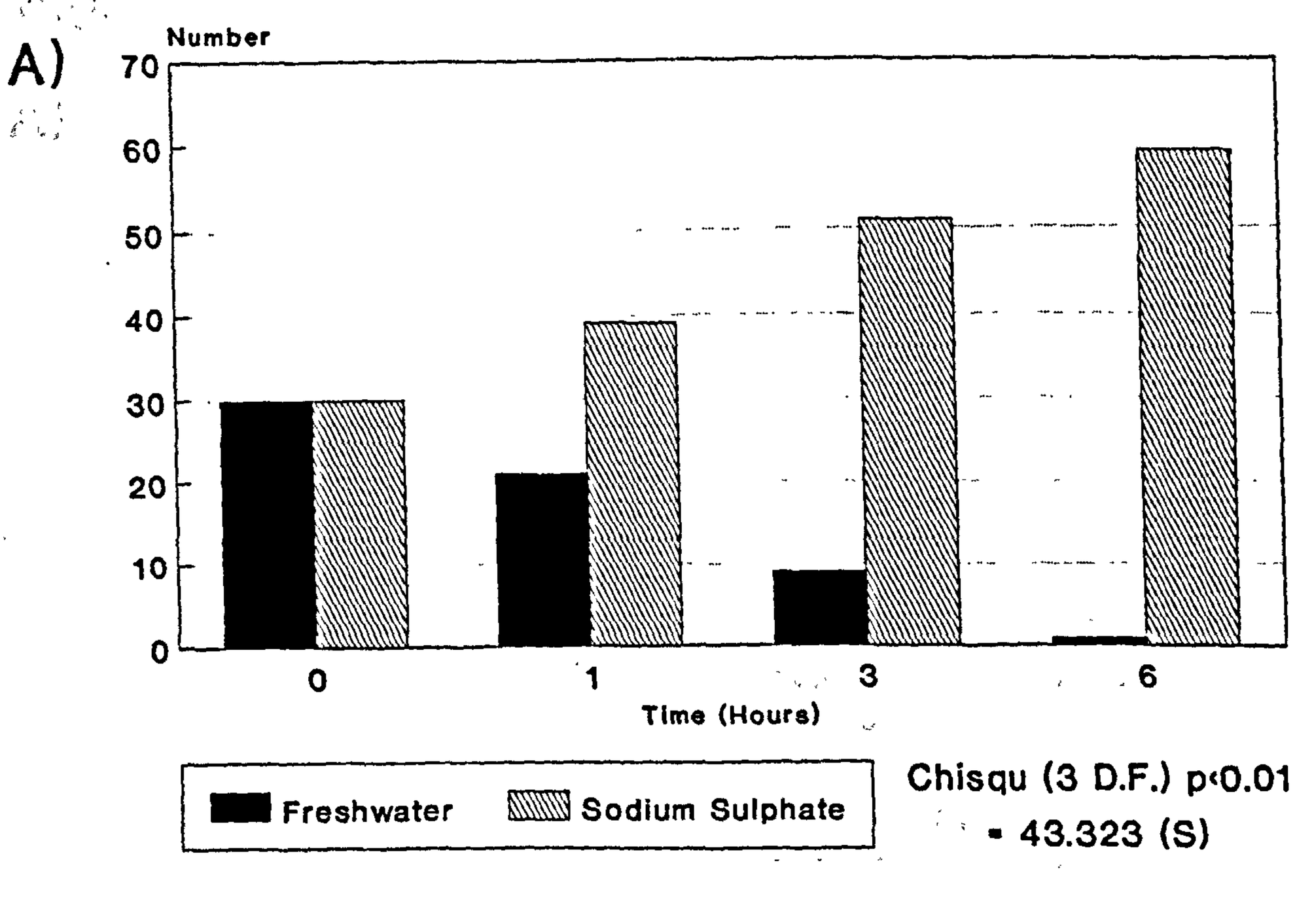


Fig. 5.4. Freshwater/Sodium Sulphate solution



Seawater/Sodium Sulphate solution

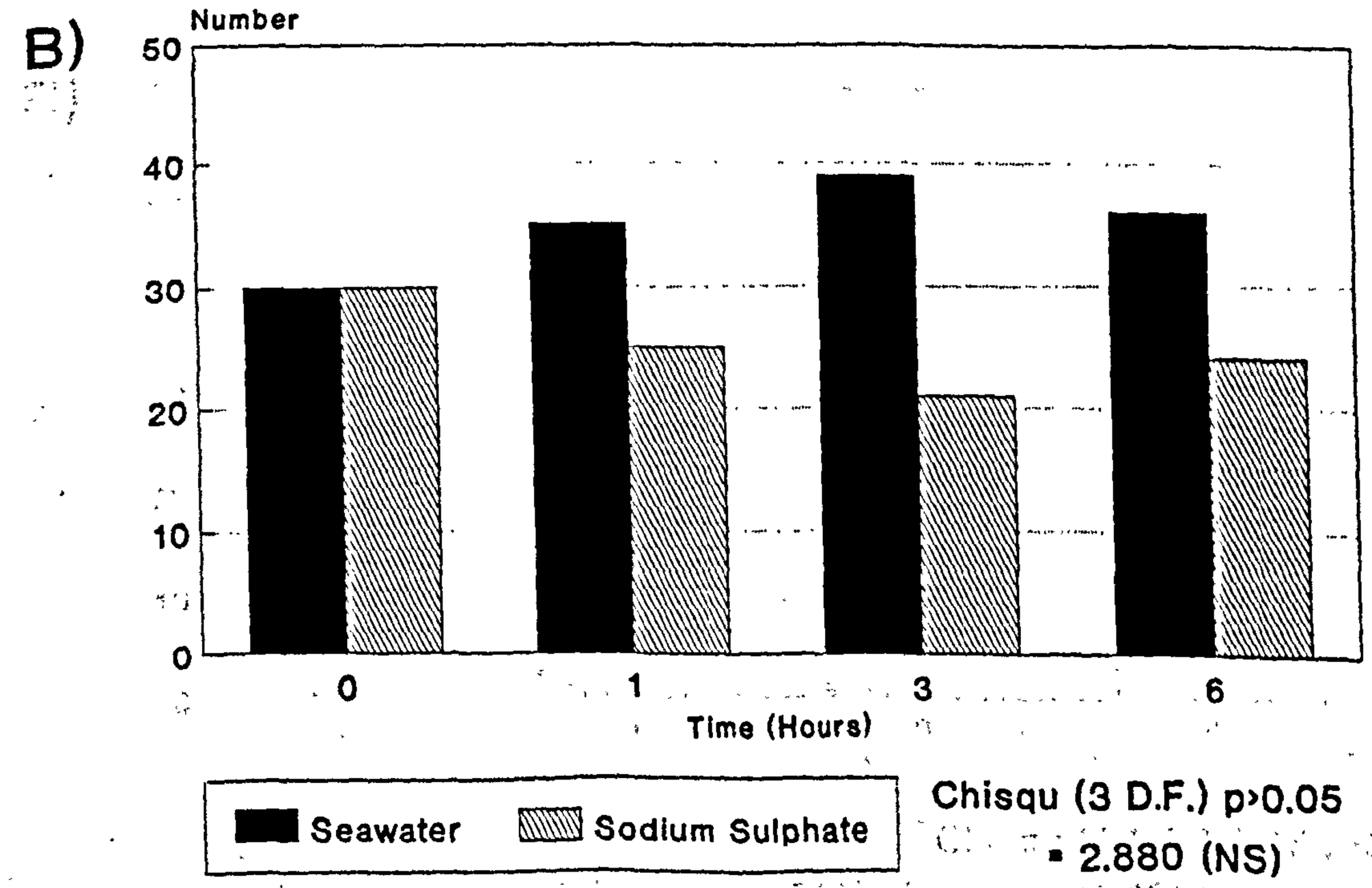
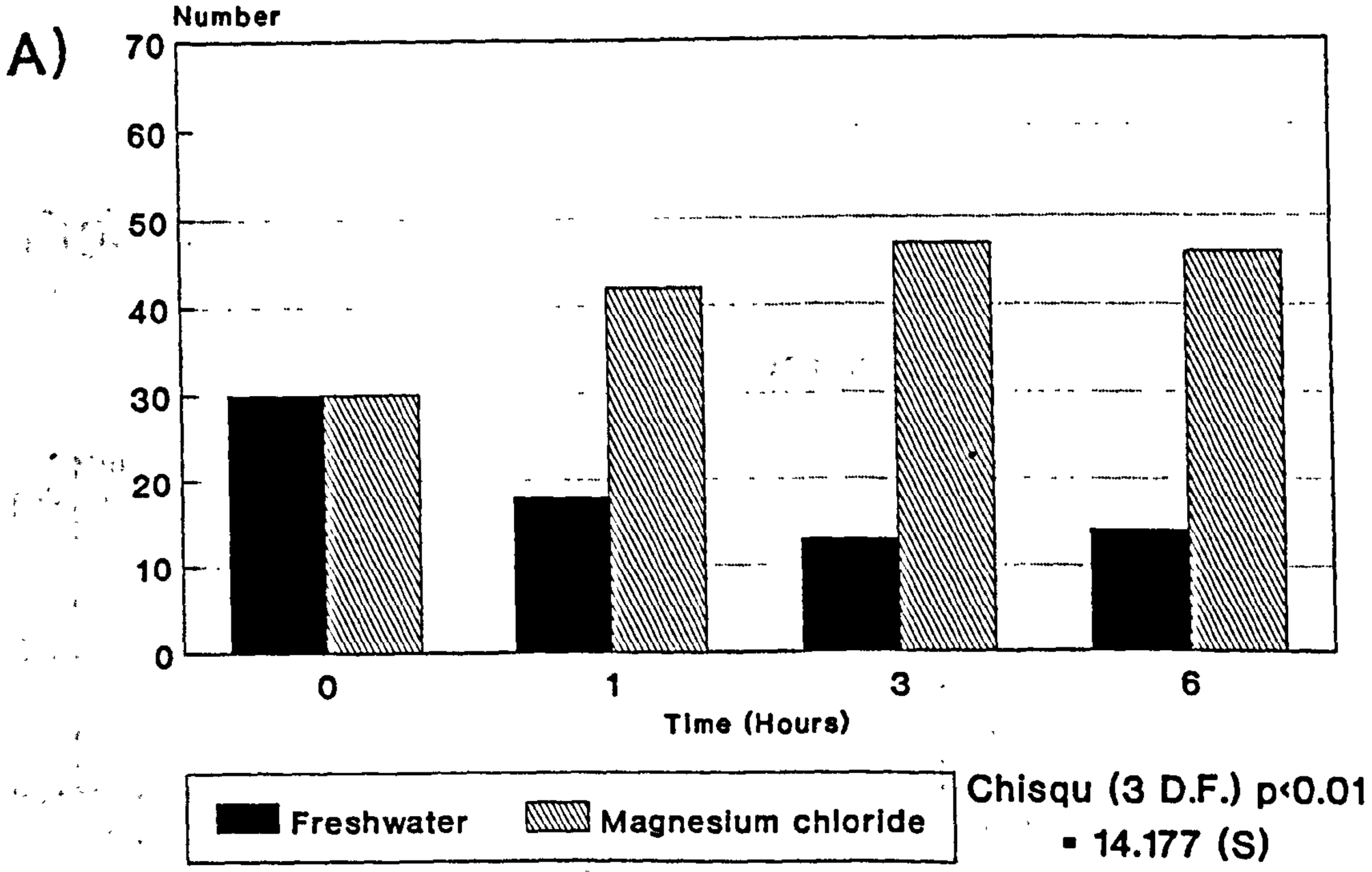


Fig. 5.5. Freshwater/Magnesium Chloride solution



Seawater/Magnesium Chloride solution

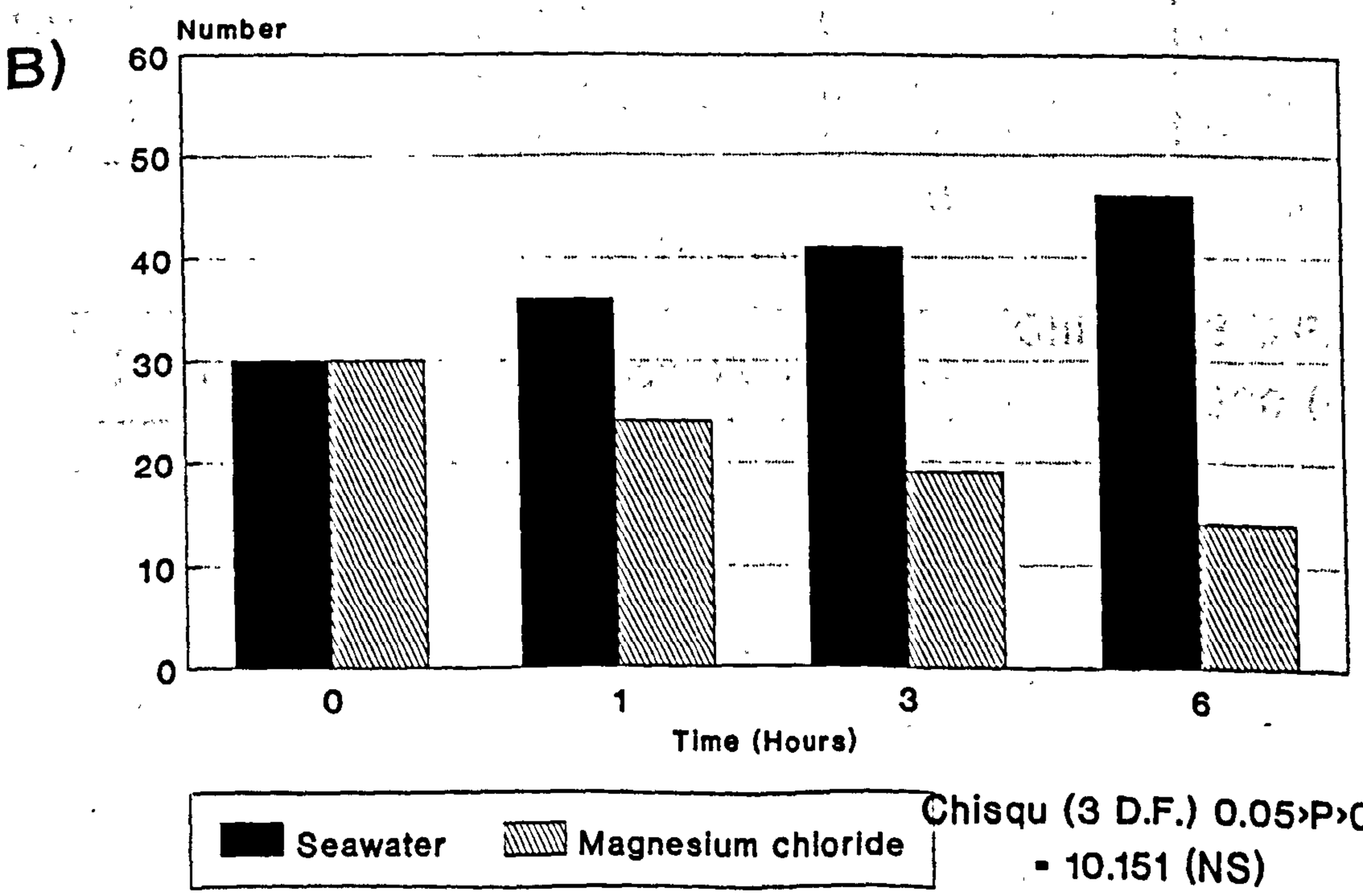
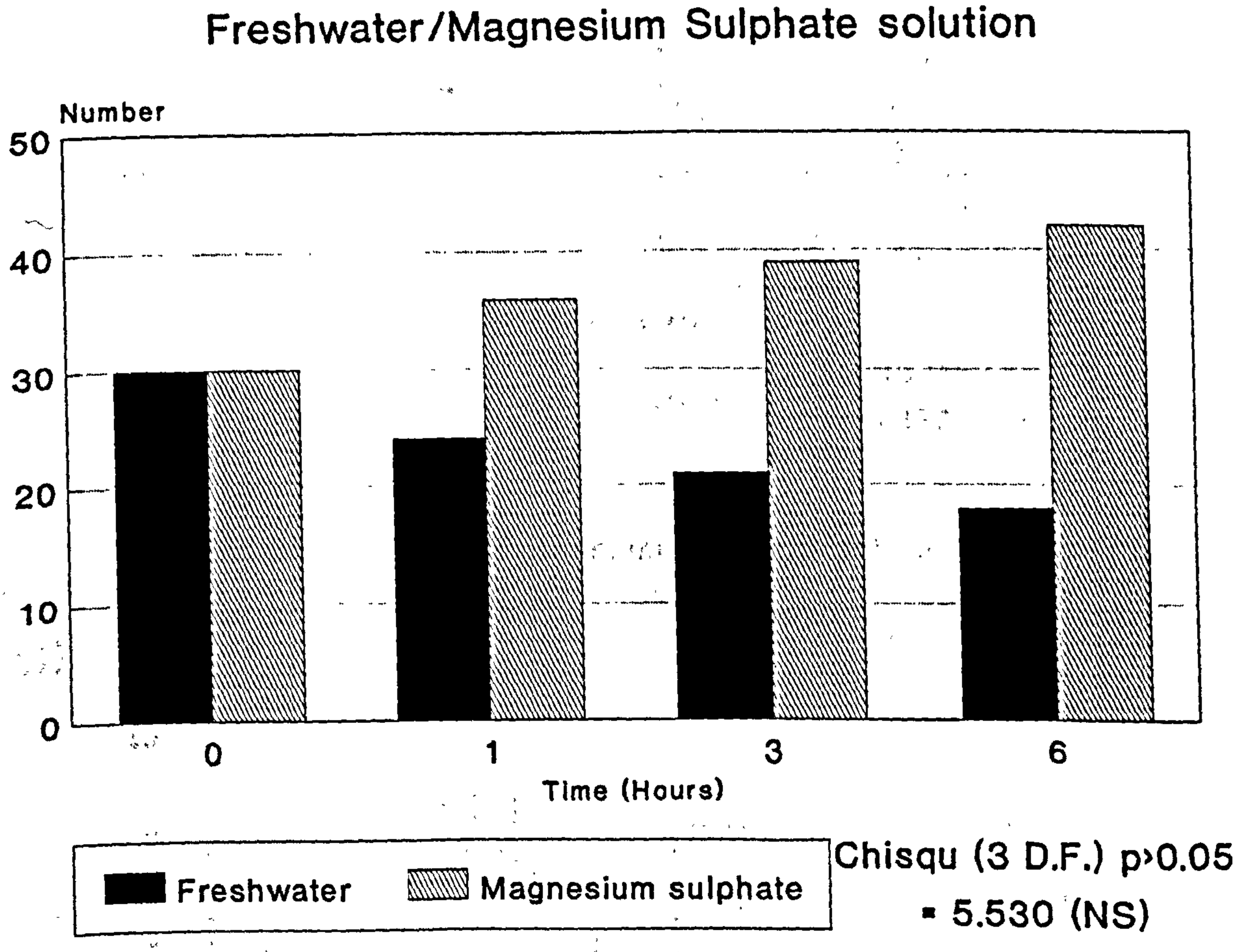
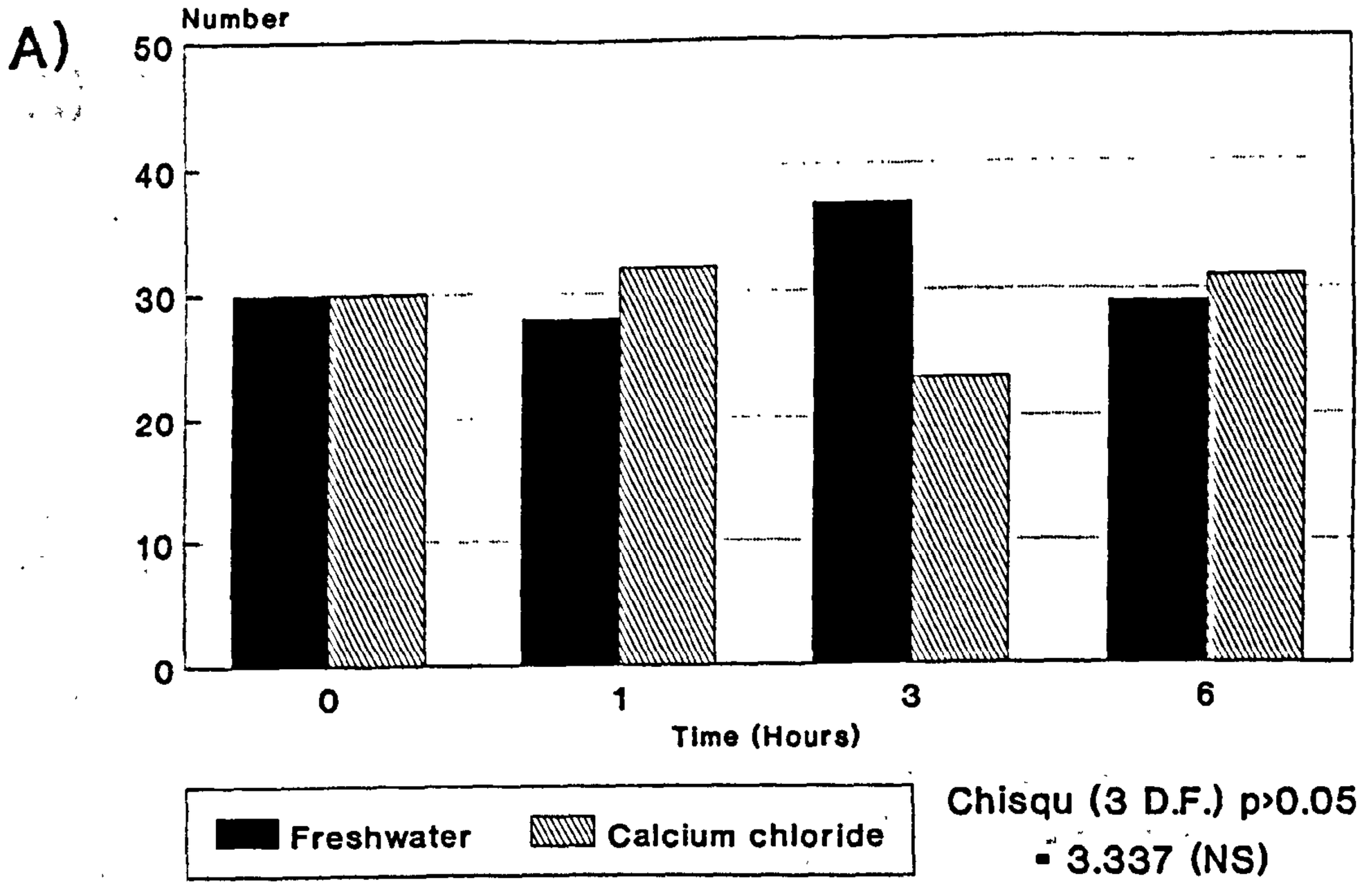


Fig. 5.6.



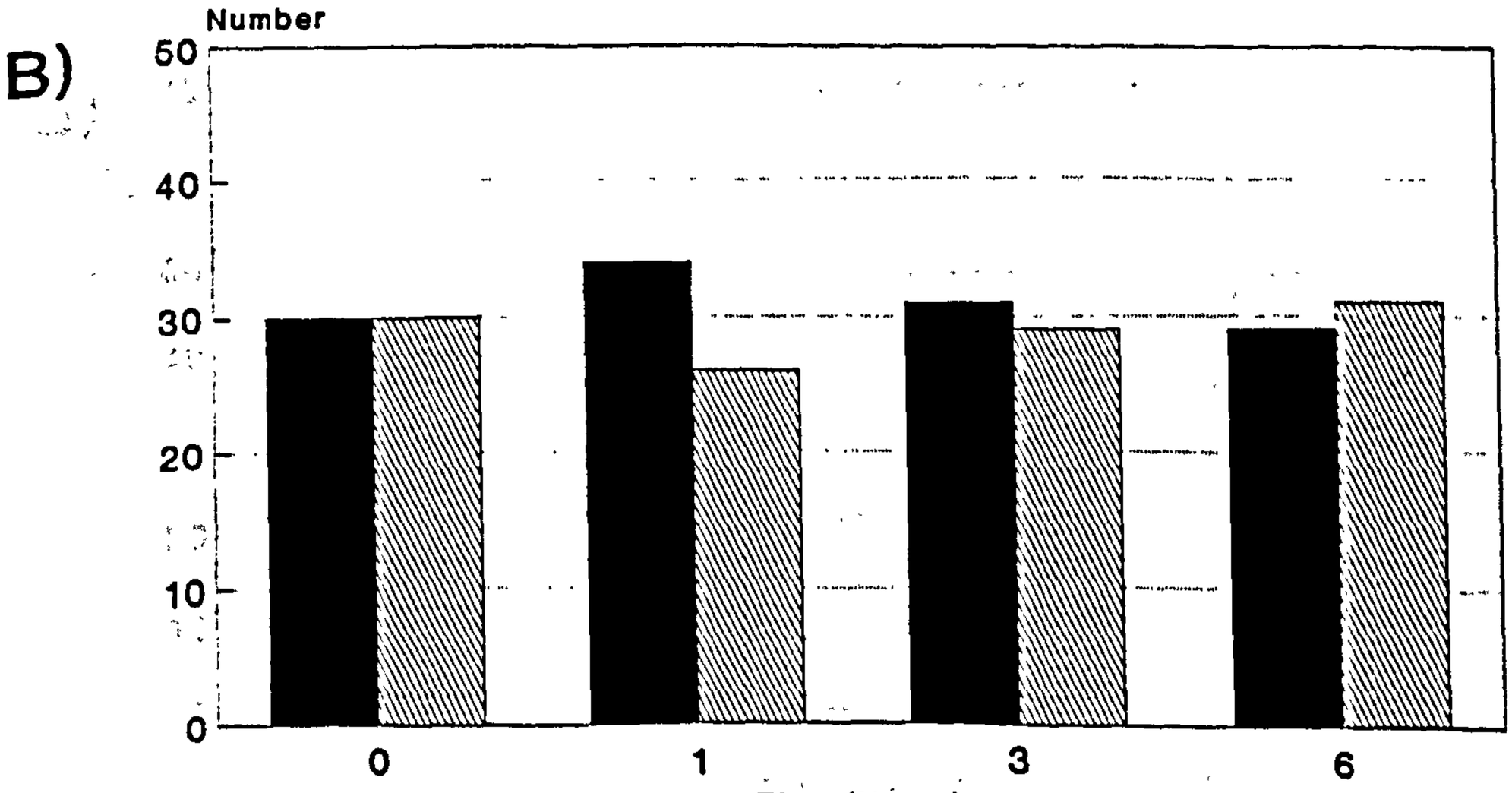
Chisqu (3 D.F.) p>0.05
= 5.530 (NS)

Fig. 5.7. Freshwater/Calcium Chloride solution



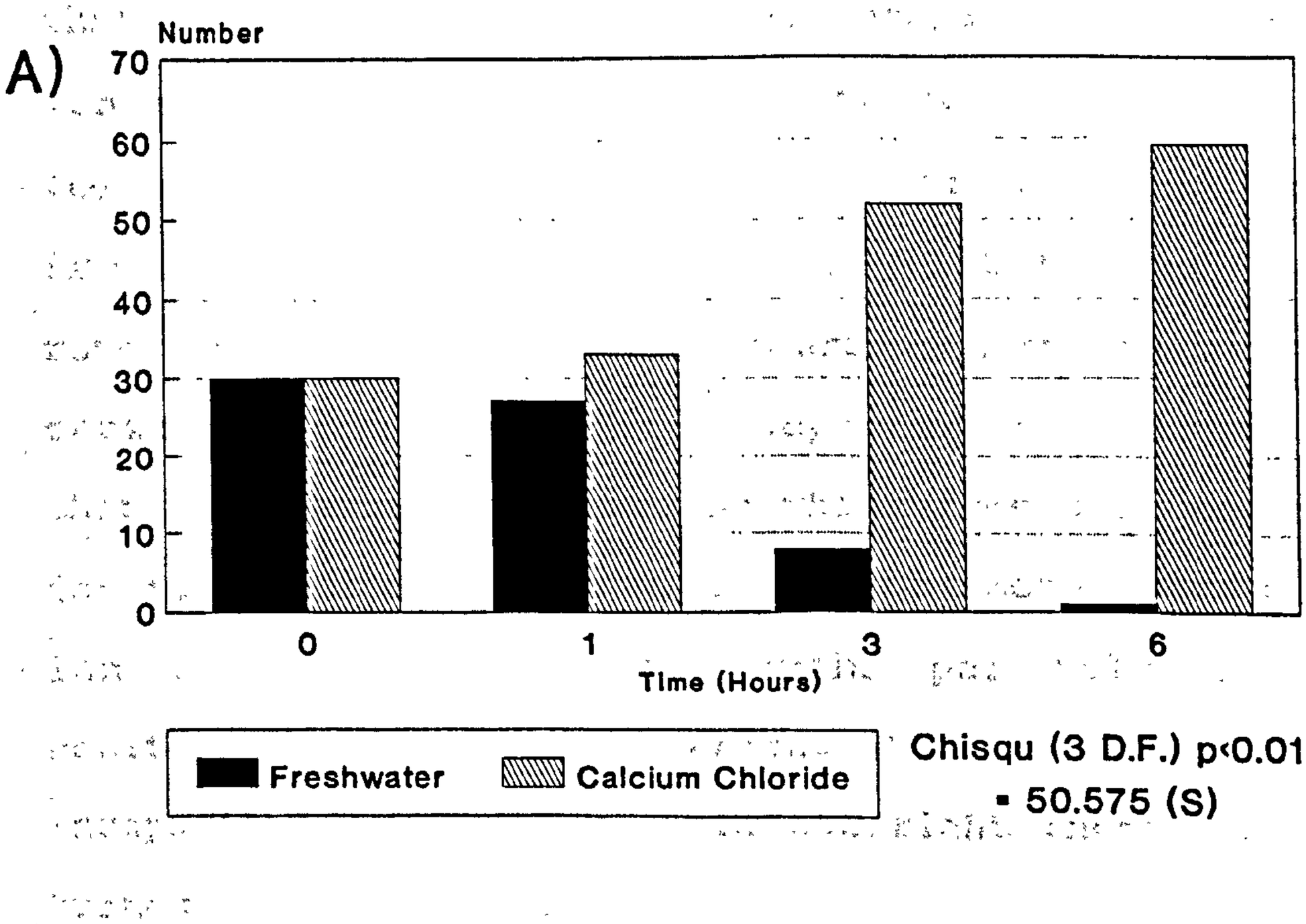
Chisqu (3 D.F.) p>0.05
= 3.337 (NS)

Freshwater/Potassium Chloride solution

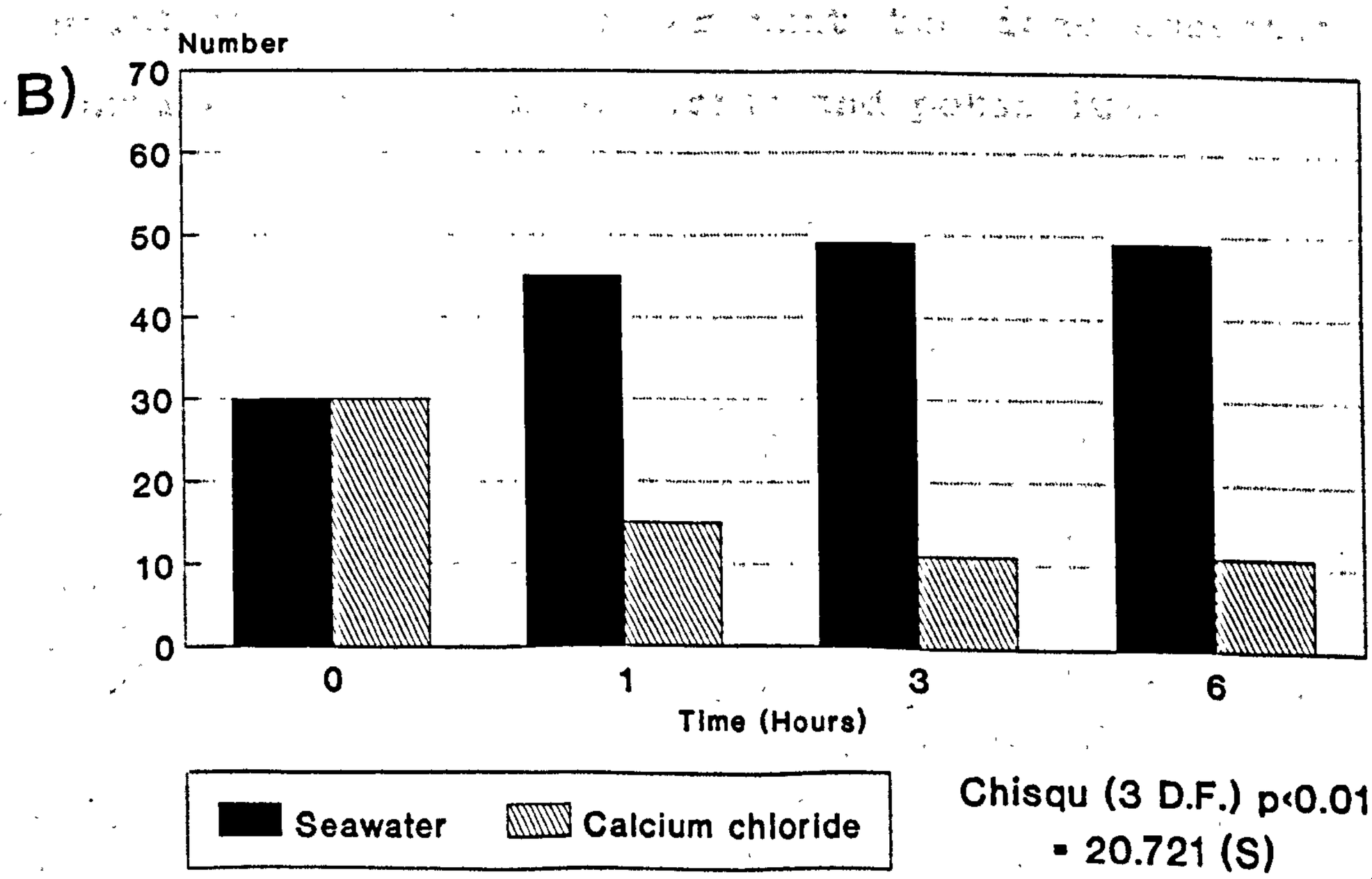


Chisqu (3 D.F.) p>0.05
= 0.718 (NS)

Fig. 5.8. Freshwater/Calcium Chloride solution (Chloride)



Seawater/Calcium Chloride solution (Chloride)



SURVIVAL IN SINGLE ION SOLUTIONS

After periods longer than 6 hours it was found that *Carcinus* started to become incapacitated in single ion solutions. However this incapacitation did not follow the normal time course for death rates in freshwater (see chapter 1, Fig.1.2.A) and it was reversible after a number of hours upon return to normal seawater. Various ion - depleted solutions were therefore tested to determine which ions were essential for survival. Solutions were made up isotonic with that ion in seawater and ten crabs per solution were monitored over a 2 day period. Crabs were considered incapacitated when they could not right themselves when turned on their backs.

Table 5.1. shows survival in various ionic solutions and it is clear that the ions essential for survival are sodium, chloride and potassium.

TABLE 5.1.

Cumulative number of deaths of *Carcinus* in ionic solutions to determine which ions are essential for survival (N=10)

Time (Hrs.)	NaCl MgCl2 CaCl2 KCl	NaCl (Isosmotic)	NaCl (Isotonic)	MgCl2	NaCl + MgCl2	NaCl + CaCl2	NaCl + KCl	NaSO4 + KSO4	KCl
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
6	0	2	1	0	3	0	0	0	0
9	0	7	9	0	0	0	0	3	2
12	0	10	10	5	8	6	0	5	6
24	0	-	-	10	10	10	0	10	10
36	0	-	-	-	-	-	0	-	-
48	0	-	-	-	-	-	0	-	-

DETECTION OF SODIUM CHLORIDE CONCENTRATIONS

The ability of *Carcinus* to detect actual concentrations of sodium and chloride ions was tested using varying strengths of sodium chloride solution (isotonic for sodium at a given salinity). These solutions were also made isotonic for chloride by the addition of potassium chloride.

Figure 5.9.A illustrates that *Carcinus* are able to detect different strengths of sodium chloride solution. Most of the crabs move to the most concentrated solutions after 5-6 hours.

THRESHOLDS OF SALINITY DETECTION

Multiple choice chamber experiments (Chapter 3 Figs. 2.2. - 2.3.) showed that *Carcinus* can detect a difference as low as 6ppt. between salinities. The choice of different seawater strengths became apparent after 4-5 hours and was well marked after 12 hours in salinity ranges below 22ppt. In seawater strengths above 22ppt. the choice between differing salinities took longer to become significant (see Chapter 3. Table 3.3.). However, it is unclear whether *Carcinus* is unable to detect small changes in salinity in the range above 22ppt. as efficiently as below that value, or whether these salinities are not as osmotically stressful.

Salinity detection threshold levels were therefore tested using salinities below 22ppt. Figure 5.9.B illustrates that *Carcinus* are able to detect a

difference in salinity of 4ppt. and a significant choice for the highest salinity is apparent after 12 hours.

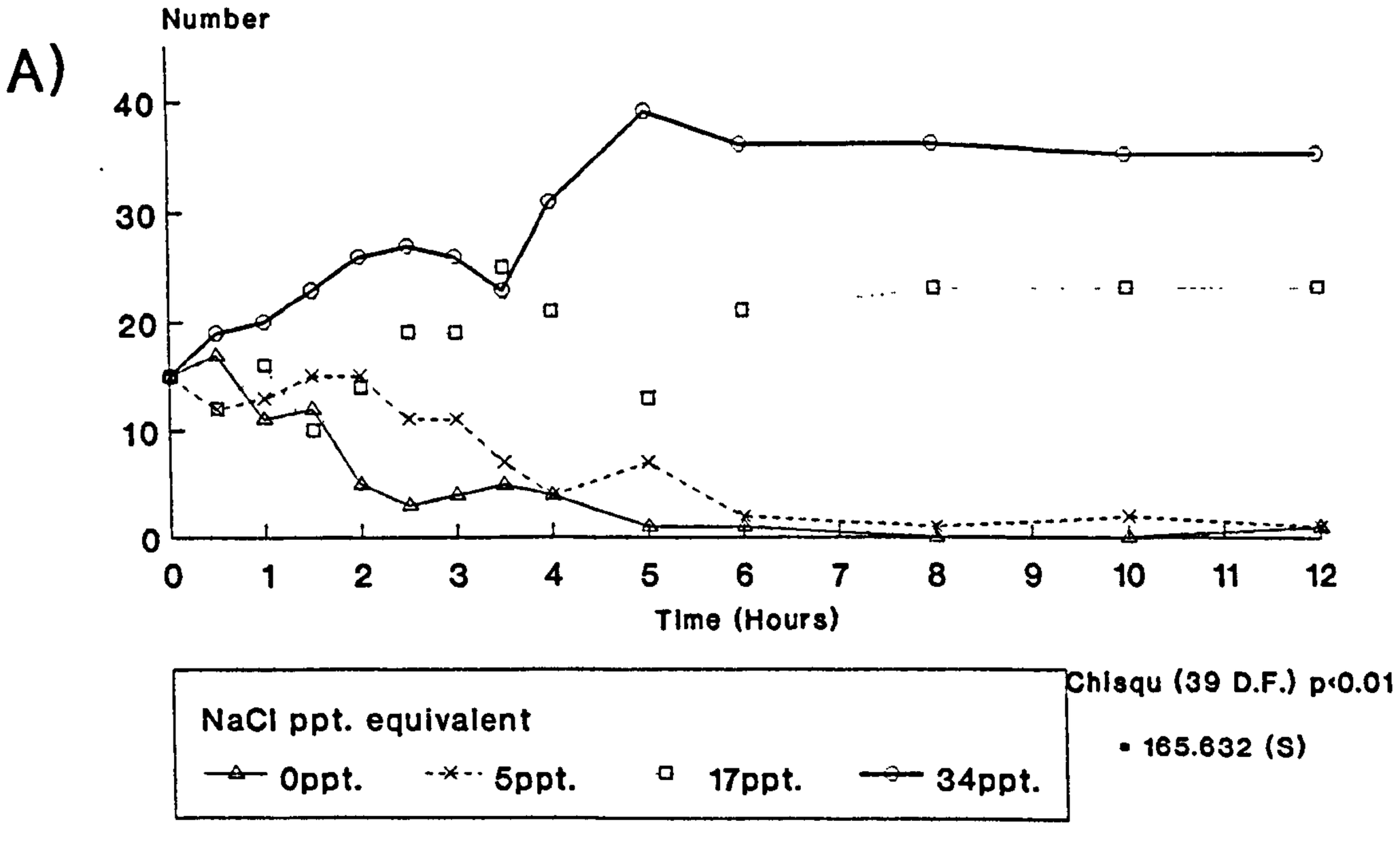
Fig. 5.10. A and B demonstrates the detection abilities of *Carcinus* when salinity choices of 2ppt. difference are offered. Salinities separated by as little as 1ppt. (Fig. 5.11.A and B) are also detected, migration from the lowest salinity towards the highest salinity occurring in both sets tested. Moreover a similar pattern occurs when a choice of salinities separated by 0.5ppt. difference is offered (Fig. 5.12.A,B); the crabs exhibit a significant choice towards the highest salinity. A choice for salinities separated by a difference smaller than 0.5ppt. was not tested due to the difficulty in maintaining these salinities at a stable level for any length of time.

Figs. 5.9. to 5.12.

Graphs (totals of 3 repetitions, each with 5 crabs at each of the 4 initial salinities)) to show the number of crabs making a choice for a particular salinity when the choice of salinities offered are separated by various increments; these are given on each graph.

Fig. 5.9.

Choice of ionic strengths
Sodium Chloride (ppt. equivalent)



Choice of seawater strengths
5ppt.-17ppt. (4ppt. difference)

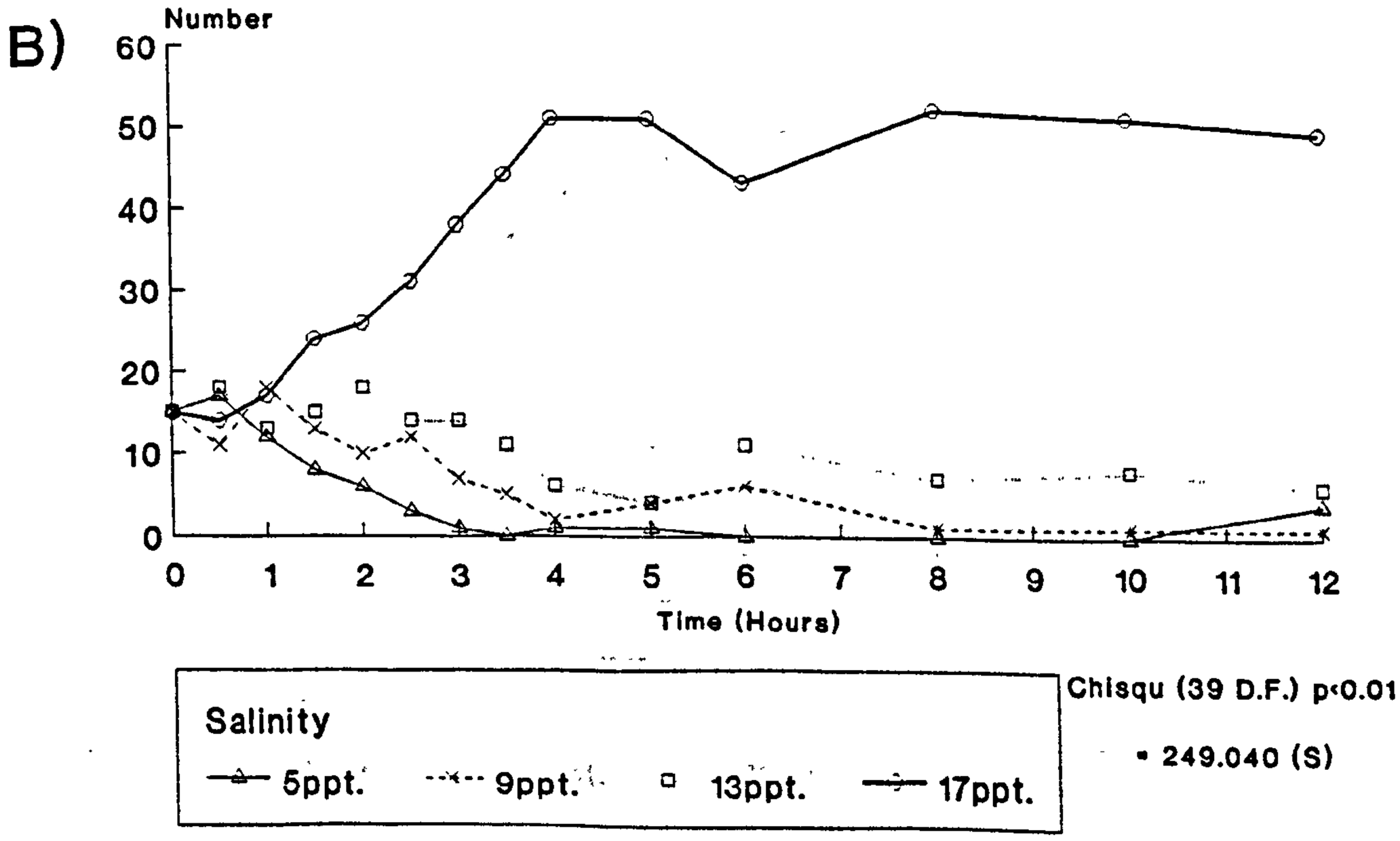
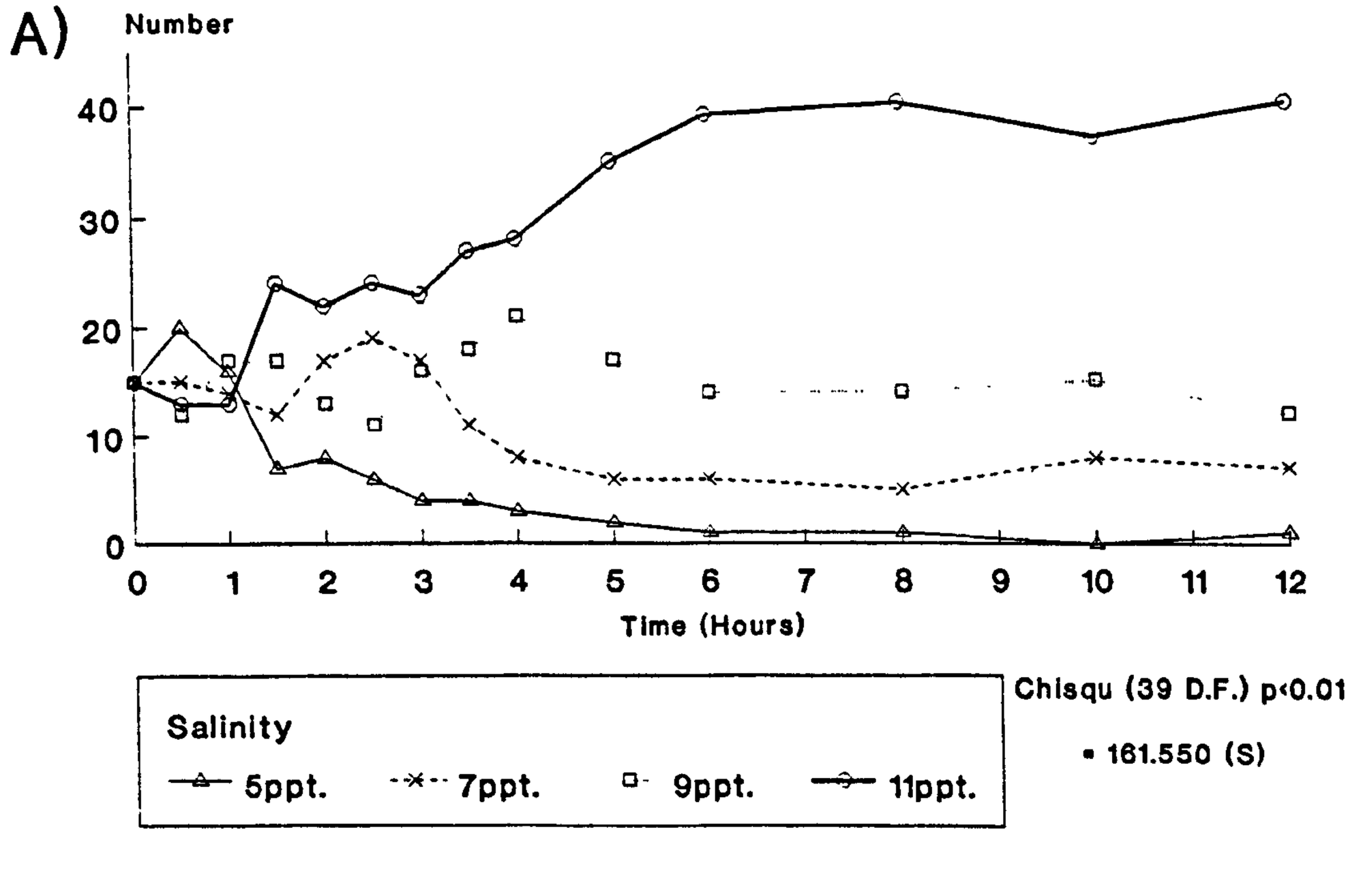


Fig. 5.10. Choice of seawater strengths
5ppt.-11ppt. (2ppt. difference)



Choice of seawater strengths
16ppt.-22ppt. (2ppt. difference)

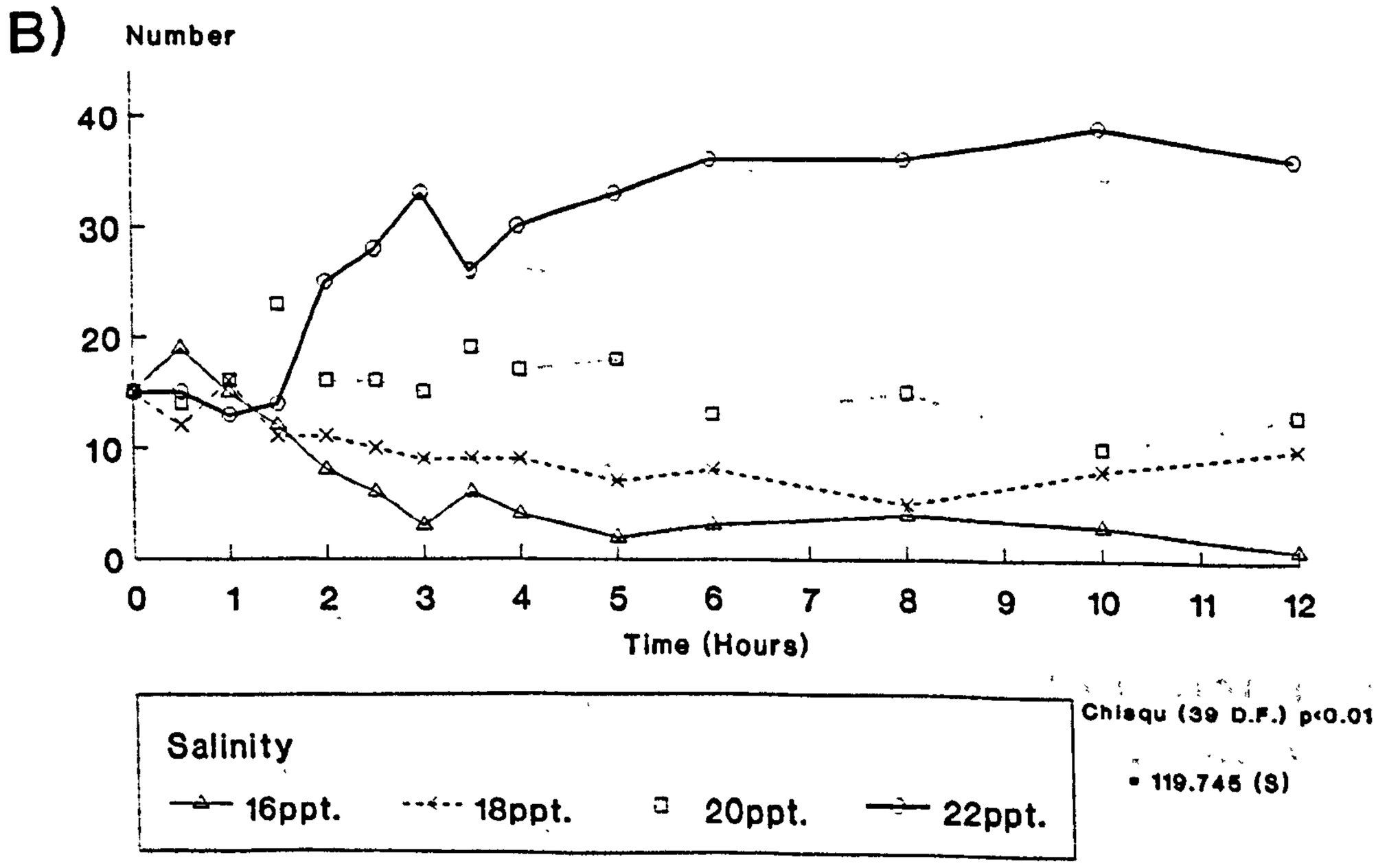
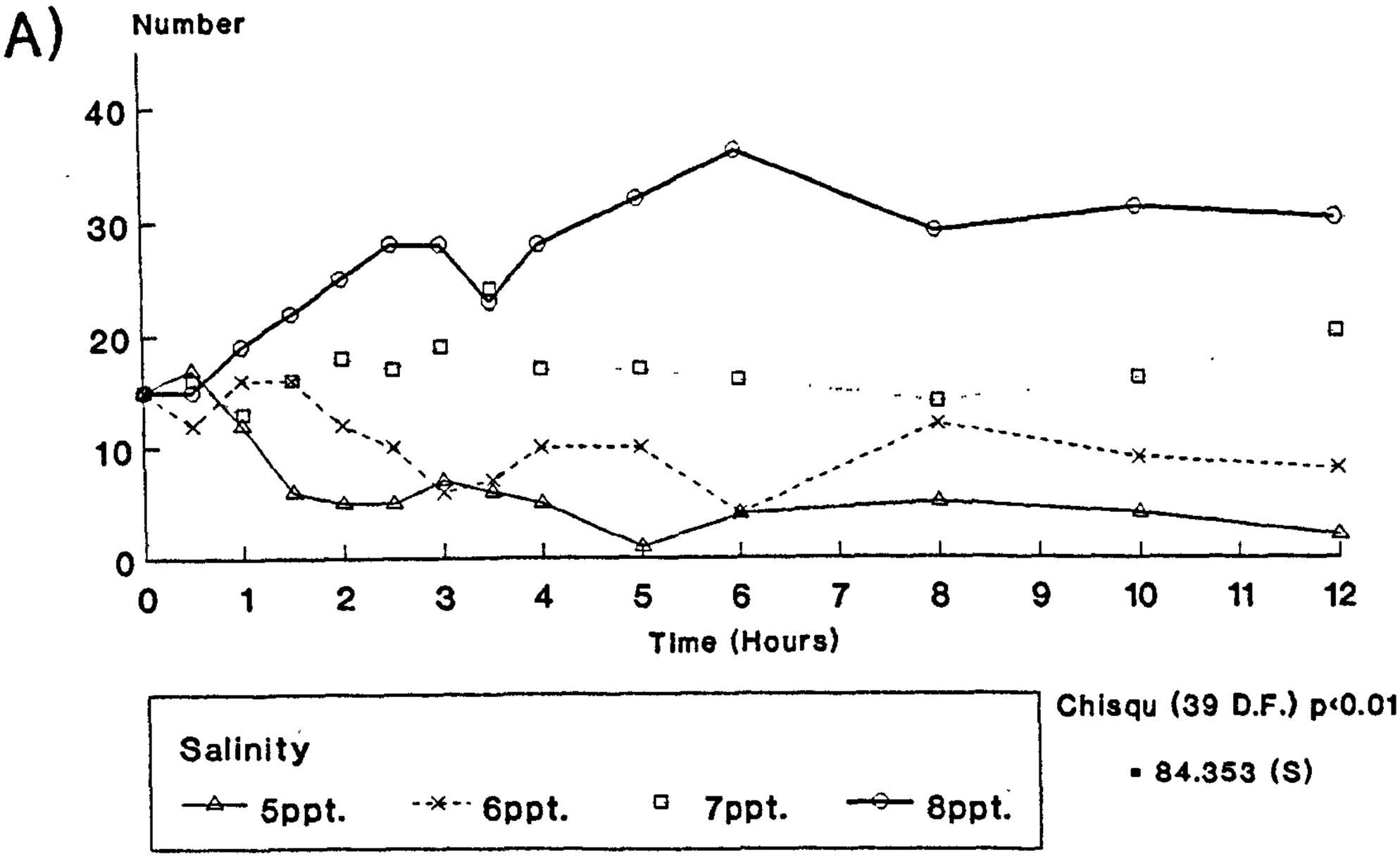


Fig. 5.11. Choice of seawater strengths
5ppt.-8ppt. (1ppt. difference)



Choice of seawater strengths
19ppt.-22ppt. (1ppt. difference)

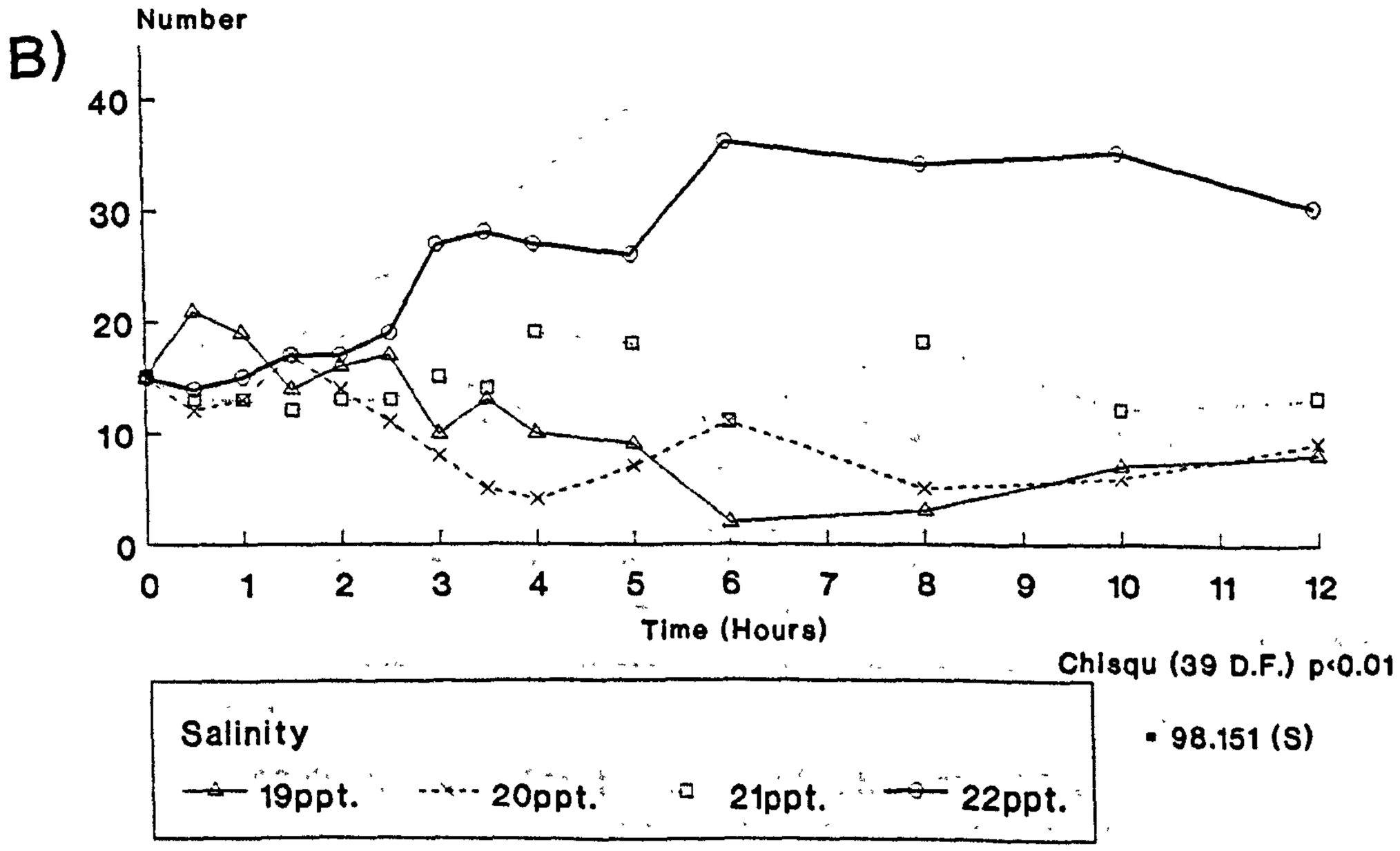
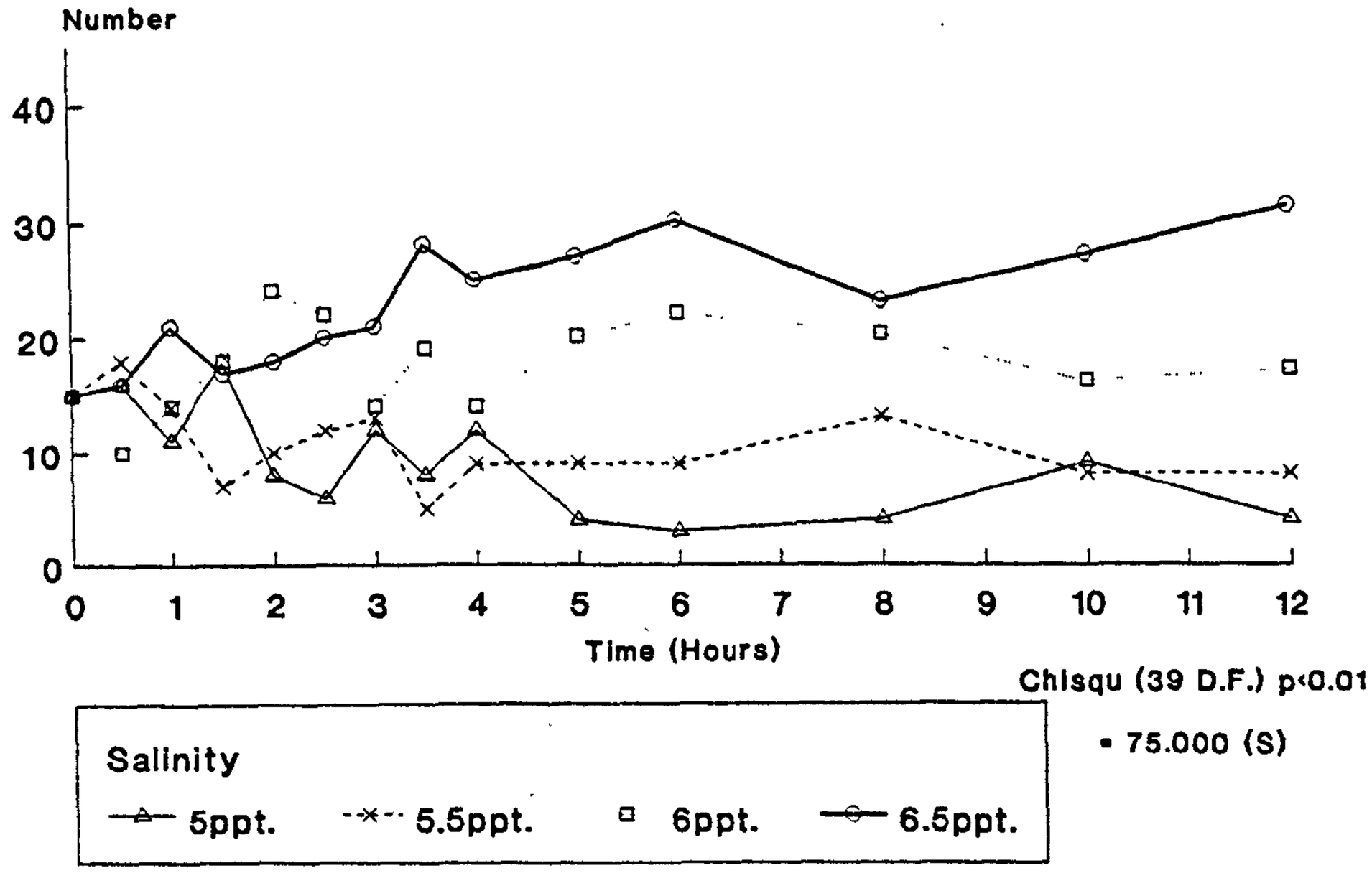
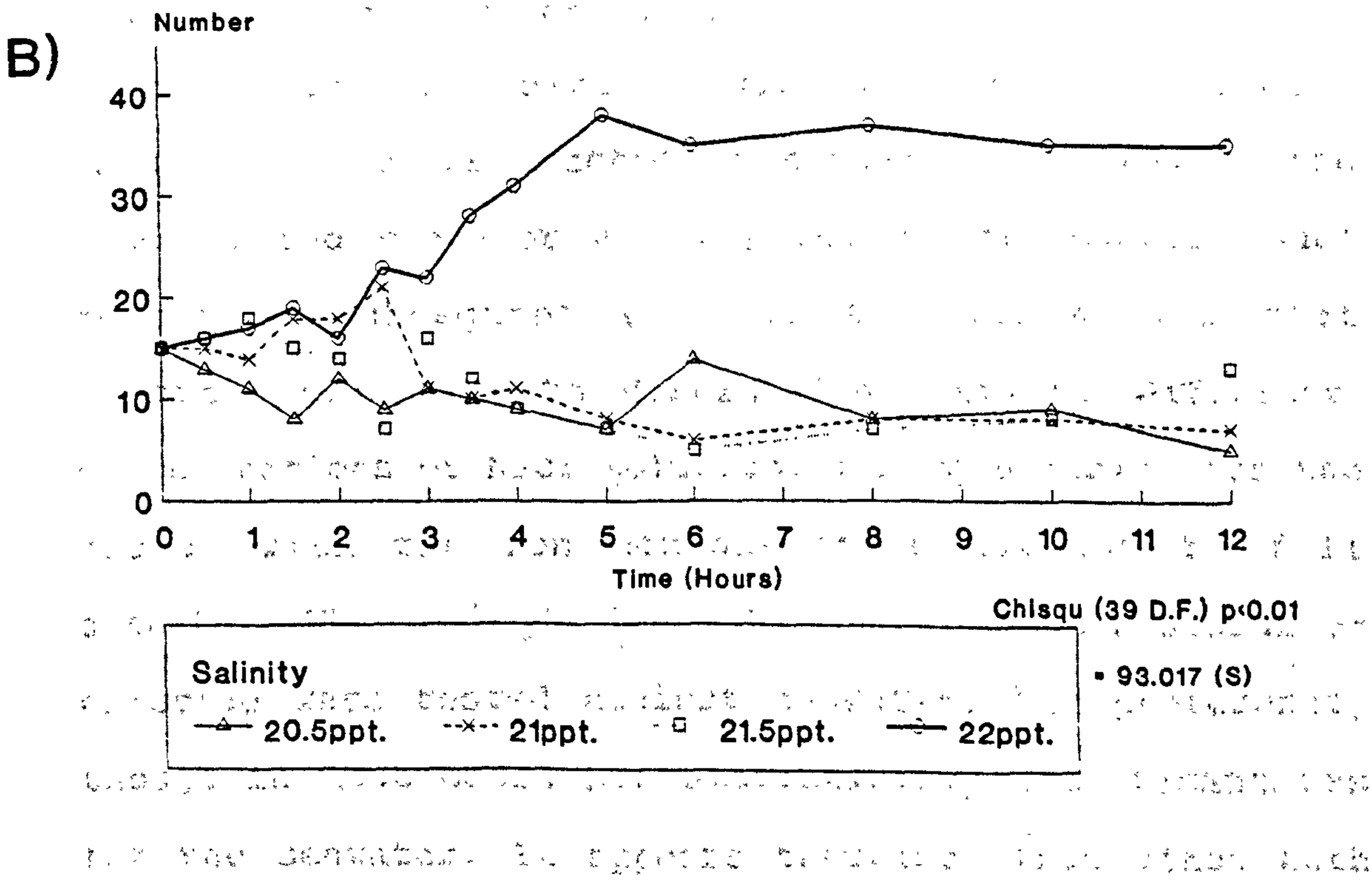


Fig. 5.12. Choice of seawater strengths
5ppt.-6.5ppt. (0.5ppt. difference)



Choice of seawater strengths
20.5ppt.-22ppt. (0.5ppt. difference)



DISCUSSION

The results of the present investigation indicate that *Carcinus maenas* detects changes in salinity by responding to the concentration of specific ions rather than the total osmolality of the medium. The critical ions were found to be Na^+, Cl^- and possibly Mg^{2+}, and the crabs were not capable of detecting either Ca^{2+} or K^+ in concentrations similar to those which occurred in seawater. Analysis of the results suggest that Na^+ may be the most important detectable ion because when offered a choice between iso-osmotic NaCl solution (which is isotonic for Cl^- (Akberali and Davenport, 1982)) and seawater, there was a slight, but statistically non-significant preference for seawater. The amount of sodium in an iso-osmotic solution is equivalent to that contained in 43ppt. seawater and, when offered a choice between seawater and 43ppt. seawater the crabs reacted in a similar fashion to the concentrated seawater as they did to iso-osmotic NaCl solution. Subsequent experiments also showed that *Carcinus* was able to distinguish between different concentrations of NaCl solution, making a choice for the medium with the ion concentration closest to full seawater. When single ion solutions of either sodium or chloride were tested against seawater, the preference, though in most cases not statistically significant was for the seawater. It appears therefore that since both

ions are detected, a solution containing sodium and chloride is preferable to a single ion solution.

Hume and Berlind (1976) observed acceleration of the heart-rate of *Carcinus* upon lowering of the salinity, and accordingly sought to determine the critical aspect of salinity decline to which the crabs responded. However receptors mediating cardioacceleration did not respond to changes in osmotic pressure and when concentrations of either sodium or chloride were decreased individually, whilst keeping the concentration of the other ion at normal levels, no cardioacceleration was observed. On the basis of present experiments this might be explained by the fact that both ions are detected in parallel and consequently both need to be lowered simultaneously to initiate cardioacceleration. The ions in seawater which *Carcinus* responds to differ from those reported for other marine organisms so far studied. *Gammarus pulex* responds to Ca^{2+} (Vincent, 1973), *Mytilus edulis* detects Na^{+} and Mg^{2+} (Davenport, 1981) and *Scrobicularia plana* reacts to Na^{+}, Mg^{2+} and Ca^{2+} ions (Akberali and Davenport, 1982). The basis for the difference in ion specificity is still a matter of speculation as no clear evolutionary or phylogenetic pattern is yet apparent (Rankin and Davenport, 1981, Davenport and Beard, 1988).

Schmidt (1989) found that chemoreceptors responsible for salinity detection in *Carcinus maenas*

were the hair peg organs located on the dactyls of each walking leg. In many other crustaceans, receptors are situated on the antennae or antennules (see Van Weel and Correa, 1967). The advantages of having the salinity receptors situated on peripheral structures are that the animal can test the medium without having to fully immerse itself (Davenport and Wankowski, 1973). However during present experiments this type of behaviour was rare, and *Carcinus* often entered lower salinity chambers, often even containing freshwater, and remained within these for up to three hours. This perhaps suggests that changes in an internal physiological parameter determine the avoidance behaviour, but the osmoregulatory physiological changes may occur independently, before crabs exhibit behavioural responses.

It was observed after a number of hours in ion-depleted solutions that crabs started to become incapacitated, even though pH and temperature remained constant and isotonic solutions were used. The essential ions were Na^+, Cl^- and K^+, whereas Ca^{2+} and Mg^{2+} were unimportant, at least for short term survival. In vertebrate tissues Na, Cl and K are known to be essential intracellular osmotic constituents and their role in volume maintenance and regulation has been extensively studied (Kregenow, 1971, Roti-Roti and Rothstein, 1973, Hendil and Hoffman, 1974). Na and K are

essential for the sodium pump, which is the enzyme Na-K ATP-ase (Rankin and Davenport, 1981). The sodium pump generates potential differences which are the driving force for active Na transport (Siebers *et al*, 1985, 1986). Chloride transport is coupled to Na/K uptake (Frizzell *et al*, 1975), but Na and Cl can be taken up separately (Zanders, 1980, Siebers *et al*, 1983). The uptake and regulation of Na and Cl are not only dependent on the concentration of each of these in the blood, but also the electrochemical balance between the two (Zanders, 1981). Na and Cl therefore need to be in balance and K is required for functioning of sodium pump. Without these, the internal fluid balance will be altered and membrane transport systems will fail, possibly also affecting part of a wider mechanism connected with acid-base balance (Zanders, 1981, Truchot, 1981a, 1988). Winkler (1986) states that Mg is critical for ATP-ase activity, but Lockwood and Riegel (1969) showed that *Carcinus* was able to survive for at least 96 hours in magnesium free seawater without ill effects. However other species such as *Pachygrapsus* were unable to tolerate such conditions for more than a few hours (Gross and Marshall, 1960).

Calcium plays an important role in ion and water transport across the gills of aquatic organisms (Ando, 1980, Winkler, 1986). Yet *Carcinus* can survive in calcium free or low calcium seawater for considerable

periods (Robertson, 1937, Greenaway, 1976). In calcium-free water calcium is lost from the body fluids and internal organs, causing the external concentration of calcium in a limited volume of external seawater to rise. Eventually an equilibrium between the seawater and the body fluids is achieved under such experimental conditions (Robertson, 1937). It is unlikely that depletion of specific ions will occur in the wild, but such experimental observations need to be borne in mind when considering the wide range of habitats that may be occupied by organisms such as *Carcinus*.

In the present study *Carcinus* was able to discriminate between salinities separated by a difference as small as 0.5ppt. within the salinity range 5ppt. to 22ppt., in contrast to observations by Thomas *et al* (1981) that though crabs were able to distinguish between salinities separated by a 25% seawater difference, they were unable to detect 10% seawater (3.5ppt.) differences. However the crabs in the earlier experiments were monitored for only 2 hours, and over a 4 to 5 hour time period *Carcinus* was certainly capable of detecting salinities separated by a 4ppt. difference (Ameyaw-Akumfi and Naylor, 1987). Salinities separated by smaller differences were not tested (Naylor, pers. comm.). The detection abilities exhibited in present experiments suggest that *Carcinus* should be capable of exhibiting orientational movement along a salinity

gradient in the form of a taxis. This would enable them to escape from rockpools or small enclosed areas exposed to freshwater runoff. However, orientation of crabs in estuaries where salinity gradients may remain constant over a relatively large area or may fluctuate rapidly on a tidal basis is uncertain. Crabs took 3 to 4 hours to exhibit directional movement towards the higher salinities within the choice offered. This was within a limited space where salinities remained constant and could be 'tested' a number of times. Such conditions are unlikely to occur within a tidal estuary, where orientation is likely also to be dependent on other factors and not solely as a response to salinity.

Future work should address the problem of detection of the medium,, to investigate similarities and inconsistencies between different species of Crustacea in order to determine any coherent pattern. The role of salinity gradients and halotaxis in the orientation of crustaceans within estuarine environments also warrants further investigation to determine whether salinity is the critical factor determining distribution or whether, as suggested here for *Carcinus*, that other factors may be involved.

GENERAL CONCLUSIONS

The aim of the present study was to examine various aspects of the behaviour of *Carcinus* when exposed to salinity variation. In addition, aspects of the animal's physiology were studied to determine any links between behavioural and physiological responses to a reduced salinity environment. Physiological and behavioural responses are best described in general terms for each colour form of crab in relation to their ecological distribution.

Red crabs On the open shore the majority of red crabs do not migrate into the intertidal zone, but tend to remain below low water mark (Crothers, 1968, Reid *et al*, 1989). Such crabs do not migrate into estuaries (Chapter 2). Present results suggest that they actively avoid contact with water of reduced salinity, which is detected by specific responses to the concentration of Na and Cl in the seawater. In reduced salinity water red crabs exhibit markedly increased locomotor activity which overrides tidal and daily locomotor rhythmicity. Red crabs are unable to survive in salinities below 11ppt. for extended periods; haemolymph concentration falls to critical levels and mortality is rapid in such conditions. At these low salinities they can detect salinity differences of as little as 0.5ppt. and may exhibit orientated movement in such subtle salinity gradients. Locomotor activity levels remain high in such

crabs at salinities below their preferred salinity regime, of between 22ppt. and 40ppt.

Green crabs Green crabs migrate intertidally and are found in estuaries (Crothers, 1968, Reid, *et al*, 1989). They can withstand lower salinities than red crabs, and are able to survive in salinities as low as 5ppt. for extended periods. When low salinity is encountered, again sensed by detecting the levels of Na and Cl in the water, they exhibit increased locomotor activity. The increase in activity is smaller than that observed in red crabs, and at low salinities their haemolymph concentration does not change as rapidly or fall as low as in the red crabs. If shelter is available green crabs often remain in the lower salinity for extended periods rather than exhibit an escape response, especially if the animal is of a small size. When such behaviour is exhibited in the estuarine environment, habituation to the salinity change takes place and crabs tend to show progressively reduced levels of locomotor activity in response to low salinity episodes. Such crabs are predominantly active at times of high tide, as in crabs on the open shore (Naylor, 1989) suggesting that for estuarine crabs high salinity is the most important factor for entrainment of rhythmicity. High salinity is certainly an important zeitgeber in open shore forms (Taylor and Naylor, 1977, Bolt and Naylor, 1985, 1986) but temperature and

hydrostatic pressure cycles are probably equally important in such crabs (Naylor, 1989). Estuarine crabs react less strongly than open shore green crabs to episodes of low salinity (Fig. 4.4.C). Such behaviour presumably conserves energy and helps the population to remain within the estuary. On the open shore low salinity will be more important since crabs do not encounter this continually, and so are unlikely to habituate to it, increased locomotor activity in such conditions acting as an escape response.

Schmidt (1989) reported that *Carcinus* can detect salinity changes by using the hair peg organs on the dactyls of the walking legs. However in the present study crabs often entered lower salinity water and remained in it for extended periods, and did not avoid such conditions immediately upon detection. Therefore changes in the internal physiological state of the crab are considered to be of more importance in effecting many of the behavioural responses observed here.

Some crabs remain in low salinity for over 3 hours (Figs. 3.2., 3.3., 3.8.), and for even longer in some cases if shelter is available (Fig. 3.13.). During this time the internal physiology of the animals undoubtedly changes. The haemolymph osmolality decreases (Margaria, 1931, Shaw, 1961, Blasco and Forward, 1988, Fig. 1.6.), heart-rate increases (Hume and Berlind, 1976, Cumberlidge and Uglow, 1977, Spaargaren 1982, Fig.

1.10.) and permeability values also change within the first few hours of exposure to low salinity (Spaargaren, 1974a, 1975a,b). All these points suggest that internal physiology of an individual may change before behavioural responses have been mediated. It seems likely then that internal change is the cue for the behavioural avoidance response, and the rate of physiological change, or change to a critical condition may be required before the crabs elicit such behaviour.

It is possible that a trade-off occurs between expending energy to change internal concentrations, and expending energy to escape. In salinities above approximately 25ppt. crabs do not expend energy regulating the osmolality of their internal fluids (Spaargaren, 1974a). In waters below 25ppt. the energy required to exit low salinity and seek a preferred salinity range may be lower than that required in regulation of the haemolymph osmolality. Indeed there appears to be a relationship between the salinities where regulation is carried out and the occurrence of choice behaviour. Both colours of crabs vacated low salinities at a faster rate when the internal regulation of body fluid concentrations occurred. However, preliminary evidence shows that, although the haemolymph concentration changed during this time there was no evidence of total evacuation at a critically low level of haemolymph osmolality.

The red and green crabs also have different physiological capabilities. Although the lower end of the preferred salinity range lies between 22ppt. and 28ppt. for both colour forms, red crabs are far less tolerant of low salinities and are unable to survive in salinities below 11ppt. Green crabs, however, can survive indefinitely in salinities as low as 5ppt. and indeed have higher haemolymph concentrations than red crabs when held within the 5ppt. to 17ppt. range. This illustrates again that physiology affects behaviour. Red crabs vacated very low salinities at a faster rate, since they are unable withstand the lower salinities tolerated by green crabs.

Prior acclimation to either high or low salinity also affects the behaviour. The lower the salinity of acclimation the faster the exit times when introduced into even lower salinities, and the higher the salinity of acclimation the longer the crabs will remain within the lower salinity ranges tested. These behavioural reactions were not due to the effect of altered magnesium concentrations in each acclimation salinity, which can affect activity levels in certain crustaceans (Katz, 1936, Waterman, 1941, Boardman and Collier, 1946, Robertson, 1953). The responses exhibited possibly occur to optimise the internal concentration. Here again changes in physiology alter the behaviour exhibited.

The links between the observed behavioural and

osmoregulatory physiological changes demonstrated here for *Carcinus* in experiments performed in controlled conditions help to explain the observed distributional differences in the Foryd estuary. Some physiological changes occur before behavioural responses are exhibited, which would suggest that they are in part responsible for mediating changes in behaviour. These responses in turn ensure that the physiological condition of an individual remains within viable limits.

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APPENDIX

SURVIVAL TEST, (Chapter 1)

Survival tests (SPSSX) produce life tables illustrating the number of animals remaining alive at each designated time period, and fit median lethal times to the data. The test performs pairwise comparisons on all possible combinations between the different groups, using the D-statistic. This is calculated from the survival scores using the algorithm of Lee and Desu (1972). The larger the D-statistic the greater the probability of significant difference.

HIERARCHICAL LOG LINEAR TEST, (Chapter 3)

Hiloglinear tests (SPSSX) were performed on a number of different experiments in chapter 3. They are a form of contingency table used for frequency data. They have a number of advantages over standard Chi-square or G tests; a large number of independent variables can be tested at once and the test allows one to impose structural sampling zeros. Chi-square goodness of fit are calculated for each of the models. The K way effects, which test the probability of significance of the highest factor interaction and the lower order interactions are also shown.

A large number of interactions proved to be significant for the results obtained for the multiple choice experiments (see Chapter 3, Tables 3.1. and

3.2.). The various interactions were fitted to the generating class (Time*colour*acclimation). In this way the most appropriate interactions for the results obtained could be deduced.