

SUBSPECIES, MORPHS AND CLINES IN THE AMPHIPOD *GAMMARUS DUEBENI* FROM FRESH AND SALINE WATERS

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Introduction

As a general rule, most species of *Gammarus* typically live in one of three major environments: near-constant salinities of the marine-littoral, fluctuating brackish waters in estuaries and saltmarshes, or the low salinities of freshwater streams and lakes. A few straddle two of these major environments, for example *G. tigrinus*, a North American coastal brackishwater species that is colonising some fresh waters in western Europe (Gledhill et al. 1993). But *Gammarus duebeni* Liljeborg is outstandingly versatile, well known for its ability to tolerate and colonise environments where salinities greatly exceed those of normal seawater, as in hypersaline supralittoral rockpools where the salt content may be doubled due to evaporation. It also occurs in fresh water on peninsulas and islands around the western seaboard of Britain, where close proximity to the sea has slightly raised the concentration of sodium and chloride. Furthermore, *G. duebeni* is widespread and indeed the common gammarid in the fresh waters of Ireland and western part of Brittany.

The exceptional wide-ranging tolerance of *G. duebeni* to salinities that encompass three orders of magnitude in salt content was known in the 19th century and has attracted considerable attention from physiologists and ecologists for much of the 20th century, including the likelihood that populations in fresh water are distinct from those living in more saline environments; some of the extensive literature is reviewed and conveniently summarised by Hynes (1954), Kinne (1959), Pinkster et al. (1970), Sutcliffe (1972, 1978) and Fryer (1978); also see MacNeil et al. (1999).

Because *G. duebeni* is widespread in the rivers and loughs of Ireland, Reid (1939) suggested that it was an a form physiologically adapted to fresh water and thus distinct from a (i form living in brackish water. Later studies showed that *G. duebeni* from Irish fresh waters low in sodium chloride is indeed distinguished by a suite of osmoregulatory features conferring improved survival at increasingly lower sodium concentrations. However, osmoregulation in specimens from fresh water on the Kintyre and Stranraer peninsulas, and from the Isle of Man, is intermediate between that of the Irish populations and those from brackish water in northeast (Budle Bay) and

northwest (Morecambe Bay) England and, although the adaptive features are most pronounced in *G. duebeni* from Ireland, it is not considered to be a separate physiological race (= subspecies). Instead, the experimental evidence indicates there is continuous selection from a common genetic pool including the populations living in brackish water on the coastline of Britain (Sutcliffe 1967a, 1971a,b, 1978).

Against this background of physiological variability I examine below the nature and extent of morphological variation in the same populations of *G. duebeni* and range of salinities.

Subspecies of *G. duebeni*: ratios of merus width to length

Stock & Pinkster (1970) and Pinkster et al. (1970) erected two subspecies of *Gammarus duebeni*, based on the relative dimensions of the length and width of the merus (meropodite) - the 4th segment (article) of pereopod 7 (the 5th "walking leg") (Fig. 1). In specimens from coastal brackishwater habitats on the continent of Europe (Pas de Calais northwards to Sweden), merus length was found to be less than twice the width (ratio < 2.0) and this was regarded as diagnostic for *G. duebeni duebeni*. In specimens from freshwater habitats (Eire and Brittany) merus length was much greater than twice the width (ratio > 2.0), i.e. the limb segment was narrower, regarded as diagnostic for *G. duebeni celticus*. However, due to allometric patterns of growth in *Gammarus*, the relative dimensions of some parts of the body change in proportion to other parts as the animal grows in size. Thus, because *Gammarus* has numerous moults and grows throughout most of its life, the variation in size (and ages) between individuals in each population sample greatly affects the dimensions, and hence ratios, in the segments on pereopod 7 of *G. duebeni*.

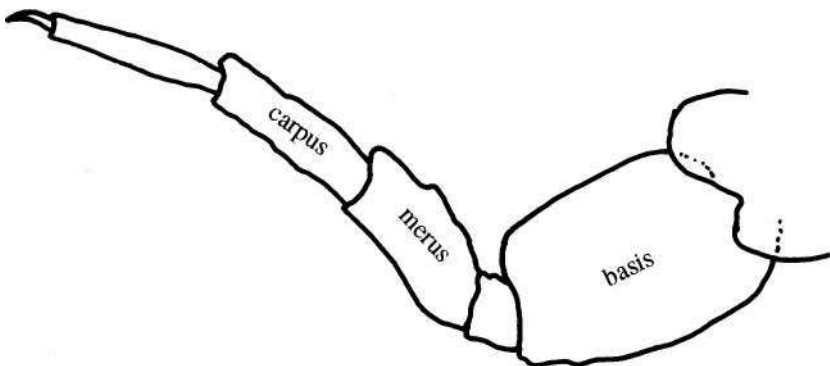


FIG. 1. Pereopod 7 of a male *G. duebeni* from fresh water in Ireland.

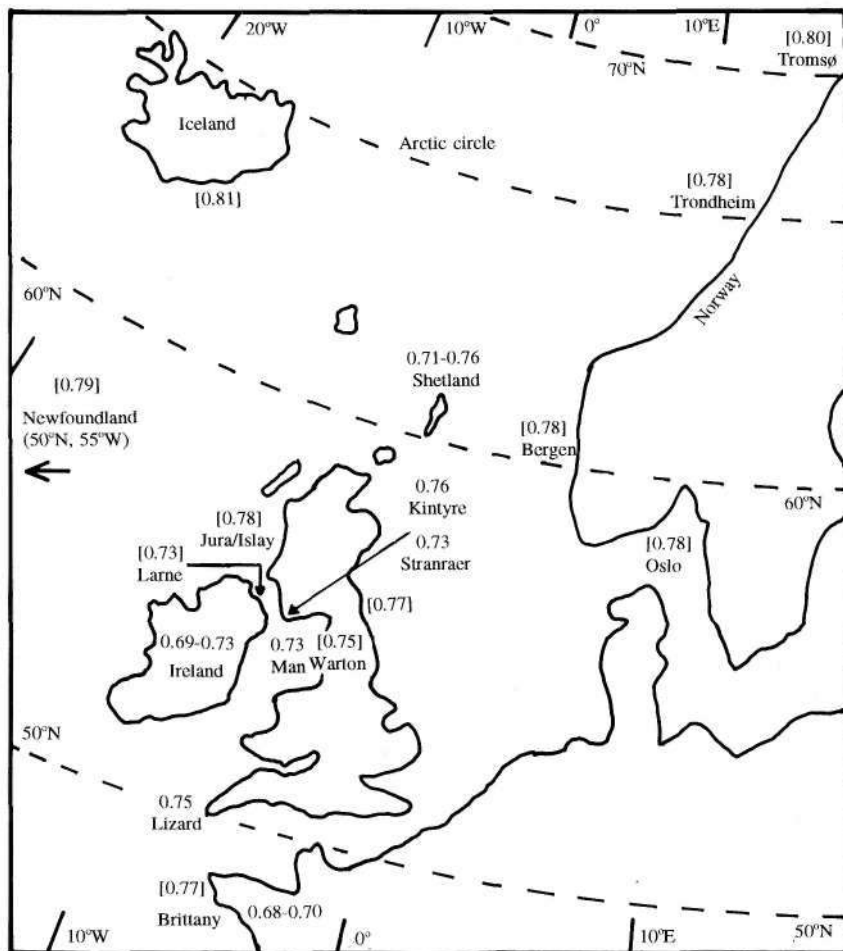


FIG. 2. A distribution map for ratios of log W/L, where W is the mean merus width and L is mean merus length for populations of *G. duebeni* sampled in various localities. Ratios for populations sampled in brackish-marine (SALINE) habitats are shown in brackets. Ratios for populations in freshwater habitats (ISLESPEN and IRISH) are not bracketed and for some a range of values is shown where several populations in streams and lakes were sampled in Ireland and the Shetland Isles. Ratios shown for Brittany and Newfoundland are from Dennert (1975). [The Canadian value is based on a small sample of 15 specimens and may be too low as Dennert calculated the mean dimensions of arithmetic measurements before transforming to logarithms. For small samples with a skewed distribution of size, this procedure yields lower values compared with my log/log measurements. For this reason other ratios given by Dennert have been ignored here].

Attempting to find a more reliable criterion for identification and to ascertain the distribution of the two subspecies, I minimised the effects of natural variation between individuals by selecting mature, relatively large males for examination. All individual measurements on limb segments were converted into common logarithms, followed by regression analysis of logio width on logio length for the merus of pereopod 7 on each specimen in a population sample. The ratio of sample mean log merus width relative to log length (i.e. mean log W/L) was then calculated. This produced two groups of values, one with mean ratios ranging from 0.74 to 0.77 in samples from brackish water on the east and west coasts of northern England, and the other group with mean ratios ranging from 0.71 to 0.73 in four freshwater populations from Ireland (Sutcliffe 1972). This, and subsequent detailed analysis of other populations (presented here and by Dennert 1975), suggested that ratios of 0.73 and below are characteristic of *duebeni celticus* in the fresh waters of Ireland and Brittany, whereas higher ratios characterise *duebeni duebeni* from coastal brackishwater habitats, ranging from Brittany to Tromsø in northern Norway, Iceland, and Newfoundland on the northeastern seaboard of Canada (Fig. 2).

However, specimens from freshwater habitats that have slightly raised sodium and chloride concentrations, on islands and peninsulas (ISLESPEN) around the western and northern coastline of the British Isles, appear to be "intermediate" forms, with merus ratios ranging from 0.71 to 0.76. Thus the mean ratios for these freshwater populations overlap the equally large variation in ratios now known to occur between populations in IRISH fresh waters (0.68 to 0.73) with relatively low salt contents, and populations in brackish-marine (SALINE) habitats (0.74 to 0.81) (Fig. 2).

The occurrence of a third overlapping group of intermediate forms in fresh water is difficult to reconcile with the original simple concept of two distinct subspecies, requiring further detailed analysis of variation in limb dimensions.

Predicted mean widths at standard lengths of the merus and carpus in populations from various localities and habitats

Ratios for mean logio width/length of the merus were again calculated from predictive regressions for pooled measurements on 73 to 270 males, representing two to five populations occupying similar habitats. The regressions also were used to predict values (expressed as μm) of the mean merus width at a standard merus length of 1000 μm , where width and length are antilogarithmic values (= geometric means) calculated from the logarithmic regressions. The standard value chosen for length was close to the actual mean values of merus length in the large population samples; the results are shown in Fig. 3. Similarly, regressions were done on logio values for width and length of the carpus on the same individuals, and also the basis

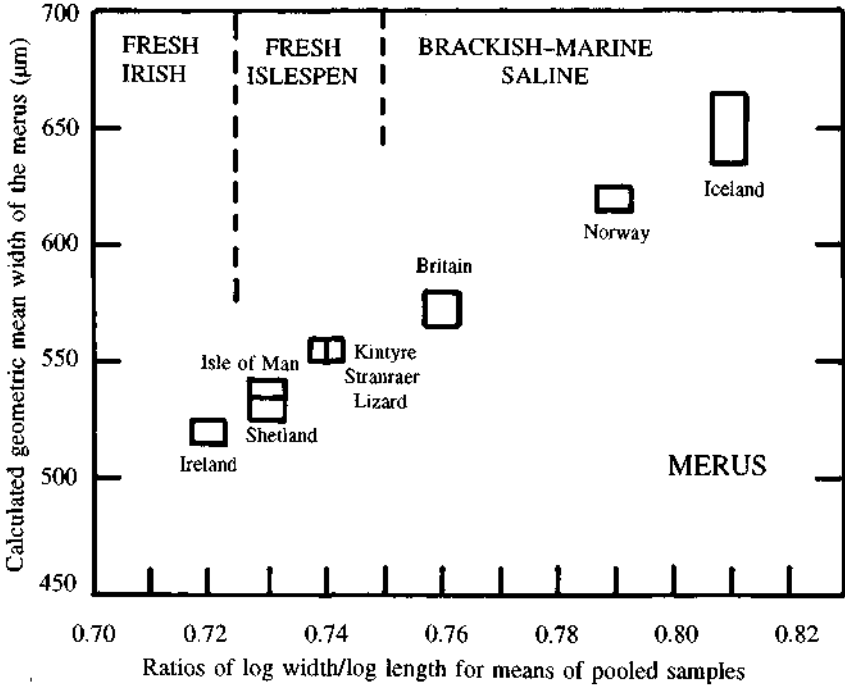


FIG. 3. Calculated mean width of the merus at a standard length of 1000 μm , plotted against the ratio of mean log W/L in male *G. duebeni* for pooled samples representing one or more local freshwater populations in Ireland (n = 255), Isle of Man (n = 74), Shetland (n = 165), Kintyre, Stranraer and Lizard peninsulas (n = 96), and SALINE habitats in Britain (n = 270), Norway (n = 139) and Iceland (n = 73).

in fewer specimens. Mean log ratios, and predicted carpus width at a standard length of 1300 μm , are shown in Table 1; values for the basis are shown later in Table 4.

In Fig. 3, predicted mean merus width at a length of 1000 μm apparently decreases linearly from right to left in six pooled populations from different localities within the three major habitats. In part this is an artefact due to plotting the same dimension on both axes of the text-figure, where width is plotted against its ratio to length. Nevertheless, predicted merus width progressively decreases by 11-12% when comparing brackish-marine populations in Iceland with those in Britain, and by another 8-9% for the Irish freshwater populations, with an overall reduction of 20%. An almost identical decrease (21% overall) occurs in the predicted width of the carpus at a length

of 1300 mm (Table 1). The predicted values shown for both limb segments are significantly different ($p < 0.01$, ANOVAR/COVAR) between all of the six pooled samples. In this way at least six distinct groups or morphs can be recognised and probably more could be distinguished by further assiduous collection and analysis.

A quantitative description of two subspecies of *G. duebeni*

Stock & Pinkster (1970) originally noticed an undoubted morphological difference between specimens from coastal brackish water (*G. duebeni duebeni*) in northwestern Europe and specimens from fresh water in Brittany and Ireland (*G. duebeni celticus*). This, and evidence shown in Figs 2 and 3, might still be regarded as indicative of two morphologically distinct subspecies, with an intermediate form of *duebeni* evolving in the third major habitat of ISLESPEN fresh waters. This view was adopted by Dennert (1975) who regarded *celticus* as a subspecies confined to the lakes and rivers of Brittany and Ireland. From the analysis summarised in Fig. 3, a taxonomic description of *G. duebeni celticus* must then specify a ratio of 0.72 or less for *mean* \log_{10} width/length of the merus in adult males from a large population sample of at least 60-100 specimens; similarly, *mean* \log_{10} carpus width/length should be less than 0.54 (Table 1). More importantly, perhaps, the predicted mean merus width at a merus length of 1000 μm should not be significantly greater than 525 μm (I am assuming that populations from

Table 1. Values for the ratio of mean \log_{10} width to mean \log_{10} length (log W/L), and 95% confidence limits for mean carpus width (μm) predicted from logarithmic regressions for a standard carpus length of 1300 μm ; n is the number of mature male specimens examined from six pooled populations, representing different habitat salinities and localities. SALINE = brackish-marine habitats; ISLESPEN = freshwater habitats with slightly raised salt contents on islands and peninsulas around the British Isles; IRISH = freshwater habitats in Ireland.

Habitat/locality	n	Mean log W/L	95% CL for mean carpus width
SALINE			
Iceland	73	0.62	486 – 504
Norway	96	0.59	444 – 463
Britain	197	0.58	422 – 435
ISLESPEN fresh water			
Kintyre + Lizard	48	0.56	410 – 424
Shetland Isles	67	0.54	392 – 405
IRISH fresh water			
Ireland	204	0.53	386 – 395

Brittany would fall well below this value). For the carpus, predicted mean width should not be significantly greater than 395 μm at a standard length of 1300 μm . (Note: the calculated 95% confidence limits for predicted merus and carpus widths of *single individuals* are considerable, at ± 10 to 20% of the predicted mean values. Also see Table 2.)

Table 2. Values for the ratio of \log_{10} merus width/length (and ranges of values for smaller population samples; see Fig. 3) obtained from predictive regressions (r^2 = coefficient of determination) for large pooled samples (n = number of specimens) of *G. duebeni*, and 95% confidence limits for the predicted population mean merus width (Population W, μm) at a standard merus length of 1000 μm , and 95% confidence limits for individuals in the same population (Individual W, μm).

Habitat	n	W/L	r^2 (%)	Population W	Individual W
SALINE (= <i>G. d. duebeni</i>)	482	0.78 (0.75–0.81)	88	591 – 601	494 – 719
ISLESPEN fresh water	335	0.73 (0.71–0.76)	90	536 – 544	479 – 608
IRISH fresh water	255	0.72 (0.69–0.73)	89	516 – 525	454 – 596
Pooled ISLESPEN + IRISH (= <i>G. d. celticus</i>)	590	0.73	88	528 – 534	466 – 606

On these criteria, all other populations would be regarded as subspecies *duebeni*, including those from Shetland and the Isle of Man (see Figs 2 and 3). But the latter two are in fact morphologically very similar to the IRISH populations, and there is no good reason for excluding them and other ISLESPEN populations from the subspecies *celticus*, particularly as they all occupy freshwater habitats on the Celtic fringes of the British Isles. When all of the ISLESPEN populations that I have sampled are included as *celticus*, the defining ratio of mean log₁₀ merus width/length for large samples alters to 0.73, with predicted mean merus width not significantly greater than 544 μm at a length of 1000 μm (Table 2). For the carpus the ratio is below 0.55, with mean width significantly below 412 μm at 1300 mm length.

For completeness here I should add that similar changes in shape occur to the merus and carpus of other pereopods in males and also occur in female *G. duebeni*, where individual variation is more pronounced and difficult to quantify in a satisfactory manner.

We therefore arrive at a more inclusive recognition of two subspecies, where *G. duebeni celticus* occupies all types of freshwater habitats in northwestern Europe and *G. duebeni duebeni* occupies all types of

brackish-marine habitats. Within each subspecies a series of morphs can be recognised, with progressive changes in limb dimensions that are evident in *duebeni* as well as in *celticus*. This variation in external morphology (and in physiological traits) between populations of *G. duebeni* (Figs 2 and 3, and Tables 1 and 2) in some respects resembles the complex of morphological and physiological "races", "forms", "subspecies" and even "species" that have been recognised at various times by numerous studies on the three-spined stickleback *Gasterosteus aculeatus*. This fish, like *G. duebeni*, also occupies both saline and freshwater habitats and displays varying tolerances to salinity in different populations and localities. Three main forms, *leiurus*, *trachurus* and *semiarmatus*, differing in numbers and arrangement of lateral body plates, are currently regarded as distinct plate morphs by researchers in Europe, but not in North America where populations in freshwater habitats are particularly complex in morphology and behaviour (Wootton 1984; Maitland & Campbell 1992; Schluter 1993; Bell & Foster 1994; Baumgartner 1995).

When uncertainty exists for the much studied *G. aculeatus*, in which there are clear morphological differences, conflicting interpretations of variation between populations of *G. duebeni* will inevitably arise, particularly as only one small morphological feature has been considered suitable for separating *G. d. duebeni* and *G. d. celticus* (Pinkster et al. 1970; Dennert 1975). After examination of 121 morphological features by ordination and clustering methods, Holmes (1975) noted that the "two forms are very similar", and then "it is hard to say whether they are distinct species, or whether they are only subspecies", but he does not say which characters were distinct; all of his specimens were from Ireland. Examination of enzymes by starch gel electrophoresis failed to separate *celticus* and *duebeni* (Bulnheim & Scholl 1981), although differences between recognised species of *Gammarus* are readily demonstrated (e.g. Siegismund et al. 1985; Skadsheim & Siegismund 1986).

Ecomorphs and clines of *G. duebeni*

Whilst accepting that it is statistically possible to recognise two subspecies in very large, pooled samples, the distribution of morphological variation in *G. duebeni* also can be interpreted either as a series of ecomorphs or as one or more clines. For the former, the change in pereopod width relative to its length is considered to be a series of discrete changes associated with selection (adaptation) within one or more population(s), correlated with the particular salinity regime of the habitats - as summarised in Table 2. The three ecomorphs correspond remarkably well with the series of physiological differences in the osmoregulatory capabilities of populations from the same three habitat groups, summarised in the Introduction and by Sutcliffe (1978). In fact, where possible my measurements of pereopod 7 were made on

specimens that had been used for experimental work on salt and water balance, so the morphological and physiological traits considered here actually apply to the same population sample.

On the other hand, progressive change in dimensions might represent a continuum between populations from progressively more dilute waters, particularly with respect to their sodium and chloride concentrations although other major ions (e.g. potassium, calcium and bicarbonate) are also physiologically important. As such the variation in shape correlated with salinity represents a cline, like other clines described for the genus *Gammams* (Holsinger & Culver 1970; Kolding 1985), whereas the terms ecotype (generally favoured by botanists, but applied to *G. minus* by Fong 1988), ecophenotype (Gooch & Hetrick 1979) and ecomorph (generally favoured by zoologists) tend to imply and stress the presence of at least some discontinuities in distribution (Merrell 1981).

Rapid morphological and physiological changes in an experimental population

The relative plasticity of adaptive alterations in osmoregulatory traits of populations from differing regimes of salinity was examined by an experiment started in 1967, when *G. duebeni* from Kintyre was successfully established in lakewater pumped from Windermere (Sutcliffe 1970). In this lake the sodium concentration (ca. 210 microequivalents or micromoles per litre) was lower than any reported for natural habitats of *G. duebeni*, including those in Ireland. Remarkably, after only two years, sodium regulation in the experimental animals closely resembled that of Irish animals, a rapid convergence due to extreme selection in the experimental ponds (Sutcliffe 1971a). Moreover, I can now report that when sampled in 1971, four years into the experiment, the shapes of both the merus and carpus had altered significantly, being ca. 3% narrower than in the parent population (Table 3). A sample taken two years later did not reveal any further significant change and, subsequently, the experiment was abandoned in favour of other research.

A 3% reduction in width for these experimental animals may seem very modest, but in fact it is similar to an overall 4% reduction between the pooled results for ISLESPEN and the IRISH populations shown in Table 2. The rapid change in shape of the characteristic used here for recognising subspecies of *G. duebeni* is, at the very least, a good reason for including the ISLESPEN populations in *G. d. celticus*.

Whilst contemplating possible reasons for this convergent morphological change it occurred to me that the slimmer pereopod in freshwater populations of *G. duebeni* looked suspiciously like that of the common freshwater shrimp *Gammarus pulex*. An analysis of specimens from a local stream provided conclusive confirmation (Table 4). Why?

Why does *G. duebeni* have slimmer limbs in fresh water?

Here, reasons for developing narrow limbs are considered to be directly related to the suite of physiological features and adaptive changes required for osmoregulation in fresh water. A 20% reduction in width of the limb-segments implies that both total surface area and volume are reduced, although the ratio of reduced area to volume presumably is increased. This would be a disadvantage for regulating osmotic ingress of water and outward loss of salts through the relatively permeable body wall. However, *G. duebeni* actively reduces its permeability when exposed to dilute media (Bolt 1983; Dawson et al. 1984), aiding the vital maintenance of blood and tissue cells at the appropriate internal concentrations, assisted by copious production of dilute urine with reabsorption of salts in the antennal gland (Sutcliffe 1971a,b). Blood volume is reduced (Lockwood & Inman 1973) and rates of oxygen uptake and heartbeat increase (Kinne 1952, 1959, 1961; Bulnheim

Table 3. Values for the ratio of logio width/length for the merus and carpus of pereopod 7 on male *G. duebeni* obtained from predictive regressions (r^2 = coefficient of determination) for n specimens from a population in Corachan Burn, Kintyre, and an experimental population of the same animals established in Windermere water with a low sodium concentration (Sutcliffe 1970, 1971a). Also given are 95% confidence limits for the predicted population mean merus width (mm) at a length of 1000 μ m, and for the carpus at a length of 1300 μ m.

Population	Merus				Carpus			
	n	log W/L	r^2 (%)	Merus width	n	log W/L	r^2 (%)	Carpus width
Kintyre 1967	35	0.76	69	560 – 580	30	0.56	66	409 – 427
Experiment 1971	38	0.74	95	548 – 563	30	0.55	63	389 – 425

Table 4. 95% confidence limits for predicted mean width (μ m) at standard lengths of the basis (when $L = 2000 \mu$ m), merus (when $L = 1000 \mu$ m) and carpus (when $L = 1300 \mu$ m) obtained from predictive regressions for n specimens of *G. duebeni* from SALINE and IRISH populations compared with a population of *G. pulex* from Cumbria.

Population	Habitat	n	Basis width	Merus width	Carpus width
<i>G. duebeni</i>					
Iceland	SALINE	73	1402 – 1433	635 – 665	486 – 504
Fromsø	SALINE	19	1289 – 1407	663 – 704	488 – 518
R. Boyne	IRISH	21	1173 – 1218	471 – 511	365 – 394
L. Corrib	IRISH	18	1115 – 1171	501 – 521	305 – 365
<i>G. pulex</i>					
Outgate	Fresh water	45	1177 – 1202	480 – 510	343 – 370

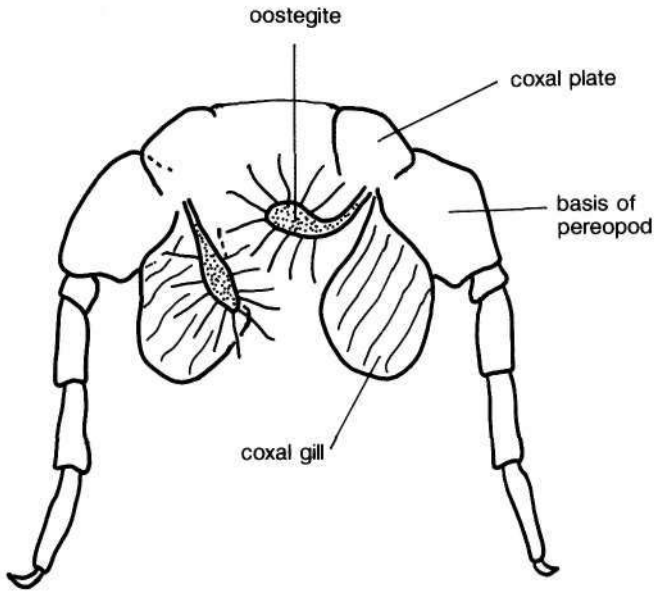


FIG. 4. Transverse section (semi-diagrammatic) through the body of a female *G. duebeni*, showing the locations of a pereopod, coxal gill and oostegite; on the latter, long setae overlap with setae on the opposing oostegite to form a ventral broodpouch for developing embryos.

1972, 1979, 1984). These adaptive features are all present in the common freshwater shrimp *G. pulex* (Sutcliffe 1967b, 1971c, 1984; Costa 1980), which has the additional adaptation of lower sodium and chloride concentrations in the blood, and even higher affinity for sodium uptake from dilute media.

In addition to the exchange of dissolved gases, active uptake of sodium, chloride and other ions also occurs at the gill surface (Milne & Ellis 1973); hence an increased flow of blood through the gills would be advantageous and assisted by the reduced blood volume and faster heartbeat mentioned above. The total surface area of the six coxal gills is ca. 50% larger in *G. pulex* compared with *G. duebeni* from brackish water (Moore & Taylor 1984). Perhaps the gill area increases in freshwater populations of *G. duebeni*? If so, a change in size of the pereopods in *G. duebeni* at low salinities might be correlated with an even more important adaptive change in the embryonic and juvenile development of the coxal gills, which lie at the base of pereopods 2 to 6 (Fig. 4). The neighbouring oostegites, forming the broodpouch in females, might similarly alter in shape and size; in brackishwater *G. duebeni* and other

marine species the oostegites are narrower than in *G. pulex* and other freshwater species (Steele 1990).

Further speculation and discussion about morphological variation must wait until new observations are made on populations of *G. duebeni* in fresh water, but it should be noted that the relative dimensions of morphological features in *Gammarus* are likely to be dependent on temperature as well as salinity. These two basic environmental variables combine to strongly affect growth rates in *G. duebeni* (Kinne 1959), and this may be crucial during embryonic and juvenile development in a euryhaline animal that exhibits allometric growth to a marked degree. Explanation along these lines is required for the puzzling and unexplained fact that the relative dimensions of limb-segments are significantly different between populations of *G. duebeni* from brackish-marine habitats in northern latitudes (Figs 2 and 3), where there is a general decline in the ratio of mean log W/L from 0.81 in Iceland to 0.77 in warmer habitats further south. Temperature and daylengths also affect the timing of reproduction, development times, size at sexual maturity and number of generations per year, with distinct differences between populations of *G. duebeni* related to latitude (reviewed by Sutcliffe 1993).

For a broader evolutionary viewpoint, comparison with the limbs and gills of *G. tigrinus* and *G. zaddachi* might throw further light on morphological changes associated with gradients of salinity, especially when also compared with congeneric species from the marine-littoral.

Concluding remarks

The existence of morphs in *G. duebeni* is open to question and requires a flexible approach for interpreting the considerable variation found in the shape of pereopod 7 and other limbs. Although highly variable both within and between populations, there is nevertheless a pronounced trend towards narrower segments (articles) of the limb in populations from fresh water. The observed trend with decreasing salinity of the habitat appears to have a northeast-southwest component that is equally evident in SALINE (brackish-marine-littoral) habitats. A reasonable explanation for this apparent decline in SALINE populations cannot be offered unless it is postulated that small differences in the salinity and temperature regimes affect growth patterns in the developing embryos and juveniles. For the populations in freshwater habitats (ISLESPEN and IRISH), the trend in changing limb shape continues with decreasing salt content of particular localities. Here, experimental studies indicate that a slimmer limb develops rapidly in response to selection for improved osmoregulation in dilute fresh waters. This selection continually operates on ISLESPEN populations that are geographically isolated from others in fresh water but are in direct contact with estuarine SALINE populations, so that the morphological and physiological differences

examined here are phenotypic expressions of a common genotype.

Until contrary evidence is forthcoming, phenotypic expression in IRISH populations also can be explained by continuous selection from a common genotype pool, shared with populations living in brackish water round the coastline of Ireland. Invasion of inland fresh waters, with easy access via the Shannon and other waterways, presumably occurred after the retreat of the maximum glaciation of the current Ice Age, i.e. less than 50 thousand years ago. At that time, extensive areas of brackish water probably existed at the edge of the retreating ice, affording conditions suitable for adaptation to increasingly dilute meltwaters and movement into fresh waters free from competition with other gammarids; Brittany could have been colonised at the same time. Later colonisation or a series of colonisations cannot be ruled out, and the time-scale for populations on the western coastline of northern Britain is probably less than 10-15 thousand years, after the last glaciation. Thus gradual evolution into discrete and readily distinguished subspecies is currently underway, eventually producing, perhaps, several new species in freshwater (and saline?) habitats.

Acknowledgements

Many people, too numerous to mention here, have contributed by collecting specimens, sometimes in remote localities, or by assisting with the rather laborious work of making slides and measurements. I thank them all; without their help I could not have undertaken this detailed study, done sporadically in the 1970s and early 1980s and funded by the FBA, grant-aided by the NERC. Apart from this valuable assistance the other precious commodity - time in which to think, analyse and write this article - has only now been available.

References

- Baumgartner, J. V. (1995). Phenotypic, genetic, and environmental integration of morphology in a stream population of the threespine stickleback, *Gasterosteus aculeatus*. *Environmental Biology of Fishes* 44, 312-223.
- Bell, M. A. & Foster, S. A. (Editors) (1994). *The evolutionary biology of the threespine stickleback*. Oxford University Press, Oxford.
- Bolt, S. R. L. (1983). Haemolymph concentration and apparent permeability in varying salinity conditions of *Gammarus duebeni*, *Chaetogammarus marinus* and *Gammarus locusta*. *Journal of Experimental Biology* 107, 129-140.
- Bulnheim, H.-P. (1972). Vergleichende Untersuchungen zur Atmungsphysiologie euryhaliner Gammariden unter besonderer Berücksichtigung der Salzgehaltsanpassung. *Helgoländer wissenschaftliche Meeresuntersuchungen* 23, 485-534.

- Bulnheim, H.-P. (1979). Comparative studies on the physiological ecology of five euryhaline *Gammarus* species. *Oecologia (Berlin)* 44, 80-86.
- Bulnheim, H.-P. (1984). Physiological responses of various *Gammarus* species to environmental stress. *Limnologia (Berlin)* 15, 461-467.
- Bulnheim, H.-P. & Scholl, A. (1981). Electrophoretic approach to the biochemical systematics of gammarids. *Helgolander Meeresuntersuchungen* 34, 391-400.
- Costa, H. H. (1980). The effects of some heavy metal pollutants on the heart beat of *Gammarus pulex* (L.). *Crustaceana Supplement* 6, 170-181.
- Dawson, M. E., Morris, R. J. & Lockwood, A. P. M. (1984). Some combined effects of temperature and salinity on water permeability and gill lipid composition in the amphipod *Gammarus duebeni*. *Comparative Biochemistry and Physiology* 78A, 729-735.
- Dennert, H. G. (1975). The variability of the dimensions of the merus of the fifth pereopod in the amphipod *Gammarus duebeni* Liljeborg, 1852. *Bijdragen tot de Dierkund* 45, 1-19.
- Fong, D. W. (1988). Morphological evolution of the amphipod *Gammarus minus* in caves: quantitative genetic analysis. *American Midland Naturalist* 121, 361-378.
- Fryer, G. (1978). A remarkable inland brackish-water crustacean fauna from the lower Aire Valley, Yorkshire: a conundrum for the ecologist. *Naturalist* 103, 83-94.
- Gledhill, T., Sutcliffe, D. W. & Williams, W. D. (1993). *British freshwater Crustacea Malacostraca: a key with ecological notes*. Freshwater Biological Association Scientific Publication No. 52, Ambleside. 173 pp.
- Gooch, J. L. & Hetrick, S. W. (1979). The relation of genetic structure to environmental structure: *Gammarus minus* in a karst area. *Evolution* 33, 192-206.
- Holmes, J. M. C. (1975). A comparison of numerical taxonomic techniques using measurements on the genera *Gammarus* and *Marino gammarus* (Amphipoda). *Biological Journal of the Linnean Society* 7, 183-214.
- Holsinger, J. R. & Culver, D. (1970). Morphological variation in *Gammarus minus* (Amphipoda, Gammaridae) with emphasis on subterranean forms. *Postilla*. No. 146.
- Hynes, H. B. N. (1954). The ecology of *Gammarus duebeni* Liljeborg and its occurrence in fresh water in western Britain. *Journal of Animal Ecology* 23, 38-84.
- Kinne, O. (1952). Zur Biologie und Physiologie von *Gammarus duebeni* Lilj. V: Untersuchungen über Blutkonzentration, Herzfrequenz und Atmung. *Kieler Meeresforschung* 9, 134-150.
- Kinne, O. (1959). Ecological data on the amphipod *Gammarus duebeni*. A monograph. *Veröffentlichungen des Instituts für Meeresforschung, Brehmerhaven* 6, 177-202.

- Kinne, O. (1961). Growth, moulting frequency, heart beat, number of eggs and incubation time in *Gammarus zaddachi* exposed to different environments. *Crustaceana* 2, 26-36.
- Kolding, S. (1985). Genetic adaptation to local habitats and speciation processes within the genus *Gammarus* (Amphipoda: Crustacea). *Marine Biology* 89, 249-255.
- Lockwood, A. P. M. & Inman, C. B. E. (1973). The blood volume of some amphipod crustaceans in relation to the salinity of the environment they inhabit. *Comparative Biochemistry and Physiology* 44A, 935-941.
- MacNeil, C, Elwood, R. W. & Dick, J. T. A. (1999). Differential microdistributions and interspecific interactions in coexisting *Gammarus* and *Crangonyx* amphipods. *Ecography* 22, 415-423.
- Maitland, P. S. & Campbell, R. N. (1992). *Freshwater fishes of the British Isles*. The New Naturalist. HarperCoWinsPublishers, London. 368 pp.
- Merrell, D. J. (1981). *Ecological genetics*. Longman, London.
- Milne, D. J. & Ellis, R. A. (1973). The effect of salinity acclimation on the ultrastructure of the gills of *Gammarus oceanicus* (Seegerstrale, 1947) (Crustacea: Amphipoda). *Zeitschrift fur Zellforschung und Mikroskopische Anatomie* 139, 311-318.
- Moore, P. G. & Taylor, A. C. (1984). Gill area relationships in an ecological series of gammaridean amphipods (Crustacea). *Journal of Experimental Biology and Ecology* 74, 179-186.
- Pinkster, S., Dennert, A. L., Stock, B. & Stock, J. H. (1970). The problem of European freshwater populations of *Gammarus duebeni* Liljeborg, 1852. *Bijdragen tot de Dierkund* 40, 116-147.
- Reid, D. M. (1939). On the occurrence of *Gammarus duebeni* (Lillj.) (Crustacea, Amphipoda) in Ireland. *Proceedings of the Royal Irish Academy B* 45, 207-214.
- Schluter, D. (1993). Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. *Ecology* 74, 699-709.
- Siegismund, H. R., Simonsen, V. & Kolding, S. (1985). Genetic studies of *Gammarus*. I. Genetic differentiation of local populations. *Hereditas* 102, 1-13.
- Skadsheim, A. & Siegismund, H. R. (1986). Genetic relationships among north-western European Gammaridae (Amphipoda). *Crustaceana* 51, 163-175.
- Steele, D. H. (1990). Comparison of oostegite shapes in some gammaroidean species (Crustacea: Amphipoda). *Bijdragen tot de Dierkund* 60, 277-282.
- Stock, J. H. & Pinkster, S. (1970). Irish and French fresh water populations of *Gammarus duebeni* subspecifically different from brackish water populations. *Nature, London* 228, 874-875.
- Sutcliffe, D. W. (1967b). Sodium regulation in the amphipod *Gammarus duebeni* from brackish-water and fresh-water localities in Britain. *Journal of*

- Experimental Biology* 46, 529-550.
- Sutcliffe, D. W. (1967b). Sodium regulation in the freshwater amphipod, *Gammarus pulex*. *Journal of Experimental Biology* 46, 499-518.
- Sutcliffe, D. W. (1970). Experimental populations of *Gammarus duebeni* in fresh water with a low sodium content. *Nature, London* 228, 875-876.
- Sutcliffe, D. W. (1971a). Sodium influx and loss in freshwater and brackish-water populations of the amphipod *Gammarus duebeni* Lilljeborg. *Journal of Experimental Biology* 54, 255-268.
- Sutcliffe, D. W. (1971b). Regulation of water and some ions in gammarids (Amphipoda). I. *Gammarus duebeni* Lilljeborg from brackish water and fresh water. *Journal of Experimental Biology* 55, 325-344.
- Sutcliffe, D. W. (1971c). Regulation of water and some ions in gammarids (Amphipoda). II. *Gammarus pulex* (L.). *Journal of Experimental Biology* 55, 345-355.
- Sutcliffe, D. W. (1972). An examination of subspecific differences in the merus of the fifth walking leg of the amphipod *Gammarus duebeni* Lilljeborg. *Freshwater Biology* 2, 203-216.
- Sutcliffe, D. W. (1978). Water chemistry and osmoregulation in some arthropods, especially Malacostraca. *Freshwater Biological Association Annual Report* 46, 57-69.
- Sutcliffe, D. W. (1984). Quantitative aspects of oxygen uptake by *Gammarus* (Crustacea, Amphipoda): a critical review. *Freshwater Biology* 14, 443-489.
- Sutcliffe, D. W. (1993). Reproduction in *Gammarus* (Crustacea: Amphipoda): female strategies. *Freshwater Forum* 3, 26-64.
- Sutcliffe, D. W. & Shaw, J. (1968). Sodium regulation in the amphipod *Gammarus duebeni* Lilljeborg from freshwater localities in Ireland. *Journal of Experimental Biology* 48, 339-358.
- Wootton, R. J. (1984). *Afunctional biology of sticklebacks*. Croom Helm, London.