

Aquatic insects in an estuarine environment: densities, distribution and salinity tolerance

D. DUDLEY WILLIAMS*† AND NANCY E. WILLIAMS*‡

†School of Biological Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, U.K.

‡Institute of Earth Studies, University of Wales, Aberystwyth, Dyfed SW23 3DB, U.K.

*Address for correspondence: Division of Life Sciences, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, Ontario, Canada M1C 1A4

SUMMARY

1. Aquatic insects were quantitatively surveyed at five sites along the tidally influenced section of a river-dominated estuary in North Wales. Site 1 was the furthest upstream and was established as a reference site as it was never inundated by salt water. Site 5 was the furthest downstream and was inundated by all incoming tides. Numerically, insects made up 32% of the estuarine invertebrate fauna.
2. Although the densities of most insect taxa decreased towards the estuary mouth, there were significant numbers present downstream for much of the year; for example, in April at site 4 (which was inundated by 81% of all high tides), a mean of 3514 chironomid larvae were recorded per m² of estuary bed. Even at site 5, which was inundated twice daily, there were 747 larvae per m². Among the larger aquatic insects, caddisfly and elm mid beetle larvae, together with stonefly nymphs, were consistently taken at site 4 (e.g. maxima of forty-eight caddisfly larvae m⁻² in December and seventy elm mids m⁻² in April), although their densities were lower than upstream.
3. There were seasonal shifts in the longitudinal distribution of several taxa, most notably the extension of chironomids down the estuary in April and July, and the concentration of simuliid larvae and mayfly nymphs at site 2 in July. The total freshwater benthos showed a downstream shift between September and December, which was maintained through April and into the summer. The latter was despite peak saltwater inundation (highest tides) in October, November and April. In June and July, when saltwater intrusion was lowest, the ranges of many aquatic insects had contracted to sites 1 and 2.
4. Laboratory experiments showed that virtually all individuals of nineteen species of insects collected from site 1 (freshwater) survived a 4-h immersion in 8.75‰ saltwater (25% strength seawater). Immersion in progressively more saline solutions reduced the survivorship of first the mayflies, followed by the caddisflies *Glossosoma conformis* and *Hydropsyche instabilis*. After 4 h in full strength seawater, all specimens of the stonefly *Dinocras cephalotes*, over half of the *Perla bipunctata*, and some individuals of nine species of caddisfly were alive. Four species of caddisfly (*Sericostoma personatum*, *Odontocerum albicorne*, *Potamophylax cingulatus* and *Adicella reducta*) survived a 24-h simulated tidal cycle of immersion. With the exception of *P. cingulatus*, a few individuals of these caddisfly species survived immersion in full-strength seawater for 24 h. For some individual species there was good agreement between their observed longitudinal distribution in the estuary and laboratory-measured salinity tolerance; however, there was no significant correlation, overall, for the fauna.

Introduction

Insects have achieved unparalleled success in both terrestrial and freshwater environments, with the numbers of species in these two habitats approximating several million and more than 30 000, respectively (Williams & Feltham, 1992). The one major environment in which they appear not to be well represented is the sea, as the number of species that can be called 'marine' amounts to only several hundred. As the oceans cover approximately 75% of our planet's surface, it is surprising that insects, with their approximately 300 million years of evolution, are not more common in saline habitats. Apart from the hemipteran genus *Halobates*, no insects are known from the open ocean, the majority of marine insects being associated with coastal habitats, such as saltmarshes, mangroves, decaying seaweed on the seashore, and estuaries (Axtell, 1976; Dobson, 1976; Cheng, 1985).

Few attempts have been made to survey and study insects in marine habitats by either marine or freshwater biologists. This has led, even recently, to statements such as 'insect dominance . . . extends no further into the marine environment than the high tide mark' (Gullan & Cranston, 1994). Although some brackishwater studies have recorded the presence of immature stages of insects (e.g. McIntyre & Eleftheriou, 1968; Healey, 1982; Mees, Dewicke & Hamerlynck, 1993), they typically have lumped insects into large taxonomic bins (e.g. Insecta, Diptera, Coleoptera) of limited use to entomologists.

The study reported here is concerned with the insects found living in a tidally influenced river in North Wales, and presents details of: (i) their distribution among sites variously inundated by the sea; (ii) seasonal changes in their population densities; and (iii) the salinity responses of individual species. The drift dynamics of these insects into the lower estuary and their consumption by estuarine consumers have been documented elsewhere (Williams & Williams, 1998).

Study site

The Afon Aber, located in the county of Gwynedd, North Wales (54°14'N, 4°00'W), arises in the Carneddau mountain range. Its catchment extends to an area of some 24.5 km² and the watercourse itself comprises eighty-two first-order streams, thirteen second-order

streams, three third-order streams and one fourth-order stream. The bedrock underlying the catchment is overlain with Quaternary drift. The upper Aber Valley is composed of boulder clay, whereas the valley floor is made up largely of fluvio-glacial sand and gravel. Alluvium is present near the estuary mouth. Soils in the area reflect a transition from upland peat to loam and silt in the lowland, and originate from Palaeozoic siltstone, mudstone and slate rocks (Brown, 1960; Bassett, 1967; Stocks, 1988). The upper reaches of the catchment are common lands supporting rough grazing for sheep. Further down there are, successively, areas of coniferous plantation, the village of Aber, flat pasture and finally saltmarsh.

The Aber Estuary is of the freshwater-dominated, stratified type, with freshwater flow rates varying annually between about 30 and 110 cm s⁻¹. Incoming tides push a visible (semi-opaque) saltwater wedge upstream along the surface of the bed and this creates a layer of saline water that envelops the benthic habitat. Monitoring of salinity levels on both rising and ebb tides showed that very little mixing occurs between the wedge and the overlying freshwater. At the extreme edge of the wedge front (typically around 5–10 cm thick) salinity ranged between 8 and 17‰. Tides along this stretch of the Gwynedd coast are of the semidiurnal type, and the maximum annual tidal range is of the order of 10 m. Benthic organisms on the estuary bed are thus subject to saltwater inundation twice daily.

Materials and methods

Five sampling stations, each characterized by a different degree of saltwater inundation, were set up along the estuary (Fig. 1). They were:

- 1 at a point 5 m upstream of the maximum intrusion of the highest spring tide (extreme high water mark, EHW, = 10.2 m); here the benthic fauna was exposed only to freshwater;
- 2 at the 9.1 m mark; here the benthos was inundated by 33.7% of all high tides;
- 3 at the 8.4 m mark which, in this region, represented the level of the mean high water mark (MHWM); here benthos was inundated by 50% of all high tides;
- 4 at the 7.6 m mark; inundated by 80.9% of all high tides;

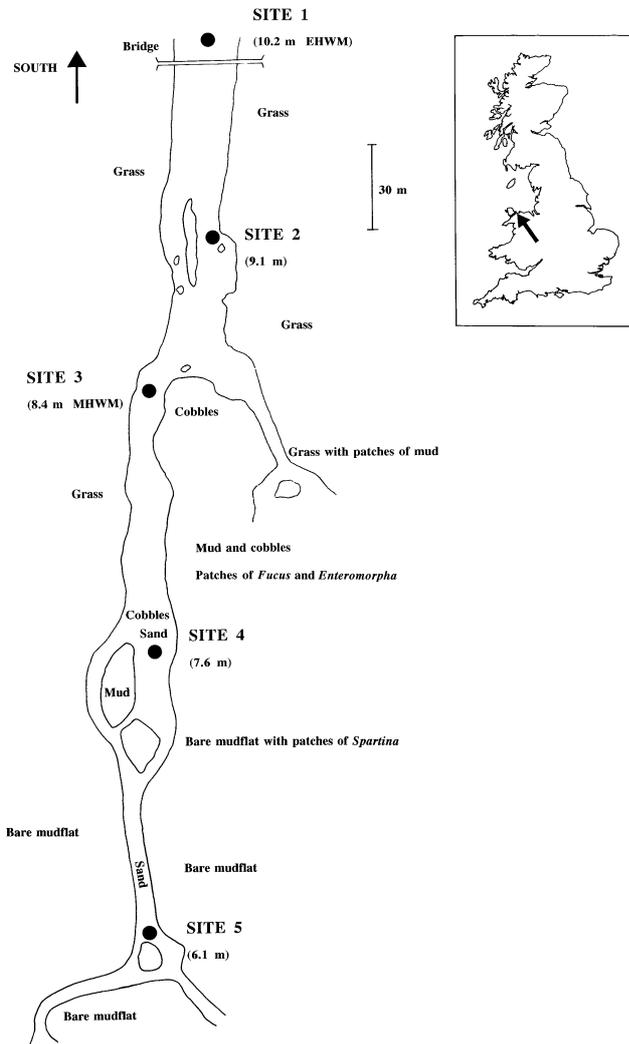


Fig. 1 Map of the Aber Estuary showing features of the bed and channel, together with the five sampling sites located at specific tidal heights (note that site 1 is above the extreme high water mark). Direction of the river flow is from top to bottom.

5 at the 6.1 m mark; inundated by 100% of all high tides.

Per cent of inundation by high tides was calculated by preparing graphs of the daily low and high tide heights over the previous year (1989–90), and choosing a range of values for which corresponding sites along the Aber Estuary could be found. Sites were physically located at the appropriate times of high tide, and marker stakes were driven into the bed. Subsequent inundation measurements made during the study year (1990–91) showed some seasonal variation around the designated sites; for example, taking site 3 (designated 50% inundation) as a reference point, 65% of all high

tides inundated this site in April, compared with only 45% in June.

At sites 1–3, the substratum was typically riverine, comprising cobbles (up to 30 cm in diameter), stones, coarse and fine gravels, and coarse sand. At site 4 there was much more sand, and at site 5 the substratum was almost entirely sand. Whereas there was evidence of occasional water-induced bed disturbance at these latter two sites, the substratum at the three uppermost sites remained largely undisturbed over the entire study period.

Each site was sampled for benthic invertebrates at 3-monthly intervals over the period September 1990 to July 1991. Samples were taken to a substrate depth of 5 cm, using a 250 µm mesh Surber Sampler with a sampling area of 900 cm². Three, randomly positioned, replicate samples were taken at each site, starting, on each occasion, at site 5 and working upstream. Samples were preserved in 95% ethanol in the field. In the laboratory, the samples were rinsed with tap-water, floated in saturated calcium chloride solution (to help separate the animals from the inorganic substrate), and examined under a dissecting microscope. Individual animals were removed from the surrounding detritus, identified and preserved in 75% ethanol.

In addition to these samples, on seven occasions during 1990–91 additional sampling of caddisflies (Trichoptera) was undertaken at the five sites. This involved two people turning over rocks and gravel in order to look for the larvae and pupae of these insects adhering to the margins and undersides. On each occasion, about 45 min was spent collecting caddisflies at each site, beginning downstream.

For the salinity tolerance trials, which were run from January to March 1991, insects were freshly field-collected from site 1 which was above the EHW. They were transported and maintained in cool (river temperature), well oxygenated, river water. Collecting test insects solely from site 1 ensured that, as far as could be ascertained and barring extensive migration from downstream, they had experienced little or no prior exposure to saltwater. Whereas this meant that it was not possible to assess the capacity for acclimation to salinity, it did serve to standardize the insects, a factor deemed to be more important in the context of this study. Natural seawater was used for the trials but as, in this region, salinity ranges between 29 and 33‰, it was concentrated (by evaporation and aeration) to provide a 35‰ stock solution.

In the salinity trials, batches of 5–10 animals per species were subjected to four salinity levels (8.75, 17.5, 26.25 and 35.0‰; prepared from the stock solution and river water) for between 1 and 4 h. The procedure was as follows: the animals were transferred into 15-cm-diameter glass bowls in which there was a 5 cm depth of water over a 0.5 cm layer of clean, coarse, non-marine sand substrate. A slight clockwise current (approximately 4 cm s^{-1}) and aeration were provided in each bowl by means of air pumped through a glass pipette fastened to the inside of the bowl wall. Because of the different salinities used, it was difficult to standardize immersion. The protocol employed was first to transfer all batches from 'pure' river water to bowls containing river water to which sufficient natural seawater had been added to create a salinity of 4.4‰. Such exposure was meant to simulate the incoming tide for the test animals. After 15 min, the insects were transferred to new bowls containing one of the four salinity trial levels. The condition (in terms of behaviour, activity and whether they were alive or dead) of each individual was monitored at 15 min intervals for the first hour and at 30 min intervals for the next 3 h. After a total of 4 h (calculated as being the average maximum time they would probably be immersed by the tide in the Aber Estuary), all animals were transferred back into river water and their condition was assessed after a further 20 h. Transfer of insects from the 15 min 'acclimatization' bowls took place, always, at around 10.00 h, and this established time 0. Throughout monitoring, the bowls were kept under constant dim light conditions (approximately $10 \times 10^{13} \text{ quanta s}^{-1} \text{ cm}^{-2}$) and at a temperature of 10–15 °C.

A few species were selected for two additional trials. In the first, insects were immersed in river water for 8 h, then in full strength seawater for 4 h, then in river water for 8 h, and again in seawater for 4 h; this was meant to approximate a diel tidal cycle. In the second trial, they were immersed, continuously, in 35.0‰ for 24 h in order to test for extreme tolerance.

The sizes of specimens used in these trials varied slightly according to collecting time (January–March) and availability in the field. In order to reduce variation in response to salinity that might have been size dependent, whenever possible, individuals from the modal size class present in the field at the time of collection were used.

Results

Whereas the densities of most insect taxa dropped substantially downstream from site 1, there were significant numbers present further down the estuary at certain times of the year (Table 1); for example, the densities of chironomid larvae at sites 1 and 3 were not significantly different in either April or July (April: 481.0 ± 106.9 and 576.7 ± 49.5 larvae 900 cm^{-2} ; July: 565.7 ± 128.8 and 507.7 ± 38.4 larvae 900 cm^{-2} ; Student's *t*-test = -0.81 and 0.43 , respectively; $P > 0.4$). Furthermore, in April, at site 4 (inundated by 81% of all high tides), a mean of 316.3 ± 41.4 chironomid larvae was recorded on only 900 cm^2 of estuary bed (3514 m^{-2}). Even at site 5, inundated by all high tides, there were $67.3 (\pm 31.1)$ larvae 900 cm^{-2} (747 m^{-2}). Among the larger aquatic insects, caddisfly and elm mid beetle larvae together with stonefly nymphs were consistently taken at site 4, although their densities were lower; for example a maximum of 4.3 ± 1.2 caddisfly larvae 900 cm^{-2} in December and 6.3 ± 1.9 elmids 900 cm^{-2} in April (forty-eight and seventy individuals m^{-2} , respectively).

Detailed consideration of the taxa

Identification of the larval chironomids revealed a decrease in species richness towards the more seaward sites. At site 1 in September for example, five species were abundant or common (defined as > 30 and 10 – 30 specimens 900 cm^{-2} of substrate, respectively), four orthoclads: *Eukiefferiella clypeata*, *Tvetenia* sp., *Orthocladius* (*Orthocladius*) sp. and *Brillia modesta*; and *Rheotanytarsus* sp. (*Tanytarsini*). Of these, *E. clypeata* and *Orthocladius* (*O.*) sp. were common also at site 3. At site 1 in April, *Orthocladius* (*Euorthocladius*) *thienemanni*, *E. clypeata*, *Corynoneura* sp., *Rheotanytarsus* sp. and *Polypedilum* sp. were common or abundant. At site 2, *E. clypeata*, *B. modesta* and *Polypedilum* sp. predominated. *Eukiefferiella clypeata* and *Orthocladius* (*O.*) sp. were abundant at site 3 but, at sites 4 and 5, only *Orthocladius* (*O.*) sp. and *Orthocladius* (*E.*) *thienemanni* persisted in any numbers.

Larvae of the elm mid beetle species *Elmis aenea*, *Esolus parallelepipedus* and *Limnius vorkman* were common to abundant at sites 1 and 2 throughout most of the year. However, only the first two species were found, in small numbers, at sites 3 and 4. The longitudinal distributions of larval caddisfly species, mayfly and

Table 1 Densities of insects ($\times 900 \text{ cm}^{-2}$, $\pm 1 \text{ SE}$) collected from the bed of the Aber Estuary on four occasions during the year

Taxa	Site 1	Site 2	Site 3	Site 4	Site 5
September					
Plecoptera	17.0 \pm 8.5	0	0	0.7 \pm 0.7	0.3 \pm 0.3
Ephemeroptera	209.0 \pm 39.4	0.7 \pm 0.7	0.3 \pm 0.3	0	0
Trichoptera	171.0 \pm 45.7	0.7 \pm 0.7	0.3 \pm 0.3	0	0
Elmidae	140.7 \pm 53.7	21.7 \pm 6.3	1.3 \pm 1.3	0.3 \pm 0.3	1.3 \pm 1.3
Gyrinidae	12.7 \pm 3.8	0.7 \pm 0.7	0	0	0
Simuliidae					
larvae	29.3 \pm 12.7	1.6 \pm 1.2	0.7 \pm 0.7	0	0
pupae	0	0	0	0	0
Chironomidae					
larvae	3892.3 \pm 454.7	915.3 \pm 261.2	349.0 \pm 23.4	5.6 \pm 2.6	1.0 \pm 0.6
pupae	18.0 \pm 10.3	26.7 \pm 7.0	16.0 \pm 1.5	0.7 \pm 0.7	0
Other Diptera	34.0 \pm 5.0	5.3 \pm 2.9	0	0	0
December					
Plecoptera	101.7 \pm 43.2	44.3 \pm 18.7	3.0 \pm 2.1	1.7 \pm 1.2	0.3 \pm 0.3
Ephemeroptera	178.3 \pm 62.6	28.0 \pm 11.0	1.3 \pm 0.3	0.3 \pm 0.3	0.3 \pm 0.3
Trichoptera	98.3 \pm 44.3	38.7 \pm 14.5	13.0 \pm 1.5	4.3 \pm 1.2	2.0 \pm 1.5
Elmidae	5.7 \pm 3.2	9.3 \pm 4.4	1.0 \pm 0.6	1.0 \pm 1.0	0
Gyrinidae	3.7 \pm 1.7	2.0 \pm 1.5	0	0.3 \pm 0.3	0
Simuliidae					
larvae	47.0 \pm 24.0	2.7 \pm 1.2	0.3 \pm 0.3	0	0
pupae	0	0	0	0	0
Chironomidae					
larvae	250.0 \pm 107.1	101.7 \pm 43.3	45.3 \pm 8.4	6.7 \pm 1.7	4.0 \pm 2.0
pupae	0	0.3 \pm 0.3	0	0	0
Other Diptera	7.0 \pm 3.1	1.3 \pm 0.9	1.3 \pm 0.9	0.7 \pm 0.7	0.3 \pm 0.3
April					
Plecoptera	43.7 \pm 3.9	58.3 \pm 28.4	7.7 \pm 0.3	1.7 \pm 0.7	0
Ephemeroptera	210.3 \pm 31.8	53.0 \pm 13.3	4.7 \pm 2.6	0	0
Trichoptera	73.3 \pm 6.7	71.3 \pm 10.2	17.3 \pm 6.6	2.3 \pm 1.2	0
Elmidae	32.3 \pm 5.2	16.7 \pm 4.3	6.0 \pm 1.2	6.3 \pm 1.9	2.0 \pm 0.6
Gyrinidae	2.7 \pm 1.2	1.7 \pm 0.9	0.3 \pm 0.3	0.7 \pm 0.7	0.3 \pm 0.3
Simuliidae					
larvae	11.7 \pm 8.3	1.3 \pm 0.3	0	0	0
pupae	0	0	0	0	0
Chironomidae					
larvae	481.0 \pm 106.9	407.3 \pm 30.5	576.7 \pm 49.5	316.3 \pm 41.4	67.3 \pm 31.1
pupae	15.3 \pm 8.6	16.0 \pm 0.6	12.7 \pm 5.7	20.7 \pm 6.5	3.3 \pm 1.8
Other Diptera	7.0 \pm 3.1	7.0 \pm 1.2	5.0 \pm 1.5	2.0 \pm 1.0	0.3 \pm 0.3
July					
Plecoptera	26.3 \pm 11.9	19.7 \pm 15.7	0	0	0
Ephemeroptera	219.0 \pm 100.1	571.3 \pm 217.7	1.7 \pm 0.7	0.3 \pm 0.3	0
Trichoptera	72.3 \pm 15.1	48.7 \pm 24.3	2.3 \pm 0.9	0.3 \pm 0.3	0
Elmidae	31.7 \pm 11.7	78.0 \pm 32.3	4.3 \pm 0.9	1.3 \pm 0.7	0.3 \pm 0.3
Gyrinidae	0	0	0	0	0
Simuliidae					
larvae	26.3 \pm 10.8	918.3 \pm 569.1	1.3 \pm 0.9	0	0
pupae	0	10.3 \pm 7.9	0	0	0
Chironomidae					
larvae	565.7 \pm 128.8	1255.3 \pm 194.9	507.7 \pm 38.4	66.3 \pm 13.8	18.0 \pm 4.6
pupae	10.7 \pm 4.7	11.7 \pm 5.2	14.7 \pm 3.2	5.3 \pm 2.3	0
Other Diptera	6.7 \pm 3.2	4.0 \pm 2.6	0.7 \pm 0.7	0.7 \pm 0.3	0.7 \pm 0.3

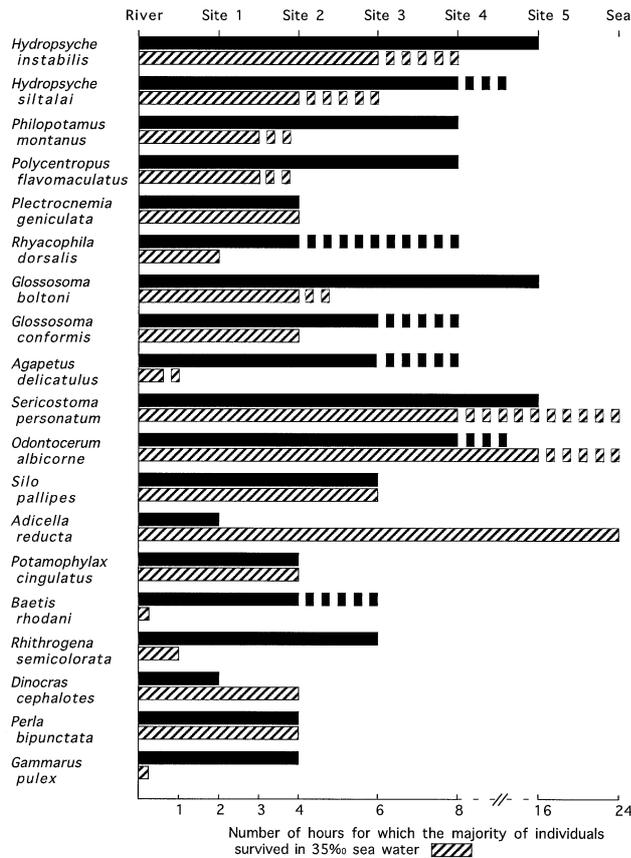


Fig. 2 Comparison of the salinity tolerance (as measured by immersion in 35‰ seawater; hatched bars) of the most abundant species of Trichoptera, Ephemeroptera and Plecoptera, with their observed distributional ranges in the Aber Estuary (solid bars).

stonefly nymphs are summarized in Fig. 2. Whereas a number of caddisflies were found as far downstream as site 5, *Dinocras cephalotes* and *Perla bipunctata* were confined to the upper estuary. Amongst the Ephemeroptera, *Caenis rivulorum* was largely confined to site 1 and *Baetis rhodani* to sites 1 and 2 (it was especially abundant at the latter site in July). *Rhithrogena semicolorata* was occasionally taken at site 3. *Simulium variegatum* occurred commonly (very abundant in July) at sites 1 and 2, and occasionally at site 3.

Salinity tolerance

Virtually all individuals of the nineteen species of freshwater insect collected from site 1 survived a 4 h immersion in 8.75‰ saltwater, although there was some indication of stress (e.g. erratic movement, thrashing of body, abandonment of case) among some

of the caddisflies (Table 2). At half-strength (17.5‰) seawater, although both mayfly species survived up to 1 h, none was alive after 4 h. There was 100% survival of the small herbivorous stonefly (Nemouridae), and of most of the caddisfly species, although again some showed definite signs of distress after 1 h. Deaths were recorded for *Glossosoma conformis* and *Hydropsyche instabilis*. In 26.25‰ seawater, there was good survival of the caddisflies *Philopotamus montanus*, *Hydropsyche siltalai*, *H. instabilis*, *Sericostoma personatum* and *Odontocerum albicorne*. After 4 h in full-strength seawater, at least some larvae of the above five species survived, together with at least one individual of four other caddisfly species. In addition, all specimens of the large predaceous stonefly *Dinocras cephalotes* and just over half of the individuals of the perlid stonefly *Perla bipunctata* had survived.

In the experiment designed to simulate immersion according to a 24 h tidal cycle in the Aber Estuary (Table 3), four species of caddisfly survived well (*S. personatum*, *O. albicorne*, *Potamophylax cingulatus* and *Adicella reducta*). Just under 20% of the *H. instabilis* larvae survived. Continuous immersion in full-strength seawater for 24 h was survived only by a few specimens of *S. personatum*, *O. albicorne* and *Adicella reducta* (Table 4).

In several cases, there was good agreement between the distributions of the most abundant lotic species in the estuary and the laboratory-measured maximum number of hours that these insects could survive in full-strength seawater (Fig. 2); for example the occurrence of the caddisflies *H. instabilis*, *S. personatum* and *O. albicorne* at downstream sites 4 and 5 matched their long immersion tolerance. Conversely, *Baetis rhodani* had a much shorter immersion tolerance and was largely restricted to upstream sites 1 and 2. However, a correlation analysis applied to the data for eighteen species showed no overall significant relationship ($r = 0.07$; $P > 0.5$) between immersion tolerance (maximum number of hours survived in 35‰ seawater) and longitudinal distribution (greatest distance recorded downstream from site 1).

Discussion

In the Aber Estuary, saltwater intrusion takes the form of a wedge that moves upstream over the surface of the substrate on a twice-daily cycle. All of the benthic insects described in this study are thus subject to a

Table 2 Results of the salinity tolerance tests on freshwater-derived insects collected from the Aber Estuary. Survival was measured after a 20 h recovery period, in river water, immediately after the test period

River taxa	Survival after 1 or 4 h in water of increasing salinity							
	8.75‰		17.5‰		26.25‰		35.0‰	
	1	4	1	4	1	4	1	4
Ephemeroptera								
<i>Baetis rhodani</i>	✓	✓	✓	D	✓M	D	D	D
<i>Rhithrogena semicolorata</i>	✓	✓	✓	D	✓	D	✓D	D
Plecoptera								
<i>Dinocras cephalotes</i>	–	–	–	–	–	–	✓	✓
<i>Perla bipunctata</i>	–	–	–	–	–	–	✓M	✓D
small stonefly (Nemouridae)	✓	✓	✓	✓	✓	D	✓	✓D
Trichoptera								
<i>Rhyacophila dorsalis</i>	✓	✓	✓	✓	✓	D	✓	✓D
<i>Glossosoma boltoni</i>	L	✓L	✓L	✓M	–	–	M	LD
<i>G. conformis</i>	✓	✓D	✓L	LD	✓L	LD	LM	D
<i>Agapetus delicatulus</i>	✓	✓	–	–	–	–	–	–
<i>Philopotamus montanus</i>	✓	✓	✓	✓	✓	✓	✓M	✓D
<i>Plectrocnemia geniculata</i>	–	–	–	–	✓	✓	–	–
<i>Polycentropus flavomaculatus</i>	–	–	–	–	–	–	✓	✓D
<i>Hydropsyche siltalai</i>	✓	✓	✓	✓	✓	✓M	✓	✓M
<i>H. instabilis</i>	✓	✓D	✓	✓D	✓	✓D	✓	✓D
<i>Potamophylax cingulatus</i>	✓	✓	–	–	–	–	–	–
<i>Silo pallipes</i>	✓	✓	–	–	–	–	–	–
<i>Sericostoma personatum</i>	✓	✓	✓	✓	✓	✓	✓	✓M
<i>Odontocerum albicorne</i>	✓	✓	✓	✓	✓	✓	✓	✓
<i>Adicella reducta</i>	✓	✓	–	–	–	–	✓	✓

(✓ = individuals were alive and moving; L = larvae had abandoned their cases—a sign of distress; M = moribund, i.e. the animals had stopped moving and had assumed an unnatural stance; D = dead; combinations indicate some individuals within the test group were in either state; – = sufficient numbers of individuals, between five and ten, were not available for a specific test).

repeated and dramatic change in the osmotic pressure of their environment, although the cycle and degree of change depends on their location along the estuary. Those at site 5, for example, were immersed twice daily, by water of 32–34‰ salinity for periods of 3–4 h, whereas those at site 2 were immersed twice daily by saline water on only approximately 10 days per month and for about 0.5–1.0 h on each occasion. Despite this salt intrusion, high numbers of aquatic insects continuously populated the Aber benthos. Indeed, overall, insects made up 32% of the numbers of benthic invertebrates collected in the tidal zone (sites 2–5). One factor deemed highly influential in promoting the persistence of larval insects in the Aber Estuary is the presence of a stable and large particle size substratum along most of the bed. Another must be physiological tolerance to salt, as discussed below.

Seasonal comparison of the density data showed

shifts in several taxa, most notably the extension of chironomids down the estuary in April and July, and the concentration of simuliid larvae and mayfly nymphs at site 2 in July. The total freshwater benthos showed a downstream shift between September and December, which was maintained through April and into the summer. The latter was despite peak saltwater inundation (highest tides) in October, November and April. Ironically, in June and July when saltwater intrusion was lowest, the ranges of many aquatic insects had contracted to sites 1 and 2—although this may have been associated with adult emergence. A more detailed discussion of seasonal variation in the longitudinal zonation of the Aber invertebrate fauna and possible causative factors is given by Williams & Williams (1998).

The salinity tolerance experiments showed that whereas the two common mayflies *B. rhodani* and

Table 3 Responses of freshwater-derived insects collected from the Aber Estuary to immersion in 35‰ seawater according to a simulated 24 h tidal cycle

River taxa	Time intervals (24 h total) of immersion cycle						
	Immersion in 35‰ seawater				After 8 h in river water	After a further 4 h in 35‰ seawater	Final condition after 8 h recovery in river water
	1.0	2.0	3.0	4.0			
Ephemeroptera							
<i>Baetis rhodani</i>	M	D	D	D	D	D	all dead
<i>Rhithrogena semicolorata</i>	✓	?D	D	D	D	D	all dead
Plecoptera							
<i>Dinocras cephalotes</i>	✓	✓	✓	✓	✓	✓	all dead
<i>Perla bipunctata</i>	✓	✓	✓	M	D	D	all dead
small stonefly (Nemouridae)	✓	M	D	D	D	D	all dead
Trichoptera							
<i>Rhyacophila dorsalis</i>	✓	✓	?	M	M	D	all dead
<i>Glossosoma boltoni</i> and <i>G. conformis</i> (combined)	L	L	M	MD	D	D	all dead
<i>Agapetus delicatulus</i>	–	–	–	–	–	–	
<i>Philopotamus montanus</i>	✓	✓	✓	?M	✓?	?	all dead
<i>Plectrocnemia geniculata</i>	✓	✓	M	M	✓	✓	all dead
<i>Polycentropus flavomaculatus</i>	–	–	–	–	–	–	
<i>Hydropsyche siltalai</i>	✓	✓?	✓?	✓?	✓D	✓D	all dead
<i>H. instabilis</i>	✓	✓?	✓?	✓?	✓	✓D	2 recovered, 9 dead
<i>Potamophylax cingulatus</i>	✓	✓	✓	✓	✓	✓	good (only 1 specimen)
<i>Silo pallipes</i>	–	–	–	–	–	–	
<i>Sericostoma personatum</i>	✓	✓	✓	✓	✓	✓	good
<i>Odontocerum albicorne</i>	✓	✓	✓	✓	✓	✓	good
<i>Adicella reducta</i>	✓	✓	✓	✓	✓	✓	good

(✓ = individuals were alive and moving; L = larvae had abandoned their cases—a sign of distress; M = moribund, i.e. the animals had stopped moving and had assumed an unnatural stance; D = dead; combinations indicate some individuals within the test group were in either state; – = sufficient numbers of individuals, between five and ten, were not available for a specific test).

R. semicolorata were generally intolerant of much salt and were restricted to the upper part of the estuary, survival of the stoneflies *D. cephalotes* and *P. bipunctata* and many of the caddisflies was much better. In particular, *S. personatum*, *O. albicorne* and *A. reducta*, survived not only a simulated 24 h tidal cycle but also 24 h of continuous immersion in full-strength seawater. In the first two species, these abilities correlated well with their observed distributions in the estuary. However, *A. reducta* was not found downstream from site 1. The reason for this, given its superior ability to tolerate salt, is not clear, although Colburn (1988) has suggested that lack of agreement between laboratory-measured salinity tolerance and field distributions may indicate overriding biotic influences, such as differential predation among field sites. There were, nevertheless, several other good correlations (e.g. *H. instabilis*, *P. geniculata*, *S. pallipes*, *P. cingulatus* and

P. bipunctata) and poor correlations (e.g. *R. dorsalis*, *G. boltoni* and *A. delicatulus*). One explanation for poor salt-tolerators occurring at downstream sites may be that individuals had been recently transported there by the current and were moribund when collected, although virtually all of the hand-collected specimens, of all species, were noticeably alive. A more plausible explanation may lie with the potential of downstream-occurring individuals to acclimatize to higher salinities – recall that all test animals were collected from site 1. Indeed, Haage (1969) has shown that the larvae of freshwater and brackishwater populations of the caddisfly *Phryganea grandis* L. exhibit a preference for the salt levels in their native waters. Larvae raised in freshwater could withstand 5‰ saline water, but not 10‰, however, larvae raised in brackish water could tolerate 10‰ for 29 days. Interestingly, a decrease in water temperature of 10 °C extended the

Table 4 Responses of freshwater-derived insects collected from the Aber Estuary to continued immersion in 35‰ seawater for 24 h

River taxa	Time intervals (48 h total) Condition in 35‰ seawater							Final condition after 24 h recovery in river water 48.0
	1.0	2.0	3.0	4.0	6.0	8.0	24.0	
Ephemeroptera								
<i>Baetis rhodani</i>	M	?D	D	D	D	D	D	all dead
<i>Rhithrogena semicolorata</i>	✓	?M	D	D	D	D	D	all dead
Plecoptera								
<i>Dinocras cephalotes</i>	✓	✓	✓	✓	✓	✓?	D	all dead
<i>Perla bipunctata</i>	✓?	✓?	✓?	M	M	M	D	all dead
small stonefly (Nemouridae)	✓	M	M	D	D	D	D	all dead
Trichoptera								
<i>Rhyacophila dorsalis</i>	✓	✓?	?M	M	MD	D	D	all dead
<i>Glossosoma boltoni</i> and <i>G. conformis</i> (combined)	L	L	L	LM	LM	LM	D	all dead
<i>Agapetus delicatulus</i>	–	–	–	–	–	–	–	
<i>Philopotamus montanus</i>	✓	✓	✓?	✓?	M	D	D	all dead
<i>Plectrocnemia geniculata</i>	–	–	–	–	–	–	–	
<i>Polycentropus flavomaculatus</i>	–	–	–	–	–	–	–	
<i>Hydropsyche siltalai</i>	✓	✓	✓	✓?	M	?D	D	all dead
<i>H. instabilis</i>	✓	✓?	✓?	✓?	M	?D	D	all dead
<i>Potamophylax cingulatus</i>	✓	✓	?	?	M	?D	D	dead (only 1 specimen)
<i>Silo pallipes</i>	✓	✓	✓	✓	?	?D	D	dead (1 specimen)
<i>Sericostoma personatum</i>	✓	✓	✓	✓	✓L	?L	?D	1 recovered, 3 dead
<i>Odontocerum albicorne</i>	✓	✓	✓	✓	✓L	?L	?D	2 recovered, 1 dead
<i>Adicella reducta</i>	✓	✓	✓	✓	✓	?	?	good (1 specimen)

(✓ = individuals were alive and moving; L = larvae had abandoned their cases – a sign of distress; M = moribund, i.e. the animals had stopped moving and had assumed an unnatural stance; D = dead; combinations indicate some individuals within the test group were in either state; – = sufficient numbers of individuals, between five and ten, were not available for a specific test).

tolerance time in all concentrations. The latter could also help explain the downstream shift in total Aber benthos that was associated with falling riverwater temperature (between September and December). It has been noted earlier that mayflies appear to be the freshwater taxon most adversely affected by high levels of salt (Short, Black & Birge, 1991). Whereas, in salinity tolerance experiments in the present study, the demise of salt-intolerant species probably provided reliable data, although in fairness it should be noted that these experiments assessed survival only over the short term. In the case of the salt-tolerant species, therefore, there is no evidence that individuals survived successfully to adulthood.

At this stage of the research, there is very little information on the behaviour of these aquatic insects *in situ*. Furthermore, there are few previous studies with which the data presented here can be compared. Sutcliffe (1961) studied the fauna of saltmarsh pools

in England and found a few species of euryhaline insects breeding there. In pools in which the maximum salinity reached was 33‰, the only aquatic insects found all year round were the midge *Chironomus aprilius*, the mosquito *Aedes detritus* and the beetle *Colymbetes fuscus*. Another midge, *Cricotopus vitripennis*, a shore fly, *Ephydra riparia*, a waterstrider, *Gerris thoracicus*, and the caddisfly *Limnephilus affinis* were found to the same salinity level, but were more seasonal in occurrence. In pools in which the salinity was < 10‰, Sutcliffe found two different gerrid species, four species of corixid bugs, three beetle species and *Chironomus riparius*. Clearly, salinity had a major effect on species distribution. Sutcliffe (1960a) suggested that the degree of osmotic independence shown by larvae of *E. riparia*, *A. detritus* and *C. vitripennis* was achievable only by a considerable degree of impermeability to water and salts. He noted that none of these three species had the functional

anal papillae characteristic of many other aquatic dipteran larvae. Salt taken in through the gut was thought to be excreted from the rectum as a hyperosmotic fluid.

Limnephilus affinis has, along with eighteen other caddisfly species, been found in the Gulf of Finland in water up to 6‰ salinity (Silfvenius, 1906). Some thirty-nine species, from several families, were recorded in Gulf of Finland waters with < 2‰. Sutcliffe (1960b) demonstrated that *L. affinis* was capable of surviving for several months in water that contained 26‰ salt, but only for a few days in approximately 30‰. It was suggested that tolerance of saltwater in this species was achieved via limited drinking of the surrounding water, combined with the ability of the lining of the gut to withstand high salt concentrations and the ability of the larvae to continue to produce hyperosmotic rectal fluid even at such high concentrations (Sutcliffe, 1962).

In a study of fourteen tidepools along the north shore of the St Lawrence River, salinity levels again influenced the assemblages of insects found (Williams & Williams, 1976). In pools with a salinity < 2.5‰ there were caddisflies (*Limnephilus tarsalis* and *Oecetis* sp.), a dragonfly (*Aeshna interrupta*), together with several species of beetle, corixid and chironomid. Whereas a few *L. tarsalis* were found in pools with salinity as high as 19.8‰, pools above 22‰ contained only the shore fly *Ephydra subopaca* and the marine midges *Halocladius* and *Cricotopus sylvestris*. Along the northern Swedish coast, populations of the stonefly *Leuctra digitata* have high drift rates from streams out into coastal areas with salinities of up to 4.2‰ where nymphal development continues to adult emergence (Müller & Mendl, 1980).

The most extreme examples of aquatic insects adapted to a marine existence include those listed below.

1 *Philaniscus plebeius*, a caddisfly from New Zealand and southeastern Australia, of which the female deposits her eggs into the coelomic cavity of starfishes. After hatching, the larvae leave their host and develop in tidepools (Winterbourn & Anderson, 1980).

2 The gerrid genus *Halobates*, which comprises some forty-four species that live on the surface of the oceans, especially in the Indo-Malaysian and Australasian region. Five of these live in the central Pacific Ocean and have no contact with land (Cheng, 1985).

3 The chironomid *Pontomyia* which completes its life

cycle at depths to 30 m on tropical coral reefs (Bretschko, 1982).

The most significant finding in the survey reported here was that, numerically, aquatic insects represented approximately one-third of the benthic metazoans in the tidal regions of the Aber Estuary. In general, as already noted, insects are believed to be poorly represented in saltwater environments. There are a variety of hypotheses to explain this, but many can be refuted on the grounds that each apparent physical, chemical or physiological barrier to living in saltwater has been overcome by at least one or two species from a variety of insect orders (see the above examples). Theories that propose the exclusion of insects, as a whole, from saltwater environments thus represent an unrealistic tenet. Undeniably, the majority of insects that have been recorded from marine situations have been collected in so-called 'bridging habitats' (e.g. estuaries, saltmarshes, intertidal areas, mangrove swamps, etc.; Cheng, 1976). It has been argued recently, therefore, that rather than insects being fundamentally unsuited to saltwater, we are currently observing the early stages in the spread of insects into marine environments (Williams & Feltmate, 1992). Clearly, in the Aber Estuary all the insect species found also occurred in purely freshwater parts of the system. In no way, therefore, can they be considered to be 'marine' species. Nevertheless, their ability to tolerate inundation by saltwater attests to changing osmotic, physiological and, possibly, behavioural capabilities. Perhaps there are genotypic differences in the abilities of subpopulations of these freshwater insects to cope with varying salinity, as has been demonstrated recently in the mosquito fish, *Gambusia holbrooki* (Congdon, 1994). The selective forces promoting such adaptations are the subject of ongoing research.

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