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*Phil. Trans. R. Soc. Lond. B* 1997 **352**, 365-379
doi: 10.1098/rstb.1997.0027

# The population biology and genetics of the deep-sea spider crab, *Encephaloides armstrongi* Wood-Mason 1891 (Decapoda: Majidae)

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## SUMMARY

Numerous specimens of the majid spider crab, *Encephaloides armstrongi*, were sampled from six stations (populations), between 150 and 650 m depth, on the continental slope off the coast of Oman. This extended the known geographic and bathymetric range of *E. armstrongi*, which is now known to occur along the continental margins of the northern Indian Ocean from the western coast of Burma to the coast of Oman. This band-like distribution is contiguous to the oxygen minimum zone in this region.

The biology and genetics of populations of *Encephaloides armstrongi* separated by depth were studied. The overall sex ratio of the *E. armstrongi* sampled was male-biased ($p < 0.01$; 3.3 males: 1 female; $S_o = 0.538$). However, sex ratio varied both between populations ($p < 0.01$) and between size classes of crabs. Size frequency analysis indicated that the male and female crabs consisted of at least two instars, one between 6 and 16 mm carapace length and one between 16 and 29 mm carapace length, which probably represented the terminal (pubertal) moult for most individuals. Accumulation of female crabs in the terminal instar probably caused the variation of sex ratio with size classes. Some male crabs grew to a larger size (up to 38 mm carapace length), possibly as a result of maturity at later instars.

Length frequency distribution was significantly different between sexes (one-way ANOVA $p < 0.001$). Within sexes, length frequency distributions varied between different populations. In both male and female *Encephaloides armstrongi* the individuals from a single population located at 150 m depth were significantly smaller than individuals at all other stations and were considered to represent a juvenile cohort. For female crabs no other significant differences were detected in length frequency between populations from 300 m to 650 m depth. Significant differences in length frequency were detected between male crabs from populations between 300 and 650 m depth.

Horizontal starch gel electrophoresis was used to detect six enzyme systems coding for eight loci for individuals sampled from each population of *Encephaloides armstrongi*. Genetic identity ($I$) values between populations of *E. armstrongi* ($I = 0.98$–$1.00$) were within the normal range for conspecific populations. Observed heterozygosity ($H_o = 0.080$–$0.146$) was lower than expected heterozygosity ($H_e = 0.111$–$0.160$), but in the normal range detected for eukaryotic organisms.

*F*-statistics were used to analyse between population ($F_{ST}$) and within population ($F_{IS}$) genetic structure. For both male and female *E. armstrongi* significant genetic differentiation was detected between the population located at 150 m depth and all other populations. Analyses of $F_{IS}$ and $F_{ST}$, excluding the 150 m population indicated that for female *E. armstrongi* there was no significant structuring within or between populations. For male *E. armstrongi* significant heterozygote deficiencies were detected within populations and significant genetic differentiation between populations.

The most likely explanations for the observations of the present study are: the population of *Encephaloides armstrongi* located at 150 m depth represented a juvenile cohort that is genetically distinct from deeper populations; female *E. armstrongi* formed a single population between 300 m and 650 m depth in the sampling area; male *E. armstrongi* were from two or more genetically distinct populations which are represented by different numbers of individuals at stations between 300 m and 650 m depth. This caused the observed significant differences in morphology (size distribution) and allele frequencies of male populations. It is likely that *E. armstrongi* exhibits gender-biased dispersal and that the crabs collected between 300 m and 650 m depth formed spawning aggregations. This also explains the bias in sex ratio of individuals sampled in the present study.

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## 1. INTRODUCTION

The majid spider crab, *Encephaloides armstrongi* Wood-Mason 1891, was first collected from depths of 110–180 m in the Bay of Bengal in the north-east Indian Ocean. Numerous specimens of this crab were dredged by the Indian Marine Survey Steamer *Investigator*. Almost all specimens were males (Wood-Mason & Alcock 1891) and the species was described as having 'greatly swollen and enlarged gill chambers which were an adaptation to low oxygen concentrations at abyssal depths' (Alcock 1902). Subsequently, a second species, *Encephaloides rivers-andersoni* Alcock 1899, was described from the south-west tip of India, off the Quilon coast (formerly Travancore coast). *E. rivers-andersoni* was found at a depth of approximately 750 m and differed from *E. armstrongi* in having a smaller size and a more elongate and narrow carapace.

This study forms part of the research programme of R.R.S. *Discovery* cruise 211 to the Arabian Sea in 1994. The cruise was undertaken in order to investigate the effects of the oxygen minimum zone on sediment biogeochemistry and the composition and distribution of the benthic fauna on the continental margin off the coast of Oman. The fauna was photographed and sampled using a combination of box cores, sledges, trawls and cameras at depths of 50 to 3400 m (see Gage 1995). During the cruise, dense populations of spider crabs from the genus *Encephaloides* were observed and collected at depths between 150 and 1000 m. Photo-sledge surveys between 600 and 800 m depth indicated that the crabs were ubiquitous, being present in all but one of 200 photographs. Calibration of the oblique view of the bottom indicated a maximum density of 20 crabs $m^{-2}$ and a mean density of 10 crabs $m^{-2}$ (Bett 1995). These crabs, along with numerous individuals dredged and trawled from between 150 and 600 m, were thought to be *E. armstrongi*. By contrast, between depths of 830 and 1230 m the crabs showed a much more patchy distribution with none present in over half the photographs. At approximately 1000 m depth, aggregations of crabs were photographed with a maximum density of up to 137 individuals $m^{-2}$ and a mean density of 70 crabs $m^{-2}$ (Bett 1995).

The large collections available of *Encephaloides armstrongi* from 150 to 700 m depth presented the opportunity of studying the biology and genetics of populations of a deep-sea spider crab, separated by depth. While there are considerable data on genetic differentiation in horizontally separated populations of deep-sea animals (e.g. Doyle 1972; Costa & Bisol 1978), especially those from hydrothermal vents (e.g. Jollivet *et al*. 1995*a*, *b*; Creasey *et al*. 1996), there is only very limited information on any depth-related genetic differentiation in crustacean populations. Two significant studies that examined genetic differentiation in populations separated by depth are Bucklin *et al*. (1987) and France (1994), which concern the lysiannassid amphipods *Eurythenes gryllus* and *Abyssorchomene* spp. respectively. Both studies showed substantial genetic differentiation in amphipod populations separated by depth, but it is possible that more than one species was present in each study (see Bucklin *et al*. 1987; France 1994). In the present paper we compare length frequencies and sex ratios in the populations of *E. armstrongi* sampled from the Oman slope and examine genetic differentiation between the populations using allozymes identified by starch-gel electrophoresis.

## 2. MATERIALS AND METHODS

### (*a*) *Sample collection and measurement*

Samples of the spider crab, *Encephaloides armstrongi*, were collected from Oman slope in the north-western Arabian Sea during R.R.S. *Discovery* cruise 211. Details of the stations, sampling gear and number of specimens sampled for electrophoresis are given in table 1, and number of specimens used in the size frequency analysis are given in table 2.

Once brought to the warm surface waters specimens for electrophoresis were immediately frozen whole at −70 °C. At Station 12696#1 only the first 203 specimens were frozen, an additional 1011 specimens were sexed and measured on board before being preserved in 10% formalin. Formalin-preserved samples were transported to the Scottish Association for Marine Science at Oban. Frozen samples were transported in dry ice to the Marine Biological Association, Plymouth, where they were sexed, measured and numbered. Carapace length and breadth were measured to the nearest millimetre using dial callipers. Individuals were stored at −70 °C prior to electrophoresis.

### (*b*) *Electrophoresis*

A 3 mm cube of visceral tissue (hepato-pancreas, muscle and heart) was dissected from the cephalothorax of the crab, while still frozen. The tissue was placed in a 1.7 ml centrifuge tube with 100 µl of grinding buffer (0.06 M Tris/HCl, pH 8.0, with 1.5 mg NADP $ml^{-1}$ buffer; Redfield & Salini 1980) and macerated with a glass pestle. Samples were then centrifuged in an Eppendorf Model 5415C centrifuge for 10 min at 14000 rpm and 4 °C. Individuals at Station 12731#1 were so small that they were homogenized whole, prior to centrifugation. The supernatant was absorbed on to filter paper wicks (Whatman No. 1) and applied to a starch gel.

Horizontal starch-gel electrophoresis was performed by standard methods (see Harris & Hopkinson 1978; Ferguson 1980; Richardson *et al*. 1986; Murphy *et al*. 1990) using 12.5% starch gels (Sigma Chemical Co. Ltd, Poole, Dorset, UK). Two buffer systems were used: buffer system I, Tris–versene–borate, continuous, pH 8.0 (Shaw & Prasad 1970); buffer system II, Tris–citric–boric–lithium hydroxide, discontinuous, electrode pH 8.29, gel pH 8.26 (Redfield & Salini 1980). Buffer system I was run at 200 V, 40 mA for 7 h, buffer system II was run at 300 V, 35 mA for 7 h.

Six enzyme systems coding for a total of eight enzyme loci were visualized using enzyme-specific stains modified from Shaw & Prasad (1970), Harris & Hopkinson (1978) and Redfield & Salini (1980):

Table 1. Encephaloides armstrongi. *Position and depth of sample sites, capture gear and numbers of specimens sampled for allozyme electrophoresis*

(Total number of specimens sampled for length and width measurements given in parentheses.)

| station no. (date) | latitude | longitude | depth (m) | capture gear | number of specimens |
|---|---|---|---|---|---|
| 12696#1 (10/23/94) | 19° 11′ N | 58° 18′ E | 650 | Agassiz trawl | 203 |
| 12697#1 (10/23/94) | 19° 09.95′ N | 58° 12.04′ E | 378 | Agassiz trawl | 39 |
| 12701#1 (10/24/94) | 19° 10.6′ N | 58° 17.9′ E | 631–643 | WHOI Epibenthic sledge | 86 |
| 12707#1 (10/26/94) | 19° 11′ N | 58° 13′ E | 350 | Agassiz trawl | 18 |
| 12731#1 (11/9/94) | 19° 13′ N | 58° 10′ E | 150 | Agassiz trawl | 38 |
| 12733#1 (11/9/94) | 19° 13.55′ N | 58° 18.32′ E | 577–625 | IOS Epibenthic sledge | 121 |

Table 2. Encephaloides armstrongi. *Mean numbers of male and female crabs and mean lengths (s.d.) of male and female crabs captured at each station*

(Sex ratio ($S_o$) as calculated by the method in Christiansen *et al.* (1990) for each station is also given.)

| | males | | females | | |
|---|---|---|---|---|---|
| sex station | no. of individuals | mean length (mm) | no. of individuals | mean length (mm) | sex ratio ($S_o$) |
| 12696#1 | 922 | 24.1 (3.7) | 292 | 21.9 (3.7) | 0.519 |
| 12697#1 | 30 | 26.6 (5.0) | 9 | 23.6 (2.3) | 0.540 |
| 12701#1 | 91 | 22.9 (4.1) | 9 | 22.9 (1.7) | 0.820 |
| 12707#1 | 9 | 26.1 (5.0) | 9 | 24.0 (2.0) | 0 |
| 12731#1 | 35 | 9.2 (1.5) | 3 | 8.0 (1.0) | 0.842 |
| 12733#1 | 95 | 20.9 (3.2) | 33 | 20.8 (2.3) | 0.484 |

aminopeptidase (AP-substrate Leu-Gly-Gly, Sigma Chemical Co. Ltd, Poole, Dorset, UK, E.C. 3.4.11 or 13, in buffer system I); aminopeptidase (AP-substrate Leu-Pro, Sigma Chemical Co. Ltd, Poole, Dorset, UK, E.C. 3.4.11 or 13, in buffer system I); esterase (EST, E.C. 3.1.1.1, in buffer system II); glutamate–oxaloacetate transaminase (GOT, E.C. 2.6.1.1, in buffer system I); general protein (GP, in buffer system II); phosphoglucose isomerase (PGI, E.C. 5.3.1.9, in buffer system II). In three enzyme systems additional loci were visualized, but poor enzyme activity or inconsistent staining resolution did not allow scoring across all individuals. Loci which were not scored included several esterase loci which migrated faster than the Est-B locus (i.e. loci Est-C, etc.) and also a single locus in each of the GOT and GP stain systems (Got-A and Gp-B, respectively).

The largest sample (Station 12696#1) was chosen as the reference population. Alleles were labelled in ascending alphabetical order according to their distance from the origin. The most common allele at each enzyme locus in the reference population was given a relative mobility of 100. Other alleles at each locus were given a mobility relative to the most common allele. Where more than one locus appeared for each enzyme system, the loci were numbered from the slowest to the fastest.

### (*c*) *Data analysis*

(i) *Sex ratio and size frequency distribution*

The overall sex ratio ($S_o$) of samples of *Encephaloides armstrongi* was determined according to the formula modified from Christiansen *et al.* (1990):

$$S_o = (M_o - F_o)/(M_o + F_o),$$

where $M_o$ is the number of males in the sample and $F_o$ is the number of females in the sample. Size class (1 mm intervals of carapace length) was plotted against sex ratio, and a trend line inserted with a floating mean of five size classes (in order to offset bias due to small sample number in some size classes). The sex ratio of the crabs at each station was analysed using a two tailed $\chi^2$ test to determine if there were significant deviations from an expected 1:1 sex ratio. The effective population size ($N_e$) was determined according to the equation given in Li & Graur (1991):

$$N_e = \frac{4N_m N_f}{N_m + N_f},$$

where $N_m$ and $N_f$ are the respective proportions of males and females in a population of $N$ individuals.

A one-way analysis of variance (ANOVA) was used to test if there was a significant difference in size distribution between sample sites for male and female *Encephaloides armstrongi* separately. Pairwise comparisons of difference in size distribution were carried out between each site, separately for each sex, using Scheffe's *F*-test. Significance levels of *F*-tests were adjusted for multiple tests according to the sequential Bonferroni procedure in Rice (1989).

Size frequency was plotted for both sexes of *Encephaloides armstrongi* using intervals of 1 mm carapace length in order to detect modal peaks which may be indicative of discrete developmental instars.

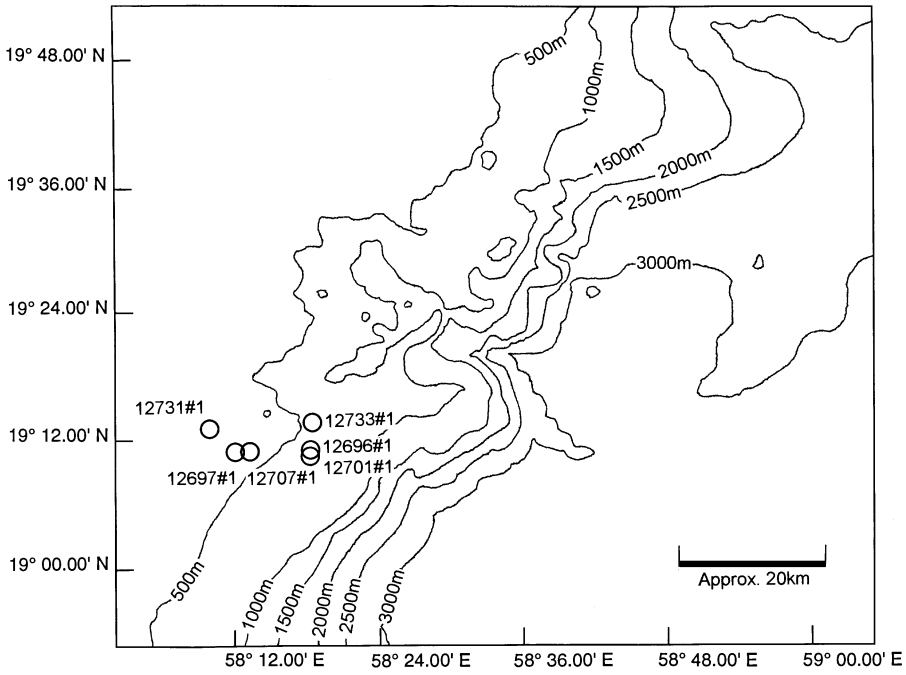


Figure 1. Map showing the distribution of sample sites for *Encephaloides armstrongi* on the continental slope south of the Island of Masira off the coast of Oman.

Carapace width was plotted to the nearest millimetre against carapace length for both male and female crabs, in order to see if there was any significant variation in the ratio of carapace width to carapace length, in relation to sex. For both sexes correlation equations and correlation coefficients were resolved and compared.

(ii) *Genetics*

Allele frequencies were calculated for all frozen samples of *Encephaloides armstrongi* over all eight enzyme loci. Genetic variability was estimated using a set of parameters including: the mean number of alleles per locus, the percentage of polymorphic loci, the observed heterozygosity and the expected heterozygosity based on Hardy–Weinberg assumptions. Genetic variability was calculated for five distinct hypotheses: (1) by assuming each station was representative of a discrete population; (2) by pooling samples into shallow- ($< 200$ m deep), intermediate- (200–400 m) and deep-living ($> 400$ m) populations (i.e. individuals were pooled into populations along isobaths in order to examine the effect of depth upon population structure); (3) by pooling samples into upper slope (150–450 m deep) and lower slope (500–700 m) populations; (4) by pooling all individuals according to sex; and (5) by separating males and females for each respective population.

Polymorphic loci, for all samples treated separately and for pooled populations, were tested for conformity to Hardy–Weinberg expectations using $\chi^2$ tests with Levene's (1949) correction for small sample size and Yate's correction for continuity (Elston & Forthofer 1977).

Overall genetic differentiation between samples was estimated using Nei's (1972) genetic identity ($I$) and genetic distance ($D$). Genetic differentiation among populations was estimated using $F_{ST}$ for all variable loci in both pooled and separate samples. Significance of departure of $F_{ST}$ from zero was calculated using the formula of Workman & Niswander (1970) given in Waples (1987):

$$\chi^2 = 2NF_{ST}(k-1); \quad \text{d.f.} = (k-1)(s-1),$$

where $N$ is the number of individuals sampled, $k$ is the number of alleles at a locus and $s$ is the number of populations.

Deviation from random-mating expectations within all samples and pooled populations was estimated using $F_{IS}$. $F_{IS}$ and $F_{ST}$ values were analysed using the FSTAT programme (Goudet 1994) to ascertain if deviations within the fixation indices were due to sample-size related bias. Significance of the deviation of both $F_{IS}$ and $F_{ST}$ from zero was also calculated using the FSTAT programme (Goudet 1994) to replicate 1000 datasets. Both $F_{ST}$ and $F_{IS}$ significance levels were adjusted for multiple tests according to the sequential Bonferroni technique given in Rice (1989).

The number of migrants per deme per generation ($N_e m$) was calculated, using the FSTAT programme (Goudet 1994), from pairwise values of $F_{ST}$ according to the following formula:

$$N_e m = ((1/F_{ST})-1)/4.$$

An alternative method of calculating $N_e m$ uses the private alleles method of Slatkin (1985):

$$N_e m = e^{-[(\ln\{p(1)\}+2.44)/0.505]}/(N/25),$$

where $p(1)$ is the average frequency of alleles found only in one population and $N$ is the average number of individuals sampled per population.

All of the above calculations, apart from Slatkin's (1985) estimate of $N_e m$ and estimates of $F_{ST}$, $F_{IS}$, and

$N_e m$ obtained from the FSTAT programme (Goudet 1994), were calculated using BIOSYS release 1.7 (Swofford & Selander 1989).

## 3. RESULTS

### (*a*) *Distribution and biology of* Encephaloides armstrongi

In this study individuals of *Encephaloides armstrongi* were sampled at depths of between 150 m and 650 m (figure 1). The overall sex ratio of these specimens showed significant deviation from a 1:1 sex ratio ($\chi^2 = 239.8$; d.f. = 1; $p < 0.001$), with a total of 1182 males to 355 females (approximately 3.3:1; $S_o = 0.538$). Between size classes (1 mm intervals of carapace length) the sex ratio varied, with males predominating in all size classes, although within intermediate size classes there was a higher proportion of females (see figure 2). There was a significant difference in sex ratio of crabs from different sampling sites ($\chi^2 = 24.61$; d.f. = 5; $p < 0.001$). The estimated overall effective population size ($N_e$) of the populations sampled was substantially lower than the actual population size ($N$) owing to the highly skewed sex ratio ($N_e = 0.71N$).

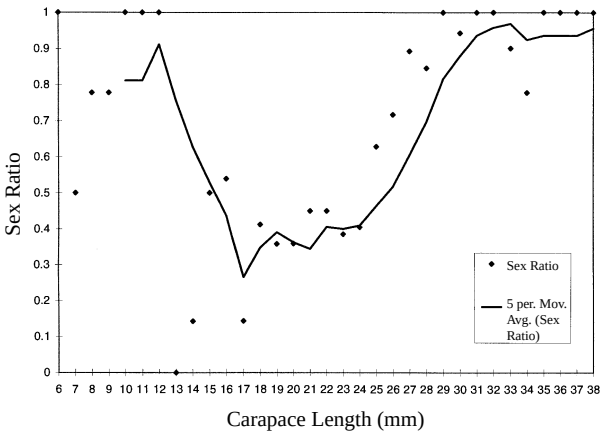


Figure 2. *Encephaloides armstrongi*. Graph of the sex ratio ($S_o$, denoted by solid diamonds) against carapace length. Trend line has a floating mean of five size classes.

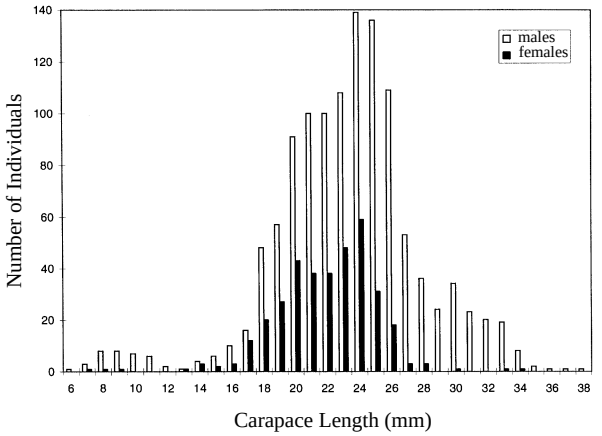


Figure 3. *Encephaloides armstrongi*. A histogram of length frequency for male (open bars) and female (solid bars) crabs from all sample sites.

Highly significant differences in the size distribution (length) of both male and female crabs were detected between each sample site using a one-way ANOVA for untransformed data ($p < 0.001$). The Scheffe $F$-test between each pair of areas showed that the differences were not significant between all sites (see table 3). For female *Encephaloides armstrongi*, only the crabs from Station 12731#1 were significantly different in size from female crabs at other sampling stations. These crabs were notably smaller than those present at all other stations and may not have been sexed accurately. For male crabs, the findings were different. Although crabs at Station 12731#1 were significantly different in size from those at all other stations, highly significant differences in size were detected between several of the other stations. Male crabs from Stations 12707#1 and 12697#1, located at 350 m and 378 m respectively, did not show a significant difference in size distribution. Male crabs from the deeper Stations 12696#1, 12701#1 and 12733#1, showed significant differences in size frequency between the deep stations and the shallower stations.

Size–frequency relationships are shown in figure 3 for both male and female crabs. Spider crabs do not

Table 3. Encephaloides armstrongi. *Probabilities for pairwise comparisons by Scheffe's* F*-test, of size distribution between samples of for male and female crabs*

| station | 12696#1 | 12697#1 | 12701#1 | 12707#1 | 12731#1 | 12733#1 |
|---|---|---|---|---|---|---|
| males | | | | | | |
| 12696#1 | 1.000 | | | | | |
| 12697#1 | 0.024 | 1.000 | | | | |
| 12701#1 | 0.115 | 0.000** | 1.000 | | | |
| 12707#1 | 0.751 | 1.000 | 0.283 | 1.000 | | |
| 12731#1 | 0.000** | 0.000** | 0.000** | 0.000** | 1.000 | |
| 12733#1 | 0.000** | 0.000** | 0.020 | 0.006* | 0.000** | 1.000 |
| females | | | | | | |
| 12696#1 | 1.000 | | | | | |
| 12697#1 | 0.727 | 1.000 | | | | |
| 12701#1 | 0.964 | 0.998 | 1.000 | | | |
| 12707#1 | 0.468 | 1.000 | 0.983 | 1.000 | | |
| 12731#1 | 0.000** | 0.000** | 0.000** | 0.000** | 1.000 | |
| 12733#1 | 0.494 | 0.268 | 0.600 | 0.123 | 0.000** | 1.000 |

* $p \leqslant 0.05$; ** $p \leqslant 0.01$.




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Table 4. Encephaloides armstrongi. *Allele frequencies for all loci for all samples of male and female crabs*

(Relative mobilities of alleles in parentheses: most common allele at 12696#1 (largest population) was nominated a mobility of 1.00 (LGG = Leu-Gly-Gly; LP = Leu-Pro))

| | station number | | | | | |
|---|---|---|---|---|---|---|
| locus | 12696#1 | 12697#1 | 12701#1 | 12707#1 | 12731#1 | 12733#1 |
| *N* | 203 | 39 | 86 | 18 | 38 | 121 |
| *Ap* (LGG) | | | | | | |
| A (0.85) | 0.020 | — | 0.023 | 0.028 | — | 0.021 |
| B (0.93) | 0.039 | 0.013 | 0.087 | 0.028 | 0.079 | 0.021 |
| C (1.00) | 0.924 | 0.987 | 0.872 | 0.944 | 0.908 | 0.942 |
| D (1.05) | 0.012 | — | 0.017 | — | 0.013 | 0.017 |
| E (1.20) | 0.005 | — | — | — | — | — |
| *Ap* (LP) | | | | | | |
| A (0.83) | 0.025 | 0.090 | 0.012 | 0.083 | — | — |
| B (0.94) | 0.074 | 0.141 | 0.122 | 0.167 | 0.079 | 0.050 |
| C (1.00) | 0.899 | 0.769 | 0.860 | 0.750 | 0.921 | 0.905 |
| D (1.04) | 0.002 | — | 0.006 | — | — | 0.045 |
| *Esta* | | | | | | |
| A (0.77) | — | — | 0.012 | — | — | — |
| B (1.00) | 1.000 | 1.000 | 0.988 | 1.000 | 1.000 | 1.000 |
| *Estb* | | | | | | |
| A (1.00) | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *Gotb* | | | | | | |
| A (0.92) | 0.002 | 0.013 | — | — | — | — |
| B (1.00) | 0.924 | 0.987 | 0.965 | 1.000 | 0.868 | 0.959 |
| C (1.08) | 0.059 | — | 0.035 | — | 0.132 | 0.025 |
| D (1.21) | 0.015 | — | — | — | — | 0.017 |
| *Gpa* | | | | | | |
| A (1.00) | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *Gpc* | | | | | | |
| A (1.00) | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *Pgia* | | | | | | |
| A (0.74) | 0.049 | 0.026 | 0.052 | 0.222 | 0.013 | 0.033 |
| B (0.84) | 0.261 | 0.115 | 0.262 | 0.278 | 0.171 | 0.219 |
| C (0.94) | 0.064 | 0.090 | 0.035 | — | 0.211 | 0.083 |
| D (1.00) | 0.596 | 0.731 | 0.640 | 0.333 | 0.553 | 0.607 |
| E (1.08) | 0.030 | 0.038 | 0.012 | 0.167 | 0.053 | 0.058 |

generally moult after sexual maturity, i.e. they have a terminal moult (see Hartnoll *et al*. 1993). Although detailed estimates of size at maturity are not available for *Encephaloides armstrongi* in this study, it is possible to interpret the size–frequency distribution of male and female crabs in terms of moult instars if they follow similar patterns to other spider crabs (see Hartnoll & Rice 1985; Hartnoll *et al*. 1993). If this is the case then we estimate that the male and female crabs sampled consist of at least two instars, one between 6 and 16 mm carapace length and one between 16 and 29 mm carapace length, which probably represents the terminal moult for the majority of individuals. It is probable that a third peak in length frequency between 29 and 38 mm for male crabs represents a minority of individuals who become mature at later instars.

There is a significant correlation between carapace width and carapace length in both sexes of *Encephaloides armstrongi*. In small ($\leqslant 20$ mm carapace length) and presumably immature individuals there is no significant difference in the relationship between carapace width ($W_c$) and carapace length ($L_c$) between male and female crabs (males: $r = 0.840$, $N = 268$; females: $r = 0.790$, $N = 114$; $p > 0.1$). However, in larger individuals ($> 20$ mm carapace length), the relationship between carapace width ($W_c$) and carapace length ($L_c$) is significantly different for each sex (males: $r = 0.936$, $N = 1182$; females: $r = 0.837$, $N = 355$; $p < 0.001$). These may be described by the following equations:

Male crabs $W_c = 0.8618L_c + 1.2831$ $(N = 1182)$

Female crabs $W_c = 0.7149L_c + 3.8651$ $(N = 355)$.

### (*b*) *Population genetics of* Encephaloides armstrongi

All enzyme loci produced well resolved staining patterns consistent with known enzyme subunit structure for all individuals. Allele frequencies between all populations are broadly similar (see table 4), though there are some differences and a number of private alleles (i.e. alleles occurring in only one population). Overall genetic differentiation between populations was estimated using Nei's (1972) genetic identity ($I$), which did not fall below 0.98 (see table 5).

Observed heterozygosity ($H_o$) for populations of *Encephaloides armstrongi* ranged from 0.080–0.146 (see table 6) and fall within the normal range of variation

Table 5. Encephaloides armstrongi. *Pairwise comparisons based on data for eight loci for male and female specimens of all samples*

(Above diagonal values for Nei's (1972) genetic identity ($I$); below diagonal values for Nei's (1972) genetic distance ($D$).)

| station | 12696 # 1 | 12697 # 1 | 12701 # 1 | 12707 # 1 | 12731 # 1 | 12733 # 1 |
|---|---|---|---|---|---|---|
| 12696 # 1 | — | 0.994 | 0.999 | 0.988 | 0.997 | 0.999 |
| 12697 # 1 | 0.006 | — | 0.995 | 0.982 | 0.991 | 0.995 |
| 12701 # 1 | 0.001 | 0.005 | — | 0.987 | 0.994 | 0.998 |
| 12707 # 1 | 0.012 | 0.018 | 0.013 | — | 0.982 | 0.987 |
| 12731 # 1 | 0.003 | 0.009 | 0.006 | 0.018 | — | 0.997 |
| 12733 # 1 | 0.001 | 0.005 | 0.002 | 0.013 | 0.003 | — |

Table 6. Encephaloides armstrongi. *Measures of genetic variability (means (s.e.)) for all samples including both male and female specimens*

(A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99 ($H_o$ = observed heterozygosity (direct count); $H_e$ = Hardy–Weinberg expected heterozygosity).)

| population | no. alleles per locus | % polymorphic loci | $H_o$ | $H_e$ |
|---|---|---|---|---|
| 12696 # 1 | 2.8 (0.7) | 50.0 | 0.101 (0.051) | 0.131 (0.069) |
| 12697 # 1 | 2.0 (0.5) | 50.0 | 0.080 (0.047) | 0.111 (0.067) |
| 12701 # 1 | 2.5 (0.6) | 62.5 | 0.097 (0.048) | 0.136 (0.066) |
| 12707 # 1 | 1.9 (0.4) | 37.5 | 0.146 (0.092) | 0.160 (0.099) |
| 12731 # 1 | 2.0 (0.5) | 50.0 | 0.135 (0.076) | 0.147 (0.076) |
| 12733 # 1 | 2.4 (0.6) | 50.0 | 0.101 (0.058) | 0.118 (0.069) |
| overall | 2.9 (0.6) | 62.5 | 0.103 (0.054) | 0.131 (0.069) |

detected for eukaryotic organisms (Nevo 1978; Nevo *et al*. 1984). In all populations of *E. armstrongi* observed heterozygosity was lower than the expected heterozygosity according to Hardy–Weinberg equilibrium conditions. This is also shown by Wright's (1951, 1965) fixation index ($F$) for variable loci (see table 7), which in almost all cases is strongly positive (indicating heterozygote deficiency), although this heterozygote deficiency is only significantly different from zero in a few cases. $H_o$ and $F$ are notably different between populations.

$F_{ST}$ values for individual populations varied from 0.01 to 0.05 (see table 8). $F_{ST}$s for all variable loci except *Esta* were significant at the $p < 0.01$ level. This is indicative of a degree of structuring between populations with limited gene flow between them. As samples were progressively pooled into populations from different depth strata, the number of $F_{ST}$ values which were significant decreased, with only the *Ap* (Leu-Pro) and *Pgia* loci significant (at $p < 0.01$ and $p < 0.05$ respectively) in the 'upper' versus 'lower' slope population comparison.

When populations were split into separate sexes, $F_{ST}$ values for male crabs were significant at all variable loci except *Esta* ($p < 0.01$; see table 9). In female crabs however, only *Gotb*, *Pgia* and the mean $F_{ST}$ were significant ($p < 0.01$). When individuals from Station 12731 # 1 were removed from the comparison, on the grounds that these were much smaller animals possibly belonging to a different cohort, no $F_{ST}$ values were found to be significant between the female populations. $F_{ST}$ values for all loci except *Esta* in the males remained significant at the $p < 0.01$ level. This indicates that if juveniles are excluded (i.e. population 12731 # 1), female populations do not show a significant degree of structuring, although male populations do.

The number of migrants per deme per generation ($N_e m$) was calculated using both $F_{ST}$ values and Slatkin's (1985) private alleles [$p(1)$] methods. Both methods indicated that between individual populations and between pooled populations at different depth strata (see table 8) gene flow was generally low (Wright 1951, 1965). $N_e m$ values given by the two methods varied, with the $p(1)$ method giving lower estimates of migration than the $F_{ST}$ method. The main reason for this is that the number of loci screened in the present study was low, compared with the number of populations, thereby limiting the number of private alleles that may be detected.

Deviation from random mating expectations measured by $F_{IS}$, was significant at all loci and also the mean $F_{IS}$, when populations were treated separately and pooled into depth strata (see table 8). However, when populations were divided into male and female subpopulations, the number of significant $F_{IS}$ values varied (see table 9). Data from all six female populations resulted in $F_{IS}$ values that were significant at the *Gotb* locus ($p < 0.01$) and the mean $F_{IS}$ ($p < 0.05$). However, when Station 12731 # 1 was excluded, no significant $F_{IS}$ values were obtained. In male crabs, all variable loci, except *Gotb*, yielded significant results when all six populations were compared. When population 12731 # 1 was excluded, significant values were still obtained for the *Ap* (Leu-Pro), *Esta*, *Pgia* loci and the mean $F_{IS}$ ($p < 0.01$) value. In individual and pooled populations, as well as in male and female populations, $F_{IS}$ values were predominantly significant owing to a heterozygote deficiency. Heterozygote deficiency was detected in populations as a low observed heterozygosity ($H_o$) compared to expected heterozygosity ($H_e$)(see table 6), and large positive values for Wright's fixation index ($F$) (see table 7).

Table 7. Encephaloides armstrongi. *Wright's (1951, 1965) fixation index* (F) *for variable loci for all individuals within each population*

(Significant deviations from expected genotype frequencies according to Hardy–Weinberg equilibrium are also indicated (** $p \leqslant 0.01$).)

| locus | 12696#1 | 12697#1 | 12701#1 | 12707#1 | 12731#1 | 12733#1 |
|---|---|---|---|---|---|---|
| *Ap* (LGG) | 0.115 | −0.013 | 0.245 | −0.043 | 0.223 | 0.034 |
| *Ap* (LP) | 0.257** | 0.258 | 0.239 | 0.172 | 0.276 | 0.110 |
| *Esta* | — | — | 1.000** | — | — | — |
| *Gotb* | 0.140 | −0.013 | 0.309 | — | 0.079 | 0.174 |
| *Pgi* | 0.264** | 0.305 | 0.282** | 0.017 | −0.022 | 0.162 |

Table 8. Encephaloides armstrongi. *Estimates of variance of allele frequencies between populations* ($F_{ST}$) *and of the correlation between homologous alleles between individuals within populations* ($F_{IS}$) *for variable loci for all samples of male and female* E. armstrongi *taken separately, for populations pooled by depth (shallow (< 200 m depth), intermediate (200–400 m depth) and deep (> 400 m depth)) and for populations pooled into upper slope (< 400 m depth) and lower slope (> 400 m depth) populations*

| | individual populations | | shallow, intermediate and deep water populations | | upper and lower slope populations | |
|---|---|---|---|---|---|---|
| locus | $F_{IS}$ | $F_{ST}$ | $F_{IS}$ | $F_{ST}$ | $F_{IS}$ | $F_{ST}$ |
| *Ap* (LGG) | 0.139** | 0.017** | 0.158** | 0.013 | 0.153** | 0.002 |
| *Ap* (LP) | 0.217** | 0.030** | 0.237** | 0.029** | 0.245** | 0.008** |
| *Esta* | 1.000** | 0.010 | 1.000** | 0.002 | 1.000** | 0.001 |
| *Gotb* | 0.133** | 0.043** | 0.104** | 0.045** | 0.155* | 0.001 |
| *Pgia* | 0.151** | 0.050** | 0.153** | 0.014** | 0.193** | 0.005* |
| Mean | 0.167** | 0.040** | 0.168** | 0.021** | 0.198** | 0.005 |
| $N_e m$ ($F_{ST}$) | 6.00 | | 16.81 | | 49.75 | |
| $N_e m$ ($p$) | 0.31 | | 11.65 | | 11.21 | |

* = $p < 0.05$; ** = $p < 0.01$.

Table 9. Encephaloides armstrongi. *Estimates of variance of allele frequencies between populations* ($F_{ST}$) *and of the correlation between homologous alleles between individuals within populations* ($F_{IS}$) *for variable loci for all samples of* E. armstrongi *with male and female crabs treated separately*

| | all sample stations | | station 12731#1 omitted | |
|---|---|---|---|---|
| locus | $F_{IS}$ | $F_{ST}$ | $F_{IS}$ | $F_{ST}$ |
| female | | | | |
| *Ap* (LGG) | 0.153 | 0.041 | 0.153 | 0.035 |
| *Ap* (LP) | 0.022 | 0.044 | 0.022 | 0.032 |
| *Gotb* | 0.692** | 0.218** | −0.057 | 0.039 |
| *Pgia* | 0.214 | 0.094** | 0.214 | 0.035 |
| Mean | 0.238* | 0.100** | 0.161 | 0.035 |
| male | | | | |
| *Ap* (LGG) | 0.114* | 0.016** | 0.086 | 0.017** |
| *Ap* (LP) | 0.229** | 0.048** | 0.225** | 0.046** |
| *Esta* | 1.000** | 0.010 | 1.000** | 0.010 |
| *Gotb* | 0.075 | 0.033** | 0.195 | 0.019** |
| *Pgia* | 0.170** | 0.060** | 0.223** | 0.064** |
| Mean | 0.173** | 0.049** | 0.209** | 0.050** |

* = $p < 0.05$; ** = $p < 0.01$.

## 4. DISCUSSION

### (*a*) *Distribution and biology of* Encephaloides armstrongi

The exact geographical range of *Encephaloides armstrongi* remains uncertain, with only Wood-Mason & Alcock (1891) and Griffin (1974) giving positions of previous capture sites. Wood-Mason & Alcock (1891) stated that *E. armstrongi* is '… characteristic … from the coast of Arrakan to the Godávari …', and Alcock (1902) states that it was '… a spider crab found on the edge of the abyssal slope all round the coasts of India …'. During their studies, Wood-Mason and Alcock (1891) and Griffin (1974) sampled *E. armstrongi* along the eastern coast of India and the southern coast of Iran, respectively (see table 10). In the present study specimens were collected off the coast of Oman, extending the previous western–southern limit of *E. armstrongi* by approximately 600–700 km.

The known bathymetric distribution of *E. armstrongi* has also been found to be greater in the present study than previously indicated. Wood-Mason & Alcock (1891) and Alcock (1899) both stated that *E. armstrongi* was common at depths of 109–182 m, and Griffin

Table 10. Encephaloides armstrongi. *Capture sites recorded previously in the literature*

| station | latitude | longitude | depth (m) | reference |
|---|---|---|---|---|
| station 76 25 miles SE, $\frac{1}{2}$ mile E. Barwa beacon (off Ganjam coast) | 19° N (approx.) | 85° 24′ E (approx.) | 169 | Wood-Mason & Alcock (1891) |
| station 81 24 miles SE Gopálpur | 18° 48′ N (approx.) | 85° 24′ E (approx.) | 162–169 | Wood-Mason & Alcock (1891) |
| station 96 | 18° 30′ N | 84° 46′ E | 178–185 | Wood-Mason & Alcock (1891) |
| station 245A Gulf of Oman | 24° 55′–24° 52′ N | 61° 10′–61° 13′ E | 170–192 | Griffin (1974) |
| station 246A Gulf of Oman | 25° 00′–24° 57′ N | 60° 57′–60° 59′ E | 298–311 | Griffin (1974) |

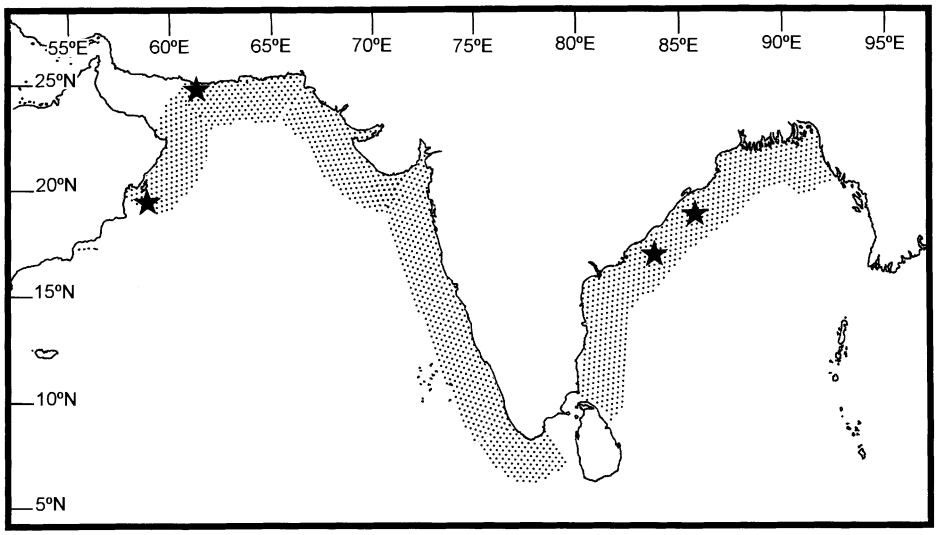


Figure 4. Map showing present and previous specific capture locations (represented by stars) and potential range of distribution for *Encephaloides armstrongi* (represented by stippling).

(1974) collected specimens at depths between 170 m and 311 m. In the present study specimens of *E. armstrongi* were sampled from 150 to 650 m (see table 1), more than doubling the previously known lower limit of distribution. It is therefore possible that *E. armstrongi* occurs in a continuous, band-like distribution, contiguous to the oxygen minimum zone, from the coast of Oman to the western coast of Burma (see figure 4) in the northern Indian Ocean (see Kamykowski & Zentara 1990).

In the region sampled during R.R.S. *Discovery* cruise 211, *Encephaloides armstrongi* was one of only a very limited number of megafaunal species found within the oxygen minimum zone between 300 and 700 m (oxygen concentration 0.268–0.414 ml $l^{-1}$; see Gage 1995). The only other common megafaunal organisms found between these depths were a cocoon-dwelling bivalve, *Amygdalum* sp. (see G. Oliver in Gage 1995) and an ascidian which has been identified as a new species of *Styela* (see Young & Vazquez 1997). Such a reduced diversity of benthic organisms within oxygen minimum zones is typical, such low concentrations of oxygen preventing all but the most tolerant species inhabiting such regions. Reduced consumption and degradation, in the oxygen minimum zone, of organic material sinking from highly productive surface waters means that there is an abundant food supply for any organisms that can survive in low oxygen conditions (Wishner *et al.* 1990) and there may also be some degree of protection from predation (Saltzman & Wishner 1997). Organisms that inhabit such regions in other parts of the world have been shown to possess highly specialized anatomical and physiological adaptations to low oxygen conditions (see Childress & Thuesen 1992 for a review). These organisms can support aerobic metabolism down to oxygen concentrations of 0.15 ml $l^{-1}$ for routine activity, but may resort to anaerobic metabolism for at least brief periods (see Childress & Thuesen 1992). One species of copepod (Childress 1977) and some species of fish (Childress 1975; Belman & Gordon 1979) are able to support their entire routine metabolic needs by anaerobic metabolism. Alcock (1902) suggested that the greatly inflated gill chambers of *E. armstrongi* were an adaptation to low oxygen concentrations. It seems likely that *E. armstrongi* may show other physiological adaptations to low oxygen conditions.

Between 800 and 1000 m depth, at the base of the

oxygen minimum zone, a greatly increased diversity and biomass of megafauna were detected (see Gage 1995; Levin *et al*. 1997). Similar increases in biomass and diversity of organisms at the bottom of oxygen minimum zones have been reported in other regions (Neyman *et al*. 1973; Wishner *et al*. 1990, 1995; Levin *et al*. 1991). At a depth of approximately 1000 m aggregations of spider crabs were photographed at densities of up to 137 $m^{-2}$ (Bett 1995). Although photographed, no spider crabs were captured at 1000 m depth. Thus, it is not possible to determine whether those observed by photography were *Encephaloides armstrongi* or another species such as *Encephaloides rivers-andersoni*. If *E. armstrongi* proves to be the dominant megafaunal species over its entire potential geographic and bathymetric distribution (150–650 m) in the numbers observed during R.R.S. *Discovery* cruise 211, then total species numbers and biomass must be enormous. The role of this species in terms of its effects on the benthic community, bioturbation and cycling of organic carbon must be highly significant along the continental margins bordering the northern Indian Ocean.

Numerous population studies have been undertaken upon majid crabs (e.g. Edwards 1980; Diesel 1986; Meyer 1992; Hartnoll *et al*. 1993). In studies of populations of *Inachus phalangium* (Diesel 1986) and *I. dorsettensis* (Hartnoll *et al*. 1993) numbers of adult crabs were heavily biased towards females, but this was only significant in the latter study. In the present study, the sex ratio is significantly biased owing to an excess of males in the populations sampled. In previous studies of *Encephaloides armstrongi*, Wood-Mason & Alcock (1891) stated that specimens captured in the Bay of Bengal were predominantly male, and Griffin (1974) found a slight excess of males in populations sampled from the Gulf of Oman. It is notable, however, that at similar depths (approximately 300 m) to the populations sampled by Griffin (1974), spider crab populations had a higher proportion of females than at other depths, although the sex ratio was still significantly biased towards males ($\chi^2 = 4.0$, d.f. $= 1$, $p < 0.05$).

Sex in crabs is thought to be determined by a pair of heteromorphic sex chromosomes (Ginsburger-Vogel & Charniaux-Cotton 1982). If so, then an equal sex ratio should occur in the eggs of crabs (Hartnoll *et al*. 1993). The large bias in overall sex ratio towards male crabs in *E. armstrongi* is very difficult to explain. Many previous examples of deviations from a 1:1 sex ratio have involved a bias towards females (e.g. Diesel 1986; Hartnoll *et al*. 1993), although in a few cases more male crabs have been captured than females (see Rodhouse 1984).

Explanations for such extreme deviations from a 1:1 sex ratio include: sex reversal, differential behaviour and differential growth. Sex reversal in spider crabs is rare and has only been detected in a single shallow-water species, apparently as a response to parasitization (see Roper 1979). There was no evidence in the specimens collected in the present study of sexually indeterminate individuals, and sex reversal is considered to be unlikely.

Differences in the movement of male and female brachyuran crabs or differences in the timing of migration have been cited as reasons for variable sex ratios (see Allen 1966; Kergariou 1976; Edwards 1980; Rodhouse 1984; McMillen-Jackson *et al*. 1994). Segregation resulting from these types of behaviour may explain the variable sex ratios found in this study, and this is supported by morphometric and genetic data (see below). Differential behaviour has also been cited as causing a variable sex ratio in spider crabs by causing differential mortality between male and female crabs. However, it is usually male crabs that suffer increased mortality, because of competition or fighting for females or as a result of predation when searching for mates (Diesel 1986; DeGoursey & Auster 1992). A difference in behaviour between males and females may also cause a difference in gear selectivity between the sexes.

Sex ratio in the present study also varied with carapace size class (see figure 2). Although male crabs were dominant in all size classes, the weighted distribution curve indicated that the proportion of females was highest in the 15–24 mm size class range. In smaller, and presumably younger individuals, the sex ratio could be biased owing to difficulties in sexing immature individuals. The distribution curve (figure 2) is similar to that termed as an 'anomalous pattern' of sex ratio distribution by Wenner (1972). Wenner considered five explanations for this pattern, which are generally the same as those used to explain the overall sex ratio distribution (see above). The longevity factor is considered as the most probable cause of the skewed sex ratio in the larger size classes in the present study. Wenner (1972) stated that if males grew to a larger size than females, a dip in the sex ratio curve would arise because of the accumulation of females in that size class. In the present study only two females were recorded with a carapace length over 30 mm, and it is probable that the instar with a modal peak at 24 mm (see figure 3) represents the terminal moult in almost all female crabs.

The significant difference in the relationship between carapace length and breadth in the two sexes is a secondary sexual characteristic. In small ($\leqslant 20$ mm carapace length), and presumably immature individuals, there is no significant difference in carapace width between male and female crabs. However, carapace breadth is larger in male crabs that are mature or close to maturity, than in female crabs. Male *E. armstrongi* probably stand over female crabs during precopulatory or postcopulatory mate guarding behaviour and a large carapace width may be advantageous. Male crabs may also exhibit agonistic behaviour during mating, as in other majid crabs (DeGoursey & Auster 1992), and a large carapace (length and breadth) may also be advantageous in conflicts between rival males.

The size distribution data for both male and female *Encephaloides armstrongi* indicate that there were significant differences in size distribution between male and female crabs at all sample sites (see results). Furthermore, the Scheffe *F*-test indicated that differences within sexes existed between sites. Crabs from the 150 m depth population (Station 12731 # 1)

were significantly smaller in size in both sexes when compared to other sites (see table 3). The 150 m station therefore probably represents a juvenile population of *E. armstrongi*. It is possible that eggs or larvae of *E. armstrongi* drift or migrate towards the surface and are swept on to the continental shelf by onshore currents and this represents a nursery area for juvenile crabs. These migrate down the continental slope as they reach maturity. Such an onshore movement of larvae has been suggested for other species of spider crab in which juveniles are found in much shallower water than adult crabs (e.g. see Roper 1979). This is in contrast to the deep-sea crab, *Geryon trispinosus*, from the northern Atlantic, in which juveniles migrate up the continental slope as they mature (Attrill *et al*. 1990).

No other significant differences in size distribution were detected between sites in female *E. armstrongi*, although sample sizes were low for some populations. In male *E. armstrongi*, however, significant differences in size distribution were detected, notably between population 12733#1 and populations 12696#1, 12697#, 12707#1 and between 12697#1 and 12701#1. One possible explanation is gear selectivity, as population 12733#1 was the only population captured using the IOS epibenthic sledge. However, female crabs showed greater similarities in size distribution between sites than male crabs even when different sampling gear was employed (e.g. populations 12697#1 versus 12701#1). It is therefore considered that sampling gear had a small and probably insignificant effect on the size distribution of the crabs in the present study. Male crabs sampled from approximately 300 to 350 m did not show significant differences in size distribution, but crabs from the deeper-water sites did show significant differences between each other and between themselves and the shallower sites.

### (*b*) *Population genetics of* Encephaloides armstrongi

Analyses of existing data on levels of genetic differentiation compared to levels of taxonomic separation indicate that there is a low level of overlap in values of genetic identity ($I$) between conspecific populations, and between separate species, and that about 97% of $I$ values between species fall below 0.85 (Ayala 1975, 1983; Thorpe 1982, 1983; Thorpe & Sol-Cava 1994). Nei's (1972) $I$ values between samples in the present study are high ($I = 0.98$–$1.00$; see table 5) and these populations are, therefore, likely to be conspecific. However, $I$ values are subject to a relatively large degree of error owing to the low number of loci screened (Gorman & Renzi 1979; Nei 1987).

Levels of genetic variability estimated for populations of *Encephaloides armstrongi* in the present study are similar to those observed in other decapod crustacea (Hedgecock *et al*. 1982) and for deep-sea crustacea in general (Gooch & Schopf 1972; Costa & Bisol 1978; Bucklin *et al*. 1987; Rasmussen *et al*. 1993; France 1994). Estimates of heterozygosity are related to both sample size and the number of enzyme loci screened (see Gorman & Renzi 1979). Because of the relatively low number of loci screened in the present study there will be a high degree of sampling error in heterozygosity estimates. Populations 12707#1 and 12731#1 also have fewer individuals than other populations, and an increased degree of error in heterozygosity estimates may have occurred in these populations (see Gorman & Renzi 1979).

Significant heterozygote deficiency is a common feature of genetic studies of marine invertebrates both in shallow water (e.g. Zouros & Foltz 1984; Singh & Green 1985; Volkaert & Zouros 1989; Borsa *et al*. 1991) and the deep sea (e.g. Bucklin *et al*. 1987; Black *et al*. 1994; France 1994; Jollivet *et al*. 1995*a*; Creasey *et al*. 1996). Explanations for heterozygote deficiency fall in to five categories: (i) as a result of inbreeding; (ii) as a result of mixing of populations (Wahlund effect); (iii) assortative mating (Karlin 1969; Wright 1969; Nei 1987); (iv) selection; and (v) the presence of null alleles or the loss of chromosomes causing incorrect estimates of genotype frequency (Zouros & Mallet 1989). Null alleles were not detected in the present study and data from previous studies on deep-sea crustacea indicate that their occurrence is extremely rare (for $Pgi < 1$ in 1000; Creasey *et al*. 1996; Creasey, unpublished data for *Munidopsis* spp.). It is therefore considered that this was not a cause of incorrect estimates of genotype frequency leading to lower than expected heterozygosities. Selection has been cited as a possible cause for heterozygote deficiencies in other deep-sea organisms (Jollivet *et al*. 1995*a*, *b*; Creasey *et al*. 1996), but in such cases deficiencies are only generally detected for a few enzyme loci among those sampled. This is also the case in assortative mating (Nei 1987). In the present study heterozygote deficiencies were detected across all variable loci sampled and this indicates that the major cause of this deficiency is likely to be at the level of populations, i.e. because of inbreeding or the mixing of two or more populations.

High levels of heterozygote deficiency are also reflected in the significant $F_{IS}$ values detected for all loci when populations are taken separately or when they are pooled by depth (see table 8). This is congruent with high levels of inbreeding or with the Wahlund effect. When male and female spider crabs are analysed separately, $F_{IS}$ values were still mostly significant (see table 9). When population 12731#1 (150 m) was removed from these analyses, on the grounds that crabs from this station are much smaller than those at all other stations and probably represent a separate (juvenile) cohort, $F_{IS}$ became non-significant for female crabs but remained highly significant for almost all loci for male crabs. Low sample sizes for some female populations may have led to an underestimate of $F_{IS}$, but this indicates a gender bias in the correlation of homologous alleles within individuals in local populations. This suggests that male crabs may be subject to a higher level of inbreeding or population mixing than female crabs. It is considered that it is not possible to generate a higher level of inbreeding in one sex than in another and so a higher level of population mixing remains the only explanation for these data.

$F_{ST}$ values are moderately high and generally significant for all populations taken separately and

pooled into different depth classes (see table 8). When male and female crabs were analysed separately, $F_{ST}$ values for female crabs were significantly different from zero at only two loci (see table 9), indicating a degree of genetic differentiation between populations. $F_{ST}$ values for male crabs were significantly different from zero for all but one enzyme locus. Omission of population 12731#1 (150 m) had little effect on $F_{ST}$ values for male populations but decreased $F_{ST}$ values further in female populations so that none were significantly different from zero. $F_{ST}$ values for each locus in female crabs with Station 12731#1 omitted are remarkably consistent, but again low sample sizes for some populations may cause an underestimation of these values. This suggests that genetic differentiation as estimated by $F_{ST}$ is higher between male populations than between female populations.

Excluding population 12731#1 which is different in terms of size and allele frequencies to all other populations (see below), a clear trend emerges from these data. Populations of female crabs do not show significant differences in size frequency distribution or significant levels of genetic differentiation from each other. It is suggested that they form a single panmictic population across the area studied (although low sample size may have led to some underestimation of genetic differentiation between samples of females). However, male crabs show significant differences in size frequency between populations along with significant levels of population differentiation over very small scales of geographic separation (approximately 30 km). High values of $F_{IS}$ also suggest that male samples originate from two or more populations (Wahlund effect). These populations may show differences in size distribution possibly resulting from differences in mortality or growth rates and in allele frequencies owing to genetic drift. Different numbers of male crabs from each of these populations at each sample site would explain differences in size frequency distribution and significant levels of interpopulation genetic differentiation and intrapopulation heterozygote deficiency.

The simplest explanation of these differences is that *Encephaloides armstrongi* exhibits male gender-biased dispersal. Gender-biased dispersal has been detected in a number of terrestrial organisms including mammals (Schaller 1972; Packer *et al*. 1991; Melnick & Hoelzer 1992) and birds (Greenwood & Harvey 1982; Woolfenden & Fitzpatrick 1984), but only in green turtles in the marine environment (Bowen *et al*. 1992). This explanation is supported by the highly skewed and variable sex ratios estimated in the present study (table 2), and fits with previous observations on sex-related differential migration in spider crabs (see above). In *E. armstrongi*, male gender-biased dispersal and variable sex ratios may be related to spawning behaviour involving aggregation, as has been observed in other spider crabs (e.g. *Maja squinado* in Nelson 1991). The majority of coastal species on the west coast of India spawn in the period from September to December (Pearse & Barksdale 1986). This coincides with the sampling period for the present study which was through October–November and which fits with the hypothesis that the high densities of *E. armstrongi* observed were a result of mating aggregations, although at the time of sampling no ovigerous female crabs were captured. It is possible that ovigerous crabs were either located deeper on the continental slope (at 1000 m; see above) or avoided capture by specific behavioural patterns such as burrowing.

When $F_{IS}$ values were obtained for populations successively pooled by depth (see table 8), $F_{IS}$ increased. This appeared to indicate that as samples from different depths were pooled, heterozygote deficiency increased, as would be expected if there was genetic differentiation between vertically separated populations. Bootstrapping these data, to eliminate the effects of different sample sizes at each site, following Goudet *et al*. (1994), caused the differences in $F_{IS}$ values to decrease markedly, and a relationship between $F_{IS}$ and pooling of populations was no longer detectable. Regardless of the analyses of $F_{IS}$ values, significant levels of population mixing and genetic differentiation between all sample sites probably obscured any depth-related effects on genetic differentiation.

Specimens of *Encephaloides armstrongi* collected at Station 12731#1 at 150 m show differences in allele frequencies from crabs collected at all other stations. Because of their small size they are thought to represent juvenile crabs from a later cohort than those sampled at greater depths. The genetic differences between this cohort and the other crabs could be a result of random genetic drift which, in the case of *E. armstrongi*, may be large from generation to generation owing to a low effective population size. Alternatively, juvenile crabs may represent settlement from a large larval pool which contains individuals from geographically distant populations with different allele frequencies to those of the resident adult population. It is possible that selection eliminates or reduces the frequency of certain alleles after settlement of larvae and/or as crabs migrate down the continental slope into the oxygen minimum zone, as with models of balancing selection (see Singh & Green 1984; Zouros & Mallet 1989). It is also possible that a few individuals of a second but closely related species of spider crab were among individuals sampled at the 150 m station.

No berried female crabs were found, so it is not possible to ascertain the type of larval development that is exhibited by this species, in the present study. Assuming that development in *Encephaloides armstrongi* follows a similar pattern to that in other majid spider crabs it would be expected that its life history includes an abbreviated pelagic larval stage (Gore 1985; Rabalais & Gore 1985). If this is the case, significant levels of genetic differentiation over the distances between sample sites in the present study, which are in the order of tens of kilometres, are remarkable. Significant levels of genetic differentiation over small geographic distances have been observed in other invertebrates that have pelagic larvae, including nudibranchs (Todd *et al*. 1988, 1994) and spider crabs (L. I. Weber, R. G. Hartnoll & J. P. Thorpe, personal communication, University of Liverpool, Port Erin Marine Laboratory, Port Erin, Isle of Man). For

nudibranchs it has been suggested that while larvae can remain competent and do not settle for extended periods of time in laboratory conditions, in the natural environment they settle rapidly to avoid being swept away from conditions favourable for their growth and survival (Todd *et al*. 1988, 1994). This may be particularly important in species which are exposed to extreme and unpredictable environmental fluctuations or which occupy a narrow ecological niche (see Rabalais & Gore 1985).

We acknowledge the support of the Natural Environment Research Council (NERC) with CASE Studentship Award No. GT4/94/247/P to S.C. Material for this study was collected during R.R.S. *Discovery* cruise 211/94 funded by NERC grant GR3/8927 to J.G. and Dr D. J. Smallman, Scottish Association for Marine Sciences (SAMS). P.T. acknowledges the provision of a SAMS bursary. C.Y. acknowledges the support of NSF grant OCE-9116560. We also thank Dr M. Whitfield for the use of the laboratory and library facilities at the Marine Biological Association of the UK, Plymouth.

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*Received 19 July 1996; accepted 30 August 1996*