Effects of salinity on the survival, growth rate, and reproductive output of *Gammarus lawrencianus* (Crustacea, Amphipoda)

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ABSTRACT: *Gammarus lawrencianus* is classed as a genuine brackish-water species on the basis of the survival of juveniles, developing eggs and of reproducing adult females. This is consistent with its estuarine distribution in the northwestern Atlantic. At low salinities *G. lawrencianus* produced larger and fewer eggs but a total brood volume equivalent to that produced at higher salinities. Young hatched from the larger eggs at low salinities equalled in length those hatched from smaller eggs produced at higher salinities.

INTRODUCTION

Salinity is an important environmental variable for estuarine organisms and its many important physiological and ecological effects have been reviewed by Kinne (1964, 1966, 1971). Most studies of crustaceans have dealt with the mechanisms by which adults adapt to varying salinities (Dorgelo 1976). According to Gilles & Pequeux (1983) relatively few studies have been made of the effects of salinity on reproduction and embryonic development, although in amphipods such studies have been made for *Gammarus duebeni* by Kinne (1959), for *Echinogammarus marinus* by Vlasblom & Bolier (1971) and for *Corophium* spp. by Shyamasundari (1976) and Mills & Fish (1980).

These studies have generally shown that the number of eggs per brood, the viability of the developing embryos and the growth rate of the young are maximal at an intermediate salinity characteristic for the species. Adults can tolerate a wider range of salinities but may not be able to reproduce at low salinities. However, there is little information available on possible effects of salinity on eggs and total reproductive output in any species, although Hynes (1954) reported that the eggs of *Gammarus duebeni* from fresh water were larger than those from brackish water.

Gammarus lawrencianus Bousfield is a small estuarine amphipod of the northwestern Atlantic (Steele &

Steele 1974) which is easily cultured in the laboratory. Previous studies have considered its life cycle in the field (Steele & Steele 1970) and its life history tactics when compared to other *Gammarus* species (Steele & Steele 1975). Its survival, growth and reproduction vary with diet (Vassallo & Steele 1980, Steele 1982) and photoperiod (V. J. Steele 1981, V. J. Steele & Steele 1986) when cultured in the laboratory.

The purpose of the present study was to determine if salinity variations influenced the growth rates and reproductive outputs (egg sizes and numbers of eggs per brood). However, it was first necessary to determine how salinity affected the survival of young and of reproducing females, since this had not yet been determined for this species.

MATERIALS AND METHODS

Three separate experiments are described and discussed here. Expt 1 in spring 1985 determined growth and survival rates to maturity and reproductive output of maturing females raised at different salinities.

Adults collected in the small estuary at Witless Bay, on the east coast of the Avalon Peninsula, Newfoundland, Canada (47° 16' N, 52° 49' W) were maintained in a container of seawater (salinity 30‰). Newly hatched young were pooled in a large container. Groups of 25

young were placed in small containers and maintained at 20 °C under 18L:6D photoperiods. Freeze-dried alga *Pilayella littoralis*, collected on the open coast at Witless Bay, was provided in excess as food.

The small containers contained water of different salinities, prepared by diluting filtered seawater with distilled water. The salinities used were 0, 2.5, 5, 10, 15, 25 and 30‰. At least 2 replicates of each salinity were used.

Cultures were examined every 3 d until mating pairs were anticipated, at which time observations were made every morning. Mating normally occurs at night and the ovigerous females were removed and preserved the following morning. The length of the extended female parent was measured from the anterior tip of the head to the end of the telson. The eggs were removed from the brood pouch, counted and their length and width measured. Mean diameter of an egg was calculated as (length + width)/2 and the volume of an egg as a prolate spheroid: $4/3\pi$ length/2 $\times$ (width/2)^2 Brood volume was determined as the mean volume of the eggs in a brood $\times$ their number. Amphipods were counted every 7 to 8 d.

Expt 2 in spring 1989 examined the duration of embryonic development, survival of developing embryos and the sizes of newly hatched young at different salinities. Observations of growth, survival and reproductive output of the maturing females were also recorded.

This experiment was similar to the first except that only salinities of 5, 15 and 35‰ were tested. Four containers of 25 amphipods were established at each salinity. Specimens in 2 containers at each salinity were processed as in the first experiment when the females matured and produced their broods. Specimens in the other 2 containers were maintained until their eggs hatched. The newly hatched young were counted and measured.

Expt 3 compared the survival rates of breeding and non-breeding adult females at different salinities. The females were induced to enter or leave ovarian diapause by exposure to photoperiods at different salinities. Adult females were established in cultures in June 1980. On July 20, groups of 30 females plus a few males were placed in small containers. One half of these containers had water with 30‰ salinity and the other half 10‰. Excess food in the form of freeze-dried *Pilayella littoralis* was provided.

Breeding status of the females was determined by the presence (breeding) or absence (non-breeding) of setae on their oostegites. The number of females in each category and the total number of surviving females was determined after 62, 102, 178 and 240 d.

The statistical significance of the differences between the means of size and age at maturity, growth rate to maturity and egg diameter was determined by analysis of variance (ANOVA) and Newman-Keuls (SNK) multiple range tests. Although excess food was provided in each container the intra-unit correlations were negative, suggesting that there may have been competition for food and thus that there is a risk of a type II error. Therefore the individual observations were used when testing for statistical significance (Hairston 1989). The number of eggs was regressed on length of the parent in grand mean regressions using power curves. The volume of the broods was similarly regressed on length (equivalent to volume of the parent), also in grand mean regressions using power curves. The regression lines were compared using analysis of covariance (ANCOVA). Correlations were determined with the product moment correlation coefficient. In all cases the accepted level of significance is 0.05.

RESULTS

Survival of immature females

Since mortality is greater in breeding females than it is in non-breeding females (V. J. Steele & Steele 1986), survival is only comparable until the females mature and commence breeding. As reproduction began after varying intervals at the different salinities, survival is shown for varying time periods in Fig. 1. Females placed in distilled water survived for a few days but all died before reproducing. Those in 2.5‰ suffered a 64 % mortality in the first 8 d but the survivors were much more tolerant of this low salinity and there was only a 23 % mortality during the next 42 d, at which time they commenced breeding

When compared for a standard time period of 21 d, which is before breeding began at any salinity, the highest survival occurred between 15 and 20‰ (Fig. 2). The low salinity LC$_{50}$ for 21 d is calculated by probit analysis to be 6.1‰. Survival times increased with salinity (Fig. 1). However, after 21 d when some started breeding, the change in LC$_{50}$ over time becomes confounded with increased mortality due to reproduction.

Survival was almost identical at 5‰ and 15‰ in Expts 1 and 2 (Fig. 2). However, survival was higher at 35‰ in Expt 2 that it was at 30‰ in Expt 1. Inspection of Fig. 1 shows that the reduced survival at 30‰ was due to a high mortality in the first week of the experiment even though the salinity was the same as that in which the amphipods had previously been held. After the first week in the small containers survival at 30‰ was similar to that observed at 35‰. Thus the survival rate plotted at 30‰ in Fig. 2 is undoubtedly too low.
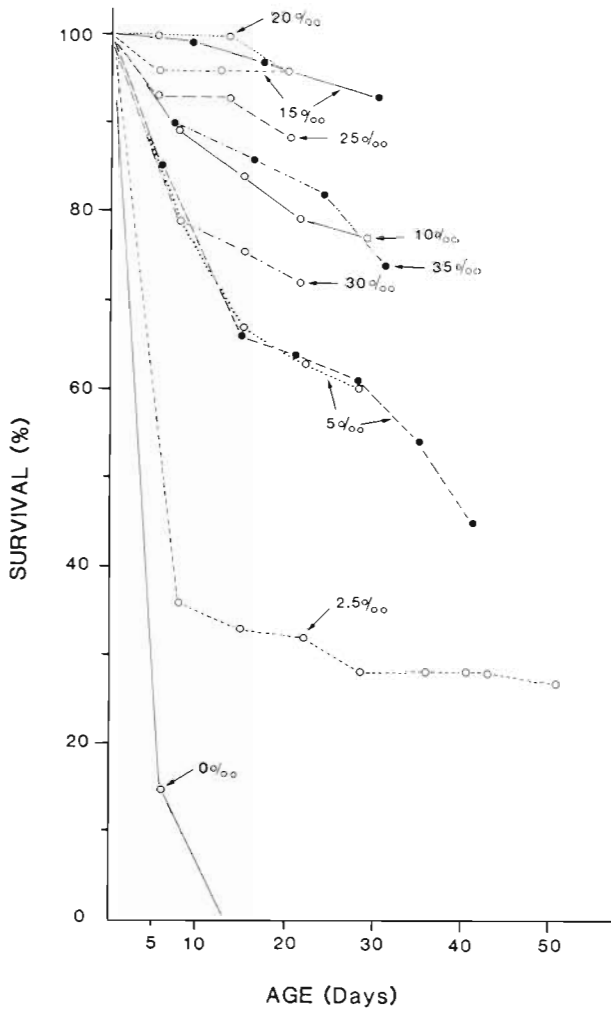
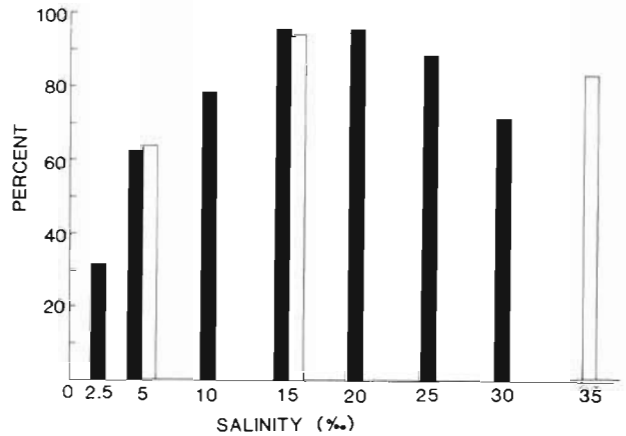Fig. 1 *Gammarus lawrencianus*. Survival rates (%) of immatures at various salinities

Fig. 2. *Gammarus lawrencianus*. Survival rates (%) of immatures for a period of 21 d at various salinities. Solid bars: Expt 1; open bars: Expt 2

Reproduction and survival of adults at 10‰ and 30‰ salinities under varying photoperiods

Salinity had no apparent effect on either the induction or termination of ovarian diapause. The mortality of breeding females was much lower at 30‰ than at 10‰ (Fig. 3) and was correlated with the percentage breeding (r = 0.96, p < 0.01). At 10‰, mortality was high and varied only slightly with the percentage breeding.

Growth rate and size and age at maturity

Expt 1. Growth rate to maturity (Fig. 4) did not differ significantly between 15 and 30‰ (ANOVA, p > 0.05), but declined progressively at salinities from 10‰ to 2.5‰ (SNK, p < 0.05).

Length at maturity (Fig. 5) did not differ significantly between 5 and 25‰ (ANOVA, p > 0.05). Those maintained at 2.5‰ were significantly longer and those at 30‰ were significantly smaller than those raised at the intermediate salinities (SNK, p < 0.05). However, lengths observed at 30‰ are suspect due to a higher than expected mortality (see below) at this salinity and since the size at maturity of females at 35‰ in the second experiment was large (see below).

Age at maturity (Fig. 6) varied inversely with the growth rates and females at 2.5‰ salinity took longest to reach maturity. The relationship between growth rate and age at maturity is non-linear and similar to that observed when diet was varied (Fig. 6; Steele 1982).

Expt 2. Growth rates to maturity (Fig. 4), age at maturity and size at maturity (Fig. 5) did not differ significantly at 15 and 35‰. At 5‰ growth rate to maturity was significantly less, age at maturity greater and size at maturity smaller than at the
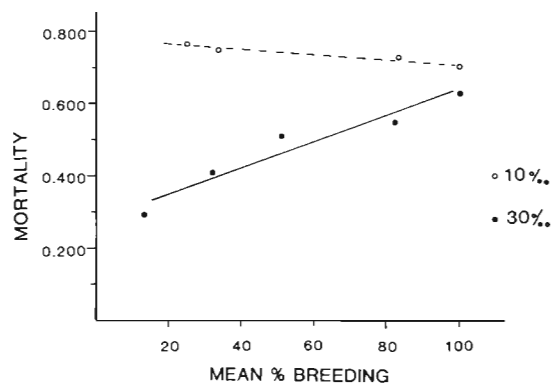

Fig. 3. *Gammarus lawrencianus*. Mortality rates of females related to percentage breeding at 10‰ and 30‰ salinity. Breeding was induced or terminated by varying photoperiods
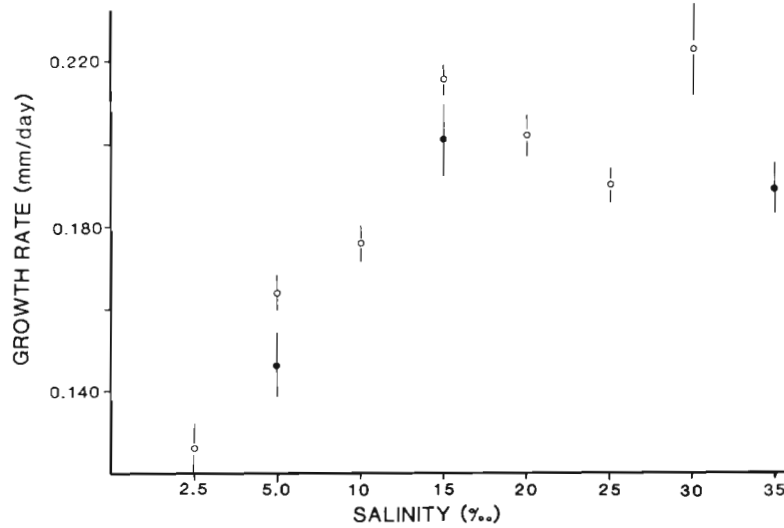
Fig. 4. *Gammarus lawrencianus*. Growth rates (mean ± SE) of females maintained at various salinities. (o) Expt 1; (•) Expt 2
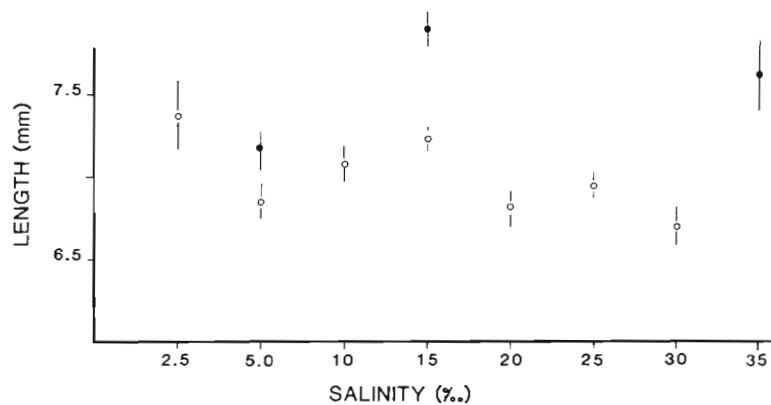

Fig. 5. *Gammarus lawrencianus*. Length at maturity (mean ± SE) of females maintained at various salinities. (o) Expt 1; (•) Expt 2

other salinities. This trend is similar to what was found in Expt 1. However, the absolute values obtained in Expt 2 all differed significantly from those obtained in Expt 1 at the same salinity. The reason for these differences is unknown, but it may have been related to differences in the quality of the *Pilayella littoralis* used as food in the 2 experiments.

Reproductive output

Expt 1. Mean egg diameter (Fig. 7) was greatest at 2.5‰ and decreased as salinities increased up to 15‰. Egg size did not differ significantly at salinities between 15 and 30‰.

The number of eggs varied directly with body length (Fig. 8A). It was significantly less (ANCOVA, $p < 0.05$) at a salinity of 2.5‰ than at the higher salinities. At 5‰, brood size was also lower than at the higher salinities but the difference is not significant (ANOVA, $p > 0.05$). Since two-thirds of the maturing females at 30‰ were 7 mm in length, it was not possible to calculate a regression with a reasonable slope at this salinity. However, the mean number of

eggs produced by the 7 mm females did not differ significantly from the numbers produced by females of the same size at salinities between 5 and 25‰. (ANOVA, $p > 0.05$).

The interaction between numbers of eggs produced and their volumes is such that the total volume of the broods show no consistent variation with salinity between 2.5 and 25‰ (Fig. 9). Thus the smaller numbers of eggs produced at 2.5‰ was compensated for by their larger size so that the brood volume was comparable to that of the other saliniities. However, at any particular salinity, the sizes of the eggs are not correlated with the number of eggs in the broods produced by individual females ($r = 0.1$, $p > 0.05$).

Expt 2. Similar trends in reproductive output were found in this experiment although the actual values differed from those in the first experiment. Mean egg diameters were larger and varied from 0.470 mm at 5‰ to 0.407 mm at 35‰. The sizes of the broods did not vary significantly with salinity (Fig. 8B) but were significantly less than in Expt 1. Brood volume also did not vary with salinity. The interaction between egg volume and numbers at each salinity resulted in brood volumes that did not differ between the 2
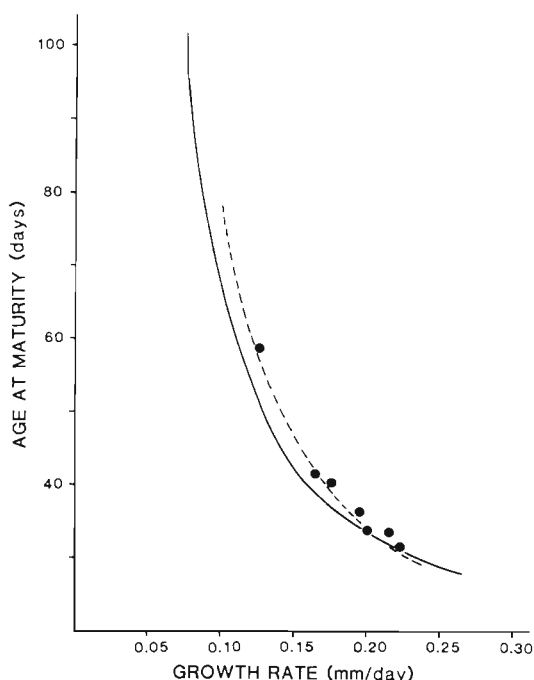
Fig. 6. *Gammarus lawrencianus*. Age at maturity of females related to growth rate. (●−−−●) [Fitted line: Age = 8.046 (Growth Rate − 0.050)$^{-0.761}$] obtained from the various salinities. (——) [Age = 11.079 (Growth Rate − 0.050)$^{-0.605}$] obtained with diet as the variable (Steele 1982)

experiments at 15‰. However, brood volumes were smaller in Expt 2 at 5‰.

Embryonic development was of the same duration at 15 and 35‰. Hatching success of the embryos varied with salinity. No embryos hatched at 5‰. At 15‰, only 57% of the broods hatched, and of those that did, the

numbers of young did not differ from the number of embryos (Fig. 10). All broods hatched at 35‰, but there were significant decreases in their sizes.

DISCUSSION

Salinity as an environmental factor has been considered mostly in terms of its effects on survival and distribution, and only to a much lesser extent as an environmental parameter affecting the reproductive strategies of marine animals. Both aspects are discussed here for *Gammarus lawrencianus*.

Dorgelo (1976) suggested that crustaceans could be divided into 5 groups on the basis of their salt tolerance. *Gammarus lawrencianus* would be considered as a 'more or less euryhaline genuine brackish-water species' (Type IV) since maximum survival to maturity occurred at salinities of 15 to 20‰. However, individual females survived and produced eggs at higher and lower salinities.

These experimental results are consistent with the distribution of this species (Steele & Steele 1970). *Gammarus lawrencianus* is normally found in estuaries and not on the open coast at high salinities (30+ ‰). The only exceptions are during periods of heavy freshwater runoff, such as the period of snow melt in the spring or after a heavy rainstorm. At these times specimens of *G. lawrencinaus* may be found on open coasts if near an estuary. *G. lawrencianus* may also be found localized at about midtide level on coarse sedimentary shores if there is freshwater seepage (Steele 1983).

Within estuaries, *Gammarus lawrencianus* is potentially exposed to varying salinities, rather than
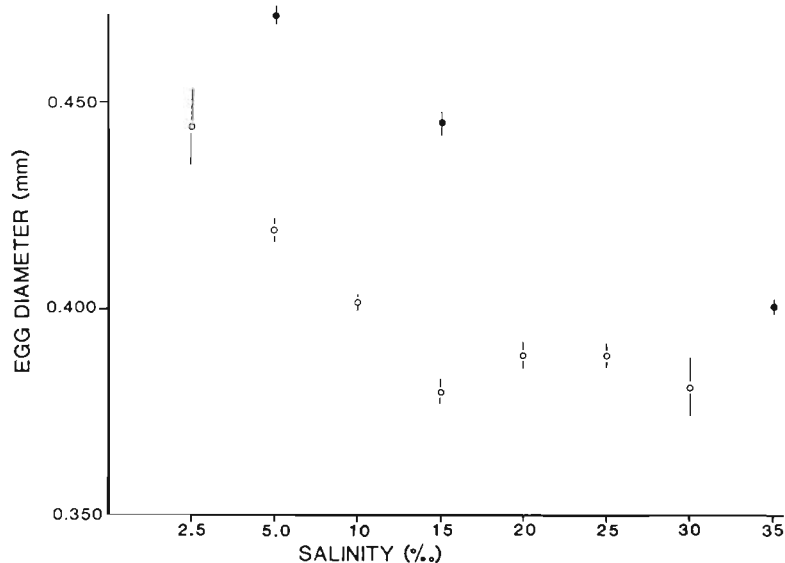

Fig. 7 *Gammarus lawrencianus*. Egg diameters (mean ± SE) at various salinities. (o) Expt 1; (●) Expt 2
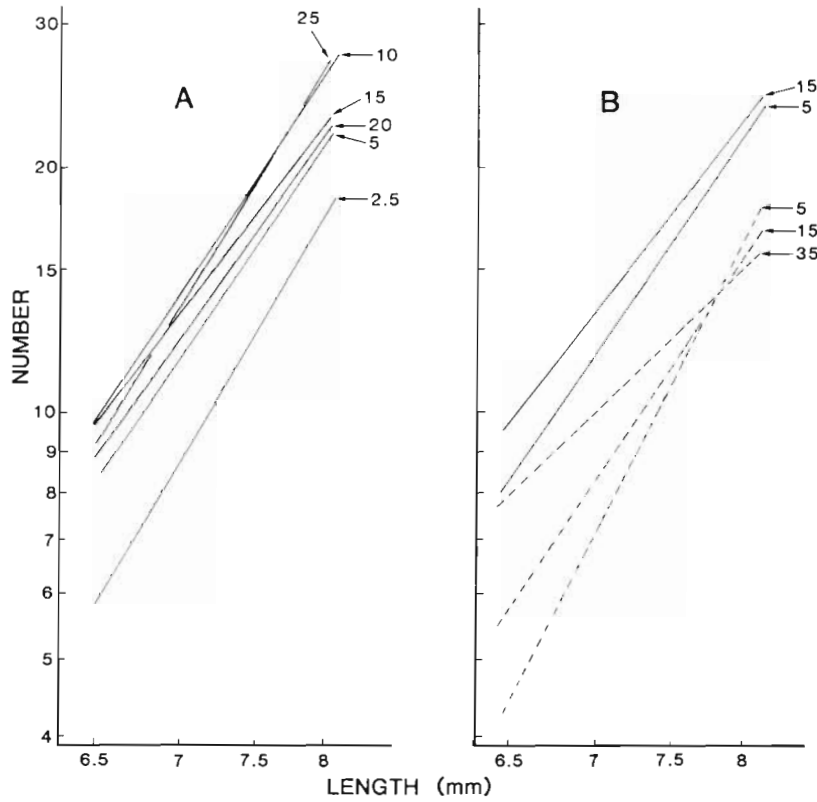
Fig. 8. *Gammarus lawrencianus*. Grand mean regressions of numbers of eggs related to female body length at various salinities. (A) Expt 1; (B) (——) Expt 1; (– – –) Expt 2

the constant salinities used in the present experiments. However, individuals can and do avoid extreme fluctuations by moving horizontally and/or vertically. During freshets they move further outwards or downwards. In the 2-layered estuaries typical of eastern Newfoundland they are found in the saline water below the surface layer of fresh water or under rocks where the residual water
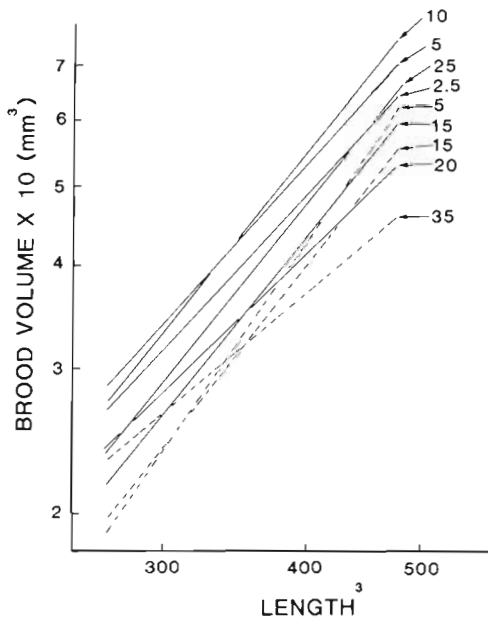

Fig. 9. *Gammarus lawrencianus*. Grand mean regressions of brood volumes related to cube of body length at various salinities. (A) Expt 1; (B) (——) Expt 1; (– – –) Expt 2
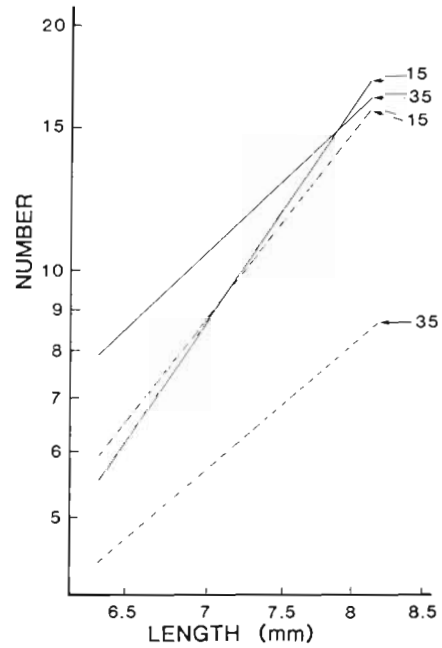

Fig. 10. *Gammarus lawrencianus*. Grand mean regressions of the numbers of eggs (——) and numbers of hatched young (– – –) against female body length at 15 and 35‰

remains saline even during low tides. The only individuals to survive the winter are found at the bottom of the 2-layered estuaries.

In New Brunswick, where *Gammarus lawrencianus* and its near relative *G. tigrinus* coexist in estuaries, *G. lawrencianus* occurs seaward of the latter species, although there is some overlap (Steele 1983). Dorgelo (1976) classed *G. tigrinus* as an 'extremely euryhaline species that tolerates the entire range from marine to limnetic conditions equally well' (Type III). However, the experimental results (Dorgelo 1974) indicate that the highest survival of adults was at salinities between 1 and 17‰ with higher mortalities at the higher and lower salinities. This would place *G. tigrinus* in Type IV with *G. lawrencianus* and its maximum survival at 1 to 17‰ is consistent with its estuarine distribution in both North America and Europe.

The higher mortalities of breeding females and of developing eggs at low salinities show that the salinity tolerances for reproduction are reduced. Reduced viability at low salinities has also been reported in *Corophium* spp. by Shyamasundari (1976) and Mills & Fish (1980). Vlasblom & Bolier (1971) likewise found a reduced viability in the eggs of *Echinogammarus marinus* at low salinities and concluded that the egg membrane offered 'only weak protection to the embryos' so that the extra-embryonic fluid of the eggs becomes isotonic in a short time. Thus at low salinities the eggs swell by imbibing water due to osmosis.

Within its estuarine habitat, *Gammarus lawrencianus* undergoes marked seasonal fluctuations in abundance. In the winter females have an ovarian diapause, reproduction stops and the population declines. However,the multivoltine (small size at maturity, short embryonic development period) and iteroparous life cycle results in the attainment of a high population density by late summer (Steele & Steele 1970).

The present study shows that growth and survival of the young are maximal at the intermediate salinities where the population is normally found in nature. This is similar to what has been observed in another estuarine species, *Gammarus duebeni*, by Kinne (1959). However, the optimal salinities for growth are higher (15+ ‰) for *G. lawrencianus* than for *G. duebeni* (5 to 20‰) and, where they occur together, *G. duebeni* is found landward of both *G. lawrencianus* and *G. tigrinus* (Steele 1983).

Kinne (1959, 1971) observed that the final body length in *Gammarus duebeni* was greatest at a salinity of 10‰ with lower values at both higher and lower salinities. In *G. lawrencianus* the size at maturity was variable but did not vary consistently with salinity.

The relationship between growth and the age at maturity observed with different salinities is similar to that found when diet influences the growth rate;

when growth is reduced by low salinity the age at maturity increases. However, the effect is not uniform over the range of growth rates. Although very low growth rates were not achieved with salinity as the variable, at salinities of less than 2.5‰ growth would be so slow that maturity would not be reached in the normal life span.

Egg size increased at salinities less than 15‰, so that eggs at 2.5 and 5‰ salinity in Expt 1 and at 5 and 15‰ in Expt 2 exceeded the mean size (0.409 mm) of eggs measured in field collections (Steele & Steele 1970).

The large eggs produced in Expt 2 at 5 and 15‰ salinities had reduced viabilities. The young hatched from the large eggs at 15‰ took the same time to develop and were not significantly larger than those from the smaller eggs produced at 35‰.

The reduced fecundities at low salinities varied inversely with the larger size of the eggs such that the total brood volume was comparable to that at the higher salinities. This non-genetic effect was produced by exposure to low salinities and is probably due to the eggs imbibing water by osmosis, since the size of the newly hatched young did not increase. The swelling of the eggs reduced the number of eggs produced per brood, but the total volume of each brood remained constant. Moreover, the large eggs produced at the low salinities did not hatch. Thus, the production of eggs at the low salinities is not an indication of successful adaptation.

Geographic and seasonal variations in egg size have often been reported in amphipods, including *Gammarus lawrencianus* (e.g. Steele & Steele 1970) and other crustaceans but they usually have been related to variations in water temperature rather than salinity. Mashiko (1990), however, reported variations in egg size in the prawn *Macrobrachium nipponense* that were related to habitat salinity. There was an inverse correlation between the salinity of the habitat and egg size. The number of eggs per brood was in turn inversely correlated with egg size but the total size of the brood measured as the ratio of the mass of the eggs to that of the female parent varied independently of the habitat. At some, but not all sites, large eggs compensated for a low number. This is similar to what was found here in the present experiments. However, the differences in *M. nipponense* are evidently genetic since young raised at different salinities 'laid eggs of unchangeable size'. In addition, larger zoea hatched from larger eggs and vice versa (Mashiko 1990).

Freshwater *Gammarus* species, in common with many other crustaceans, produce larger eggs than the marine *Gammarus* (Steele & Steele in press). These differences in egg size must also be partly genetic, but the reasons remain obscure.

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