Process and consequence of a local invasion of *Chelicorophium curvispinum* (Crustacea:Amphipoda) into a small estuary in South-East England

By

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xiv

Abstract

The tube dwelling amphipod *Chelicorophium curvispinum* is a native of estuaries in the Northern Ponto-Caspian region. Over the last hundred years, *C. curvispinum* has invaded rivers throughout Western Europe. *C. curvispinum* was first reported in Britain in 1935. A population of *C. curvispinum* was discovered in the Kentish Stour Estuary in 1998.

The invasion of *C. curvispinum* into the Kentish Stour Estuary was detected by a routine monitoring survey carried out by the Ecology Research Group of Canterbury Christ Church University. Archive data from this survey covering the time-span 1996 to 2003 were examined and the process of the invasion was chronicled. Between 1996 and 2003, three distinct time periods were identified with respect to the invasion; Before, During and After. The period Before the invasion contained no records of *C. curvispinum*. The period During the invasion was characterised by the first reports of *C. curvispinum* in the Estuary, followed by a population spike. The period After the invasion was characterised by an initial population collapse followed by a stabilisation of numbers of *C. curvispinum*. Examination of physico-chemistry data collected in concert with biological samples found that, During and After the invasion, there was a drop in suspended solids, nitrites, nitrates and phosphates in the river. Multiple regression analysis found no direct relationship between any physico-chemical determinands and *C. curvispinum* abundance. Examination of the native fauna found no evidence of a weakening of the native fauna found no evidence of any impact of the *C. curvispinum* invasion on the native fauna.

Data obtained from the Environment Agency showed that the national distribution of *C*. *Curvispinum* had changed drastically between 1985 and 2003. The data describe a steady increase of sites occupied by *C. curvispinum* that continued until the mid 1990s. Experimentation indicated that *C. curvispinum* was eclectic in its requirements.

XV

Chapter 1

Invasions: opportunities, mechanisms and consequences

1.1 Overview

In June 2000, in my first month working for the Ecology Research Group in Canterbury, I overheard two researchers discussing an exotic amphipod that had been found in the estuary of the River Stour in Kent. This amphipod, known as *Chelicorophium curvispinum*, had been linked with biological and physico-chemical changes in rivers across North-Western Europe. Its appearance in the Stour estuary raises a number of questions, which will be considered by this thesis.

1.1.1 A short account of invasion biology

Invasion biology emerged as a discipline in the latter half of the last century. The seminal work on invasions was carried out by Elton (1958) and later works have sought to identify *when* an animal can be considered an invader. Ehrlich (1984) proposed that an invader is 'a species that can easily cross a barrier, with or without the direct aid of humans, and expands its range on the other side'. This definition will be the one used throughout the thesis when considering invasive species.

There are other definitions apart from the one suggested by Ehrlich (1984). Colautti and MacIsaac (2004) found a wide number of definitions of terms such as 'invasive' or 'nuisance species'. Often there was an element of bias towards calling a species 'invasive' based on whether it adversely affected humans. Examples included species that caused aesthetically displeasing damage, as well as species that caused damage of some kind in one region, but not in others. In addition to the ambiguity of the word 'invasive' Colautti and MacIsaac (2004) also found that a number of terms, such as 'introduced', 'naturalized' and 'weedy', had a similar meaning in the invasion biology literature.

A criticism of the sub-discipline of invasion biology is that it works in isolation from other sub-disciplines. Davis *et al.* (2001) argued that several decades of research into invasions had failed to produce reliable general theories. The main reason given for this lack of progress was a lack of information flow into invasion biology from other related disciplines, such as succession ecology. Colautti and MacIsaac (2004) suggested that nonindigenous species could be viewed simply as long distance colonisers.

All cosmopolitan organisms have, at one time in their evolutionary history, been colonisers. On a geological time scale, worldwide colonisation of terrestrial species within its niche limits was until 90 million years ago, the normal state of events, due to a lack of ocean barriers to a colonisers progress (Elton 1958). In the early Tertiary period, however, continents became sufficiently separated by plate tectonics and fluctuating sea levels for something resembling the modern segregated and specialised faunal regions (known as Wallace's Realms) to come into being (Elton 1958).

At first sight, the rich literature on invasions detailed below seems to show few common features. However, common themes can be tentatively discerned. To give structure to the consideration of alien species, the rest of this chapter has been divided into four areas; the role of humans in contemporary invasions, attributes of invasive species, the origin of invasive species and the interactions between invasive species and the habitats they invade.

1.2 The role of humans

A striking feature of most invasions is the overwhelming contribution of human beings as an invasion vector. Di Castri (1989) noted the link between the emergence of civilisation and the increase in distribution of some species, citing the Mediterranean Aleppo Pine (*Pinus halepensis*), which reached its greatest old world distribution during the time of the Roman Empire.

Some invasive species prosper where humans have disturbed the environment, but it is possible that these species are responding to disturbance, irrespective of its cause. Di Castri (1989) identified several similarities between man-made and natural disturbance conditions; firstly, he associated early agriculture with the natural creation of open spaces due to occurrences such as frost and aridization. He also noted the similarity between early man's use of fire and the incidence of natural fires. Finally Di Castri noted the equivalence between grazing pressure by large wild herbivores and the effects of early livestock breeding. These parallels were intimated to have a synergistic effect on natural selection mechanisms that were already in place.

To consider anthropogenic processes as merely an extension of processes that took place in pre-human times is, of course, an oversimplification. However, such a consideration is a useful heuristic. In order to make this heuristic ecologically meaningful, certain types of 'invader' must be excluded from consideration, namely invaders that are actively supported by man, for example, domesticated livestock such as cows and sheep, and crop plants like wheat. These organisms could be considered more a product of social conditions, with animals and plants being supported by husbandry in habitats where, without such support, they would be unlikely to persist. These organisms confuse an already complicated phenomenon and will not be considered further in this thesis.

It is probable that if humans didn't exist, invasions would still take place, although admittedly at a much reduced rate. Evidence for this can be found in the fossil record: the collision of North America and South America is known to have caused invasion by species such as the Tapir (Elton 1958). In Eurasia, invasion waves from the Pleistocene to the present by the amphipod *Echinogammarus ishnus* were identified by examining genetic divergence of different populations in isolated parts of the Ponto-Caspian basin (Critescu *et al.* 2003). Evidence such as this makes it possible to view invasions as an ecological or evolutionary, rather then merely a social anthropological, phenomenon. In fact it has been emphasised that the invasion of, for example, one continent by species of another is a true evolutionary process, akin to speciation and extinction (Di Castri 1989).

Having argued that the impact of man can, with some reservations, be considered as a multiplier of mechanisms already present in natural ecosystems, it must be conceded that synanthropic mechanisms are comprehensive in their effect on world biota. Alien introduction has been classed as the second biggest threat to global biodiversity after habitat destruction (Convention on Biological Diversity 2005). Alien introduction is likely to be a particularly severe issue for freshwater aquatic systems in the future (Sala *et al.* 2000). The British interpretation of the recently formulated European Water Framework Directive lists alien species as potentially 'significant anthropogenic pressure' (United Kingdom Technical Advisory Group, 2004).

Di Castri (1989) proposed that the rate and type of invasion could be split into four distinct time phases. During the first phase, dubbed the Geological phase, invasions were caused by tectonic movement (such as the collision of North and South America), and climatic changes (such as ice ages). The second period was termed the Pre-historic phase, during which the influence of humans began to emerge, along with synchronous climatic and tectonic changes. The third period was dubbed Historical to 1500, and was distinguished from the earlier two by the 'stronger and

more extended man-made perturbations' and mass movement of humans within biogeographical realms. The Final period stretching from 1500 to the present charted the mass opening-up of routes between biogeographical realms by humans.

It is often possible to discern specific routes, or migration corridors which facilitate the spread of invasive species (Bij de Vaate *et al.* 2002, Critescu *et al.* 2003). These corridors can be physical linear objects, such as roads or canals, or conceptually linear, as is the case with inter-continental trade routes and shipping lanes. The Oxford Ragwort *Senecio squalidus* illustrates the use of corridors by an invader (Figure 1.2.1). This plant was brought to the Oxford Botanical Gardens from Sicily around 1700. In 1794 it was observed to have escaped from the botanical gardens and was found growing on the city walls. Subsequently the plant spread to the railway network, where it flourished in the clinker on which the tracks were laid (http://www.ragwortfacts.com/oxford-ragwort.html).



Figure 1.2.1.Oxford Ragwort (*Senecio squalidus*) on the platform at Bangor Station in North Wales. This mediterranean alpine plant is found along railway lines across Britain.

However, simple opportunity does not explain why some organisms are successful invaders, whilst others are not. Bohning-Gaese *et al.* (1998) compared long distance migratory birds with non-migratory birds and non-flying mammals, and found no obvious link between latent dispersal ability and colonisation success. Richardson *et al.* (2000) stated that human mediated transport was likely to be more important in determining ranges of non-indigenous species, whereas biological processes, such as competitive ability and lack of natural enemies might determine the eventual density of the invader once it had reached a new habitat. It is possible that the non-invasive nature of the long distance migrants examined by Bohning-Gaese *et al.* (1998), could be due to a lack of appropriate biological processes.

Not all organisms that are introduced to a habitat turn out to be successful invaders. It is difficult to quantify how many invasions are successful. Marchetti *et al.* (2004) remarked that 'the literature on animal invaders focuses largely on successful invasions over broad geographical scales, and rarely examines failed invasions'. This is understandable, as a failed invasion is more difficult to detect than a successful one. For example, an organism that reaches a habitat may fail to establish and become extinct without being recorded. On the other hand an organism might become established in an area that is not examined by biologists, and the resulting successful invasion of Hastings old town by the antipodean land amphipod *Arcitalitrus dorrieni*, which was discovered after a chance invitation to a house warming Party (personal observation, identification kindly confirmed by Dr P Smithers, Plymouth University).

Only around 10% of species that are introduced to a habitat develop populations dense enough for them to be regarded as a pest (Williamson 1996). Williamson does equate success with species population density, when maybe species persistence in a habitat would be a more useful measure, but it does generate some

questions, such as: what it is about the 10% that makes them so successful and why are the remaining 90% less successful?

1.3 What makes an invasive organism so successful?

All invaders are different, but it is possible to suggest some traits that are found in organisms that prosper when introduced to novel environments. Several researchers have compiled lists of these traits (Elton 1958, Ehrlich 1984, Bij de Vaate *et al.* 2002). These include:

- an abundance in their original range
- a non-specific, or wide feeding niche
- eclectic ecological requirements
- relatively short life span/generation time
- rapid growth
- early maturity
- high fecundity
- dispersal by larvae
- protection of juveniles.

There might also be a disparity in size between the invader and related taxa. Finally Bij de Vaate *et al.* (2002) observed that in general, invaders tended to be more successful in regions with a climate that is similar to their home range. Gaston and Spicer (2001) suggested that local species density was a good indicator of global range size. Thus it might be possible, if somewhat simplistic, to predict invasiveness by looking at species with high local densities, but small global distributions.

A possible factor determining the success of an invasion is the propagule pressure exerted by the invader. Propagule pressure is defined by Colautti *et al.* (2006) as

'the number of individuals introduced and the number of introduction attempts'. Crowel (1974) states that a propagule is the number of individuals needed to form a viable population. Invasions are usually accidental in nature, so propagule pressure can be difficult to estimate once the invasion becomes evident. Furthermore, deliberately introducing invasive species in varying numbers in order to determine their success is unethical.

One way of estimating the effect of propagule pressure is to examine studies carried out before such ethical considerations became evident. For example, Ebenhard (1989) introduced varying numbers of bank voles to small islands of the Stockholm archipelago, just off the coast of Sweden. He found that the more voles he introduced to an island, the greater the success of the population on that island. A second way to assess the effect of propagule pressure on the establishment of a species is to examine instances where a species has been introduced deliberately for conservation purposes. For example, Berggren (2001) found a positive correlation between propagule pressure and eventual population size for Roesel's bush cricket Metrioptera roeseli. Overall, studies into propagule pressure seem to indicate that it is a significant factor in determining the success of an invader (Tillman 2004, in Alpert 2006), although the number of individuals needed for a successful establishment seems to be species specific (Crowel 1973, Berggren 2001), and in some cases sub-population specific (Ebenhard 1989). It is possible that a more successful invader might need fewer propagules in order to establish itself in a habitat. In one study, the european rabbit, Oryctolagus cuniculus which is a successful invader in many habitats, needed only one male and one female to form a viable breeding population (Armstrong 1982, in Berggren 2001).

McMahon (2002) argues that successful invaders like *Corbicula spp.* and *Dreissena polymorpha* are less able to cope with harsh conditions than other species (for

example freshwater unionid bivalves) that invest relatively more energy in growth than reproduction. Erhlich (1984) lists eclectic ecological requirements as a feature of a successful invader. A positive relationship between range size and niche breadth was found by Gaston and Spicer (2001) when they examined estuarine species of the amphipod Genus *Gammarus*.

Most of the attributes listed above, such as a short life span and high fecundity, suggest that the strength of most invaders lies in their ability to adapt quickly by micro-evolutionary mechanisms to fare better in their new environment. This can lead to sub-populations of an invasive species having quite different ecological tolerances. An example of this is the invasive bivalve *D. polymorpha*, which exhibits local adaptation to both salinity level and temperature (Sykes 2003). It might be the case, therefore, that if the ecological tolerance of several sub-populations was pooled, then a wide tolerance might appear to exist where local adaptation was in fact the phenomenon being observed.

1.4 Where do invaders come from?

Many of the traits outlined above, such as high fecundity, short generation time, and investment in offspring instead of adult development, suggest an r-selected life history strategy (Cole 1954, Lack 1954). Such strategies are thought to help a species cope with harsh and potentially ephemeral conditions (Skelton 1993). Thus, it might be reasonable to assume that the place to look for particularly invasive species is an area that has, at present, or at some time in the geologically recent past, been a relatively unstable and harsh environment. This simple hypothesis is difficult to test for the following two reasons:

1. An area that was formerly disturbed may have in geologically recent times become stable, for example the Rift Valley in Africa.

 Past invasive processes further confuse the fluid nature of organisms' distributions over time. An animal that exemplifies this problem is the Tapir, which evolved in North America, but after invading South America became extinct in its home range (Elton 1958).

It is possible that invaders evolve their useful traits through either natural selection or It might be possible to see which traits could be caused by random genetic drift. genetic drift by examining which traits prove useful in the invaders native range. If genetic traits that increase the fitness of the organism in its home range are the same as those that increase its fitness in its extended range, then the trait is probably a product of contemporary natural selection. If, however, the organism uses different traits in its native and extended range, these traits are either stochastic, unpredictable factors, or a relic of past selection pressure. I propose that the mechanism of trait development is relevant to invasive species for the following reason: if traits are primarily acquired through natural selection then the place to look for new invasive species would be anywhere that there is intense competition for resources and/or an ephemeral unpredictable environment (for instance the side of a volcano, or a sand dune system). If genetic drift contributes a significant number of traits that prove useful in invaders, then this increases the chance of invasive species originating in small, stable populations, and means that invaders could come from almost anywhere in the world.

An aspect that could give clues to the potential range of invaders is their past range size. In the 'Old World' (also known as the Palearctic biogeographic region), the relatively high ratio of land to sea ensured that the effects of the Quaternary ice ages was greater than in any other region. Fauna that could not survive life on the ice sheets were reduced to scattered 'massifs de refuge' from which they could emerge during periods of thaw, in order to re-colonise their former range (di Castri 1989). Evidence for this is provided by discoveries such as that of Miller *et al.* (1979) who

found remains of *Corbicula* (a clam thought native to Asia) underneath Trafalgar Square in central London. The remains were dated to the last interglacial period. The ephemeral nature of species distribution makes speculation about the influence of past environmental change on the invasive potential almost a redundant exercise. However, there is geologically recent evidence which suggests that a suitable environment can produce a whole raft of invasive species. This evidence is provided by the invasive activities of the Ponto-Caspian fauna.

1.5 The Ponto-Caspian fauna

The Ponto-Caspian region comprises the Caspian, Black and Aral seas (Critescu *et al.* 2003). Ponto-Caspian fauna have been responsible for some of the most notable invasions of recent times. This in itself would not single them out for consideration, were it not for the large number of species endemic to the Ponto-Caspian that have become successful invaders.

At present the Black Sea and the Caspian Sea are both brackish water bodies, with salinities ranging from <1 ppt to around 13 ppt (Reid & Orlova 2002). The Ponto-Caspian basin is a relic of the ancient Tethys Sea. This archaic water body once divided the northern and southern super-continents, and provided a corridor linking the great eastern and western oceans (Elton 1958). Mountain formation caused by continental collisions separated the Tethys into several smaller water bodies, one of which, the Sarmatian Sea, was a precursor of the modern Ponto-Caspian basin. Since this period the Ponto-Caspian basin has been periodically cut off from saline influence, leading to drops in salinity due to freshwater input. These periods have been interspersed with periods of re-connection to the Mediterranean, which created saline (and according to some sources, hypersaline) conditions (Reid and Orlova 2002).

Bij de Vaate *et al.* (2002) proposed three possible invasion corridors used by Ponto-Caspian species to expand into northern and western Europe: a southern corridor from the Danube to Rhine river basin, a central corridor connecting the Dneiper to the Rhine via the Vistula, Oder and Elbe rivers, and a northern corridor linking the River Volga to the Baltic sea (Figure 1.5.1).



Figure 1.5.1. Invasion corridors used by Ponto-caspian species to disperse across Europe. From Bij de Vaate *et al.* (2002)

Many of the Ponto-Caspian fauna that have extended their range in recent times display r-selected traits, such as short generation time, high fecundity and early maturity, for example *D. polymorpha* and *D. bugensis*. These traits are not exclusive to the Ponto-Caspian fauna. However, the unique haloclinically fluctuating status of their original environment could provide clues to their success. McMahon (2002) argued that, invaders have little tolerance to environmental change and rely instead on their ability to swiftly re-colonise areas after an environmental disturbance has passed. However Harris and Bayliss (1990) found that individuals of the Ponto-Caspian amphipod *C. curvispinum* could acclimatise to different levels of salinity.

This single trait could give an advantage to a Ponto-Caspian invader by allowing it to colonise areas of fluctuating salinity (for example near estuaries or industrial sites), faster than fresh-water or brackish-water native species.

Harris and Bayliss (1990) found that *C. curvispinum* expended a greater amount of energy in maintaining homeostasis with respect to sodium ions than other freshwater crustaceans. In an oligotrophic environment, this extra energy burden could put the organism at a disadvantage. However, most lowland rivers are affected in some way by eutrophication. Thus energy is easier to obtain, possibly conferring an advantage to this organism over other energy conserving but less adaptable organisms.

I believe one possibility is that marine species became adapted to freshwater in the Ponto-Caspian basin by virtue of the slowly fluctuating salinity. The first colonisation of the land/freshwater by marine organisms could be considered as a sort of 'trait filter' with only the traits carried by the colonising species being carried into these habitats. Therefore, any marine forms that were subsequently forced by selection pressure to adapt to freshwater could potentially bring to the freshwater/brackish biota a whole raft of useful traits not previously found in these habitats. An example of this is the planktonic stage of the freshwater bivalve *D. polymorpha*. This animal can colonise without the aid of a vector, unlike most freshwater unionoids, which are obligate parasites when in their glochidia stage. This trait could enhance its invasive capabilities.

1.6 The invaded ecosystem

The final aspect of invasion biology to consider is the habitat that has been invaded, which in this context could be termed the sink habitat. Several factors can potentially impact an invading organism when it is introduced to an ecosystem. Alpert (2006)

suggested that an invader might benefit from being introduced into a new habitat in several different ways: the invader might be introduced in sufficient densities to swamp competing natives. Potential invaders could also be filtered, intentionally or unintentionally, by human action to ensure its suitability for the sink habitat. The invader could also escape natural enemies, enabling it to proliferate more readily in the sink habitat. Finally the invader might bring with it a novel disease, or possess a toxin to which native species had no resistance, which would give the invader a competitive edge. Alpert (2006) suggested that the invader could also suffer negative consequences when invading a habitat. The invasive organism could miss out on mutualistic relationships with other species in the source habitat. The sink habitat could contain diseases and potential predators that the invader was poorly adapted to resist. Finally, Native specialists could be better adapted to their environment and directly out-compete an invader.

The invader, if it settles successfully in the new habitat, can subsequently change the sink habitat in a number of ways. The relationship between these factors could be seen as a three pointed triangle, with the three points representing the invader, the physico-chemistry, and the native biota. The lines between the three points would represent the two way relationship between each of these variables (Fig. 1.6.1).



Figure 1.6.1 The relationship between an invader and the invaded habitat

1.6.1. The ecosystem response to an invader

Elton (1958) suggested a negative correlation between diversity of biotic assemblies and the likelihood of that assembly being invaded. This has been termed *invasion resistance*, the implication being that diverse native communities were more likely to fend off settlement by exotics than less diverse species assemblages.

Some empirical evidence supports the concept of a positive correlation between diversity and resistance to invasion. Stachowitz *et al.* (2002) studied the effect of diversity on the ability of a sessile, hard substratum dwelling, subtidal invertebrate community to resist invasion by three species of exotic ascidians (sea squirts). They found a strong negative correlation between native species richness and the number and frequency of non-native invaders. The reason suggested for this was that the different native species grew at different rates and occupied space in such a way that different species dominated at different times. At maximum native species richness, space on the hard substratum (the limiting resource for this type of community) was covered more frequently, leaving less space for the non-natives to settle on.

Consumption of exotics by native heterotrophs is another mechanism that can contribute to the resistance of a habitat to invasion. Reusch (1998) reported that after four months, 95% of an experimental population of an Asian mussel, *Musculista senhousia* was consumed by native predators. Moloy *et al.* (1994) found that in the short term, numbers of *D. polymorpha* could be reduced by native predators.

Other studies attribute less importance to the native biota in determining invader success. Fine (2002) found no evidence to attribute the low invasibility of tropical rainforests to either "their high species richness, or their high diversity of functional types". Instead he provided evidence for a variety of other mechanisms, one of which was propagule pressure. According to Fine (2002), exotic species propagules did not generally get into an undisturbed forest in quantities great enough to establish

themselves. He also suggested there could be research bias, stating that most studies examined shade intolerant invaders that would not survive under a dark, undisturbed tropical forest canopy.

Levine (2000) suggested that scale was an important factor in determining the existence of invasion resistance. He found that on a small scale (in his case the assemblage of plants supported by a single tussock of a riparian sedge), greater species richness conferred resistance to invasion. As the scale was enlarged to look at a whole community, other effects that co-varied with species richness led to an increase in habitat invasibility.

It is possible that community resistance occurs when the native and invasive species compete for the same limiting resource. With a large number of different habitats and an almost infinite number of interactions there could easily be a circumstance where an invader competes with a native species or a guild of native species for a resource, and the invader is out-competed. However, this outcome would depend on several factors, such as propagule pressure, competitive ability of invader, health of native competition and prevailing physico-chemical conditions. This brings into question whether the idea of invasion resistance is a useful heuristic at all, or whether it is just a 'catch all' concept that obscures more relevant underlying processes. Levine and D'antonio (1999) reviewed the studies carried out on the link between native species diversity and invisibility, and came to two main conclusions:

- recent theoretical studies consistently found a negative relationship between native diversity and habitat invasibility
- empirical studies showed both negative and positive relationships between native diversity and habitat invasibility.

They further argued that the any negative relationship found between invasibility and native diversity could have been due to physico-chemical factors that co-varied with diversity.

1.6.2. The effect of an invader on an ecosystem

Some invaded eco-systems seem be minimally affected by invasive organisms. For example, the antipodean land amphipod *A. dorrieni* seems to have no significant effect on leaf litter communities that it invades, despite becoming the numerically dominant species (Smithers, 2003 personal communication)(Fig. 1.6.2.1). Burger *et al.* (2001) failed to find any negative association between native and invasive spiders in Californian coastal sage scrub. They also found a positive correlation between some native and exotic spider species.



Figure 1.6.2.1. Third year undergraduates collecting the exotic terrestrial amphipod *Arcitalitrus dorrieni* from a wood near Cardiff, South Wales. This amphipod is predicted to only invade parts of Britain with mild winters, with mean monthly temperature staying above 1.4 °C, and high salinity (greater then 5 mOsmol. I⁻¹). (Cowling *et al.* 2003).

Obviously the association between an invader and its new habitat is not always so benign. A rather grisly example of an impact is provided by the Ponto-Caspian freshwater predator *Dikerogammarus villosus*. This amphipod has invaded throughout Europe, and populations of benthic invertebrates have declined after its introduction. In the laboratory it was found to not only attack and consume other invertebrates but also to administer fatal bites to animals that it did not subsequently consume (Dick *et al.* 2002).

Invaders have been found to out-compete natives as well as preying on them. Invaders can also change the physico-chermistry of a habitat, and this in turn can impact on the native fauna. A number of different effects of invaders on the environment can be seen in the Laurentian Great Lakes:

The Great Lakes have been invaded by more than 145 exotic species (Ricciardi and MacIsaac 2000). The Ponto-Caspian Zebra mussel (D. polymorpha) was discovered in the Great Lakes in 1988 (Ricciardi and MacIsaac 2000). Since this time it has been reported to: increase water clarity through filtration, create reef habitat from the shells of dead mussels, smother native mussels, and change the composition of the plankton, through selective rejection of colonial cyanobacteria in psuedo-faeces (Vanderploeg et al. 2002). Ricciardi et al. (1998) found that native freshwater mussels in the Great Lakes were extinct within 4-8 years subsequent to an invasion by D. polymorpha. Another Ponto-Caspian import, the amphipod E. ischnus was first identified in the Great Lakes in 1995 (Ricciardi and MacIssac 2000). This freshwater shrimp has become the dominant macro-invertebrate on rocky shores in western Lake Erie. It is also reported to out-compete the native Gammarus fasciatus (Dermott et al. 1998). The New Zealand mud snail Potamopyrgus antipodarum was found in Lake Ontario, having become the dominant species in several surrounding waterways (Zaranko et al. 1997). Its dispersal may have been aided by its apparent ability to pass unharmed through the alimentary canal of some fish species (Haynes et al. 1985, in Zaranko et al. 1997). Taylor and Hebert (1993) found evidence of hybridisation between introduced and native populations of Daphnia. This kind of hybridization could lead to a global loss of genetic diversity for a species.

There are more serious implications of hybridization between native and introduced species. Around 1870, native saltmarsh cord grass *Spartina maritima* hybridized with a species of chord grass from North America called *Spartina alterniflora*. The resulting hybrid, *Spartina anglica*, grew more vigorously, was more fecund then either of its parent species, and colonised aggressively (Benham 1990, in Eno *et al.* 1997). This species spread from Southampton water to many salt marshes in the south of England. *S. anglica* accretes more sediment then native species, has more productive photosynthetic pathways, and is particularly tolerant of submergence in seawater (Thompson 1991).

Invasive species can also transmit disease to natives. The native white clawed crayfish *Austropotamobius pallipes* is catastrophically affected by crayfish plague. This disease is caused by a fungus, *Aphanomyces astacii*, which is carried by the introduced signal crayfish, *Pacifastacus leniusculus* (Guan and Wiles 1997).

Invasive species can also indirectly impact on native organisms. In South Africa the invasive argentine ant *Linepithema humile*, has out competed the native ants *Anoplolepis custodiens* and *Pheidole capensis* in several areas. This has had a deleterious effect on several species of protea, particularly *Mimetes cucullatus*, which depends on the native ants for dispersal of its seed (Beeby and Brennan 2004).

As well as considering invaders in a geographical sense, an invader can be examined in a more taxonomic context. It is possible that certain taxonomic groups might exhibit a greater tendency towards invasive behaviour than other groups. Closely related groups of animals are likely to exhibit similar traits, and this factor could make them more successful invaders.

The taxonomic group of most relevance to this thesis is the amphipod family Corophiidae. Several species are thought to be invasive, or exhibit cosmopolitan distributions that are consistent with past invasive activity.

1.7 The Family Corophiidae

Amphipods of the Family Corophiidae are tube dwelling filter/deposit feeders. There are more than 100 species of Corophiidae, living in a range of habitats, from marine and intertidal, through to fresh-water (Lincoln 1979). Three species of Corophiidae are reviewed here; *Chelicorophium curvispinum, Corophium multisetosum*, and *Corophium volutator.* These species were all suspected inhabitants of the Kentish Stour Estuary.

1.7.1 Chelicorophium curvispinum

C. curvispinum (Fig. 1.7.1.1) is native to the rivers Volga, Danube, Dneiper and Don (Pygott and Douglas 1989). This amphipod has been extending its range since 1900 (Jazdzewski 1980), and was first recorded in the British Isles, near Tewksbury, in 1935 (Crawford 1935).

C. curvispinum builds its tubes on hard substratum and vegetation, although it can live in low densities in benthic sediment (Den Hartog *et al.* 1992). Pygott and Douglas (1989) examined a section of the bottom of the Shropshire union canal after it had been drained for engineering work to be carried out. They found *C. curvispinum* to be the numerically dominant invertebrate in the canal, occurring in high densities on horizontal concrete surfaces. *C. curvispinum* was found in noticeably lower densities on vertical surfaces. A small number of individuals were also found attached to vegetation. Several other authors have remarked on the correlation between *C. curvispinum* abundance and presence of hard substratum (Van den Brink *et al.* 2002, Musko *et al.* 1998, Musko 1992, Pygott and Douglas 1989, Sebestyen 1937).
C. curvispinum has a lifespan of approximately one year, although the number of generations seems to vary. Rajagopal *et al.* (1999) reported three generations a year in the Lower Rhine. In Lake Balaton in Hungary, however, only two generations a year were traced (Musko 1992). The eggs are stored by the female in a brood pouch (Lincoln 1979).

Several case studies have examined the ecology of C. curvispinum in European rivers. In the river Meuse, C. curvispinum became the dominant macro-invertebrate five years after it was first discovered in the river (D'udekem D'acoz and Stroot 1988). C. curvispinum was first discovered in the lower river Rhine in 1987 (Van den Brink et al. 1993). Two years later it was the dominant macro-invertebrate in the river's lower course, increasing in population density from 2 to 200 000 individuals per m² (van den Brink et al. 1993). Possible reasons for this population explosion were suggested by Den Hartog et al. (1992): firstly, the lower course of the Rhine had suffered several major pollution incidents. The most famous pollution event was the 'Sandoz incident' of 1986, which effectively cleansed the river of its biotic communities, including vertebrates that prey on C. curvispinum and other possibly competing invertebrates. The salinity of the lower river was also increased by discharge from salt mines in the area, while water temperature was raised by thermal pollution from industry. Secondly, the river had been highly modified to prevent flooding. This modification provided C. curvispinum with concrete and large stones on which to build its tubes.

Rajagopal *et al.* (1999) found that number of eggs per brood of *C. curvispinum* on the Rhine was one of the highest recorded (although an exact figure was not provided). They also found a positive correlation between eggs per brood of *C. curvispinum* and concentration of chlorophyll-a in the lower Rhine.



Figure 1.7.1.1. *Chelicorophium curvispinum.* The top animal is a female. The bottom animal is a male.

In the United Kingdom, *C. curvispinum* has been reported from many locations, mainly large rivers and canals. However it has only been reported from one river in the U.K. that is not navigated by boats (Pygott and Douglas 1989). Furthermore, Bij de Vaate *et al.* (2002) stated that the main inland vector of *C. curvispinum* was boat hulls. This species is the main subject of the thesis.

1.7.1.1 C. curvispinum as a euryhaline organism

There is evidence that *C. curvispinum* is a euryhaline organism, even though it is mostly found in freshwater. It was first described as a brackish water species (Sars 1895). Harris and Bayliss (1990) found that *C. curvispinum* expended a greater amount of energy in maintaining homeostasis with respect to sodium ions than other freshwater crustaceans, implying that it was a recent addition to the freshwater fauna. Lincoln (1979) stated that *C. curvispinum* is found in salt, brackish and freshwater.

1.7.1.2 The trophic status of C. curvispinum

As mentioned previously, *C. curvispinum* is a filter feeder. Van den Brink *et al.* (1993) examined the intestinal tract of *C. curvispinum* and found several species of planktonic diatom and green alga.

Eleven species of fish are recorded as predators of *C. curvispinum*, including perch, European eel and Bullhead. Musko (1992) reported that male *C. curvispinum* were more often found in stomachs of European eels then females. The reason given for this was that males were more likely to be found outside their tubes, searching for females, thus making them easier prey.

1.7.1.3 A taxonomic note concerning C. curvispinum

The genus *Chelicorophium* was originally created by Bousfield and Hoover (1997), as part of a paper that broke the genus *Corophium* into several smaller genera. In 2005, Gary Poore, at the Sixth International Crustacean Congress, presented evidence which cast doubt on the phylogenetic rationale behind Bousfield and Hoovers' revision (Poore 2005, personal communication). For the sake of consistency, and because the work of Poore has yet to be published, I have used the taxonomy of Bousfield and Hoover (1997). It would seem possible however, that the name might well revert back to it's original name, *Corophium curvispinum*.

1.7.2 Corophium multisetosum

Corophium multisetosum (Fig. 1.7.2.1.) is a brackish water species, which normally builds its tubes in sand and clay. It occasionally constructs tubes on hard substrata, but this only occurs when *Corophium lacustre* is also present (Ingle 1963). *C. multisetosum* has been found in salinities ranging from fresh water through to 30 $^{0}/_{00}$ in Canal de Mira in Portugal, although the highest densities (>80 000 individuals per m²) were found at salinities ranging from 2.5 to $10^{0}/_{00}$ (Queiroga 1990). Sediment particle size was also found to be important in determining *C. multisetosum*

settlement, with the species preferring sediment grades above $125\mu m$ (Queiroga 1990).



Figure 1.7.2.1. A large female Corophium multisetosum.

1.7.3. Corophium volutator

C. volutator (Fig. 1.7.3.1) is generally an inhabitant of intertidal mud flats and the silty mouths of river estuaries. This species is predominantly a deposit feeder, consuming benthic diatoms and bacterial films on sand particles (Creach *et al.* 1997). Distribution of *C. volutator* seems to be limited by sediment type, salinity and the presence of macro-algal mats (Lawrie *et al.* 2000). *C. volutator* travels by both swimming in the water column and by crawling across the sediment (Lawrie and Raffaelli 1998). Swimming activity tends to takes place on the flood tide, dispersing individuals uptide/upstream (Hughes and Gerdol 1997).

The life cycle of *C. volutator* is based on two generations a year, one over-wintering generation that dies over the summer, and a summer generation that reproduces until October (Fish and Mills 1979).



Figure 1.7.3.1. *Corophium volutator* from the Stour Estuary. The top animal is a male. The bottom animal is a female.

1.8 The aims of the thesis

This thesis considers the subject of invasion, using the Ponto-Caspian amphipod *C. curvispinum* as an heuristic. This examination mostly focuses on a long term dataset of the Stour Estuary, in Kent, in the United Kingdom.

One of the aims of this thesis was to investigate the question 'How did the Corophiidae, specifically *C. curvispinum*, invade the Kentish Stour Estuary?' The literature review above has revealed that many factors can potentially influence the occurrence and eventual success of an invasion. The two main factors were:

(i) Opportunity.

The opportunity to invade could be created by changes in geographical distribution of the invader, an increase in propagule pressure on the invaded habitat or an increase in the susceptibility of the receiving ecosystem to invasion.

(ii) The suitability of the invading organism

The organism invading the habitat would need to be able to successfully adapt to its new surroundings. The traits and attributes of an invader would have to match the invaded habitat in order for the opportunity mentioned above to be exploited by the invader.

The first factor will be investigated by examining a case study of a *C. curvispinum* invasion and by analysing *C. curvispinum* data from across the country. The second factor will be investigated by experimentation on *C. curvispinum* itself.

A second aim was to look at the effect that the *C. curvispinum* invasion had upon an invaded habitat. Literature quoted above has revealed that a tube-building filter feeder invading a habitat in large numbers has the potential to affect both the native fauna and the physico-chemistry of the invaded habitat. This aim would be investigated by examining the changes in the physico-chemistry and fauna of a case study habitat before and after the invasion.

Specific objectives that address these aims in more detail are revealed in context within the thesis as each chapter unfolds.

Chapter epilogue

In order to fulfil these aims it was necessary to make extensive use of archive datasets from a monitoring project for the Stour Estuary in Kent, U.K. However, before these datasets could be used it was necessary to learn more about how to get the most information from them, with respect to the *C. curvispinum* invasion.

Chapter 2

The Stour Monitoring Dataset

2.1 Overview

The River Stour has the second largest catchment in Kent. The river rises from two main tributaries: the East Stour rises just to the north of Folkestone and flows to Ashford, where it meets with the Upper Great Stour, which rises at Lenham, near Maidstone. Downstream of this confluence the river is known as the Great Stour (Environment Agency 2005). From Ashford the river flows through an alluvium channel carved from the chalk of the North Downs (Coleman 1967), reaching the silted up line of the former Wantsum channel near the village of West Stourmouth. The Stour is joined by the Little Stour at Plucks Gutter, after which it follows the line of the former Wantsum channel to the remains of the Roman Fort at Richborough. At this point the river channel changes direction south and describes a large loop taking in the town of Sandwich at its southerly point before switching direction and heading north to discharge into the sea at Shell Ness. This shape is due to the prevailing currents, which at some time in the past led to a silting of the river mouth, thereby moving the entrance progressively in a southerly direction. At some point the direction of these prevailing currents changed, and the river mouth started to move again, this time to the north. It was this process that gives the Stour estuary its characteristic 'U' shape.

Most of the water in the river comes from chalk springs, and so to an extent the discharge of the Stour is dependent on groundwater levels. However, part of the catchment area includes impermeable Gault clay and London clay formations. This makes the river, particularly the upper catchment, prone to floods following heavy rain (Environment Agency 2002). The average annual rainfall is 750 mm per year in

the upper catchment, and 600mm per year in the lower catchment. In 2001, 476 400 people lived in the Stour catchment area. There are abstraction licenses for the catchment that permit up to 187,719 megalitres a year to be removed from the river and associated groundwater (Environment Agency, no date).

The tidal limit of the river is just below the old Cinque Port of Fordwich, 35 kilometres from the river mouth. The limit of saline penetration into the Stour is just above the town of Sandwich (Figure 2.2.1). The suspended solid load of the water column is high in the estuary (Buckley *et al.* 2004). The name Stour comes from the Latin *Stauro*, meaning strong, or powerful (Environment Agency, no date).

The Ecology Research Group (ERG), at Canterbury Christ Church University, has studied aspects of the River Stour in Kent since the early 1980s. In the Early 1990s the ERG was approached to initiate a monitoring programme of the Estuary, to run before, during and after the commissioning of two effluent treatment works (ETW). One ETW was commissioned by Southern Water at Weatherlees Hill, and was due to be operational in March 1994 (Rees-Jones 1998). A second ETW was commissioned by the pharmaceutical company Pfizer and was due to be fully operational by April 1998 (Trigwell 2001).

A major part of this study was based on archive data from these surveys, conducted between June 1996 and September 2003. The June 2000 survey was the first survey I was involved with. All previous surveys were carried out by other researchers. The Ecology Research Group started surveying in 1993 in order to investigate the impact of the two sewage works being built on the Stour estuary. Surveys carried out before June 1996 were less comprehensive than those carried out afterwards and were not included in any of the analyses. The partial dataset from before June 1996 contained no mention of any Corophiidae at any of the sites examined on the river.

2.2 Survey methodology

Four surveys were carried out each year, in June, September, December and March/April. Each survey was started at the turning point of the high tide in order to standardise the haloclinic state of the estuary at the time of sampling. Each survey covered ten sites (Figure 2.2.1) from Plucks Gutter (site 10), at the upper end of the estuary, to Shell Ness (Site 1) near the river mouth. Each entire survey took approximately 6 hours.



Figure 2.2.1. The Kentish Stour Estuary, showing sample sites along the estuary. Map produced under sub-license using Digimap.

Invertebrate samples were taken from the upper five sites: Plucks Gutter, Minster, BR Bridge, Richborough and Haffenden using a standard rigid pond net for surface samples and an Eckman grab for benthic samples taken from the riverbed (Figure 2.2.2). The pond netting was carried out amongst reeds growing out of the water at the littoral fringes of the river. The reeds were vigorously agitated for one minute by the pond net, at the same time the net was moved slowly in an upstream direction in order to capture dislodged invertebrates. Two net samples were taken at each site. The Eckman grab was used in the centre of the river channel, being lowered carefully until the bottom was felt, and then stabbed sharply downwards until the mechanism was tripped and the jaws closed. The grab sample was then sieved through a 1mm mesh sieve using river water, to remove as much mud as possible, then washed into a five-litre plastic ice cream tub using 200ml of river water. Two grab samples were taken at each site.



Figure 2.2.2. The dredge, Casella deep water sampler, Eckman grab and pond net used to collect fauna from the Kentish Stour Estuary. The mouth of the dredge measured 144 cm², the bottle in the cassella held 500 ml of water, the grab collected a sample of benthos 225 cm² in area, and the mouth of the pond net was approximately 750 cm² in area.

After March 2000, trowel samples were taken instead of grab samples. This involved inserting a standard garden trowel on a pole into the mud of the bank, at an angle of 90 degrees to the bank, just below the waterline. The trowel was then manipulated to remove a portion of mud equivalent to a level trowel in volume.

The five sites downstream of the Haffenden survey site lacked suitable vegetation for net sampling, so only grab samples were taken. One additional grab sample was taken from all ten survey sites for analysis of particle size, organics and heavy metals.

A surface water sample was collected for biochemical oxygen demand (B.O.D.), by first rinsing a 250ml B.O.D. bottle in the river, and then filling it with river water, being careful to ensure that no air bubbles remained in the bottle. A second surface water sample was taken in an acid-washed 1 litre bottle. This water was later analysed in the lab for chloride, sodium, potassium, nitrates, nitrites and phosphates.

Dissolved oxygen, pH and conductivity were all measured using one of three devices; a Solomat multi-probe, a WPA kit or a set of Toledo probes. When using the WPA kit or Toledo probes, water samples were taken from the surface using a glass jar, and from the bottom of the river using a Casella deep-water sampler. The Solomat probe measured both surface water and water at depth directly. Temperature was recorded using a mercury thermometer.



Figure 2.2.3. Approaching site two. The remains of a First World War railhead that transported supplies destined for the Western Front to Richborough Port can be seen on the left of the picture.

Back in the lab, each net and grab sample was preserved by adding 200 ml of 8% formaldehyde solution, containing one spatula (approximately 500mg) of Borax glass powder per litre of preservative, to the 200ml of river water already present in the sample. The B.O.D bottles were placed in a dark cupboard for five days at 20°C before the sample was taken out of the cupboard and measured for dissolved oxygen. This was then compared with the dissolved oxygen results from the river to give the five-day biochemical oxygen demand of the sample.

The 1 litre surface water was then filtered through a weighed 70 mm diameter Whatmans GF/C filter paper. The filter paper was dried, and then weighed again to determine suspended solids. The filter paper was then placed in a furnace, before being weighed a third time. This third result was then subtracted from the result for suspended solids to give the weight of suspended organic material at each site.

A sample of the filtered water from each site was analysed for sodium and potassium using a Jenway p7 flame photometer. A separate sample was analysed using a Merck chloride test kit. Further samples were examined using a Tecator Aquatec auto-analyser to determine nitrite, nitrate, and phosphate levels in the river. All physico-chemical determinands were measured according to Standard Methods (Clesceri *et al.* 1989) or protocols supplied by the manufacturers of the equipment being used.

Invertebrates from the net and grab samples were separated from detritus under a Cambridge Instruments Z30E microscope at between X10 and X30 magnification. Sorted animals were then identified to the level of family, with the exceptions of oligochaetes and ostracods, which were identified to class, and cladocerans, which were identified to order.

2.3 The sample sites

For orientation in the next and subsequent sections of the thesis, the following conventions were adopted. The descriptions left bank and right bank apply when looking upstream while the river is ebbing. The descriptions upstream and downstream were also applied as if the water in the estuary was running out to sea. The Sites are described in reverse order from site ten, which is the most upstream, to site one, which is closest to the river mouth.

Site ten – Plucks Gutter TR2688 6345

Plucks Gutter is the site of the confluence of the Little Stour and the Great Stour (Figure 2.3.1). According to a plaque attached to the bridge, a road bridge that crossed the river at this point was built in 1900 and was replaced by the present bridge in 1995. Plucks Gutter is also the site of a rowing club.



Figure 2.3.1 The Bridge and slipway at Plucks Gutter, photograph taken from the right bank. The Dog and Duck inn can be seen through the arch of the bridge.

The Little Stour and the Great Stour meet approximately 200m upstream of the bridge. The substratum is made up of fine silts and gravel. Under the bridge the substratum is dominated by pebbles and cobbles.

Approximately 100m downstream of the bridge is Ash Level pumping station and discharge point, which protects the low lying Ash Levels by pumping drainage water from the land into the river (Marshall 2005, Environment Agency, personal communication). Sampling was carried out 10m upstream of the bridge, just below the confluence of the Great Stour and the Little Stour.

Site nine – Minster TR 30976287

The Minster sample site is situated immediately upstream of an active sewage outfall. This outfall discharges treated effluent from the village of Minster (Figure 2.3.2). The right hand bank is dominated by reeds upstream of the discharge. In some places channels are cut through the reeds to allow access to the river for anglers.



Figure 2.3.2. Minster Outfall. The out-fall itself is blocked with sediment, and effluent bubbles up through the reeds on the left of the picture.

Downstream of the discharge the right hand bank gives way to closely cropped grass. On the left bank the grass is closely cropped and generally serves as a cattle field. Samples were taken from just upstream of the out-fall.

Site eight – British Rail Bridge TR 32376240

This sample site (Figure 2.3.3) lies five hundred metres upstream of the discharge point for Wetherlees sewage treatment works, which is run by Southern Water. A portion of the original wooden bridge that crossed the river at this point can be found on the river bed. The Wetherlees treatment works currently treats wastewater from the towns of Sandwich, Deal and Ramsgate.

There are plans to expand Wetherlees to enable it to treat wastewater from Margate and Broadstairs as well, but under normal circumstances this effluent will not be discharged into the Stour (Southern Water 2006). Samples were taken from approximately five metres downstream of the present bridge.



Figure 2.3.3. British Rail Bridge looking upstream. The emergent remains of the original bridge can be seen on the right hand bank. A recent diving expedition here found remains of a wooden structure on the river bed, as well as a spent artillery shell.

Site seven – Richborough Power station TR 33186196

The presence of pulverised fuel ash lagoons at Richborough power station points to a coal-fired past, although it is listed by the National Grid as an oil-fired power station. Richborough Power Station was granted a licence in 1992 to burn the controversial fuel 'orimulsion'. The station had the capacity to burn 126 tonnes an hour of this fuel (U.K. Parliament 1992). In 1995 Powergen closed Richborough power station (U.K. Business Park 1995). Samples were taken from the portion of the river directly adjacent to the cooling tower nearest to the bank.

The pulverised fuel ash lagoons were subsequently allowed to fill with water and are now managed as part of a small nature reserve in the grounds of the power station.



Figure 2.3.4. Richborough Power Station, looking downstream. The concrete embankment that protected the power station has mostly been covered with silt and mud.

Site six – Haffenden outflow TR 33076044

The Haffenden outflow (Figure 2.3.5) discharges primarily domestic waste and runoff from rainfall (Haffendens textiles, personal communication 2005). Most of the effluent comes from premises on the Sandwich industrial estate, via a small effluent treatment works owned by Haffendens Textiles. The outflow discharges intermittently whenever the volume of effluent reaches a prescribed level. The site itself comprises several settlement ponds as well as constructed beds dominated by stands of bullrush (*Typha sp.*). There are also several open un-vegetated ponds which appear to be highly eutrophic. Sampling was carried out opposite the out-fall.



Figure 2.3.5. Haffendens Out-fall. This out-fall services a small sewage works that mostly processes surface runoff from a local industrial estate.

Site five - Sandwich marina TR 3385 5845

Sandwich Marina is one of the main berthing areas for boats on the Stour Estuary. Sampling was carried out 10 metres upstream of the green sign pictured in Figure 2.3.6. Figure 2.3.6. The upper limit of Sandwich Marina viewed from upstream.

Site four – Pfizer TR 3371 5990

Site four is situated outside the Pfizer complex (Figure 2.3.7). It is approximately half a kilometre upstream of the discharge of the Pfizer effluent treatment works. Samples were taken approximately a third of the way down this particular reach, opposite a portacabin on the right bank that was raised to first floor level.



Figure 2.3.7 Pfizer pharmaceutical works on the right bank of the river. The discharge point for the Pfizer effluent treatment works is approximately half a kilometre downstream of this site

Site three – Landfill TR 3354 6101

Site three is opposite an old, defunct landfill site (Figure 2.3.8). It is immediately upstream of Stonar cut. Stonar cut is a channel that bypasses the loop of the river that flows past Sandwich. It was built in 1776, to help drain the land to the north of the cut (Thanet Archaeological Society 2006). During high flows the cut is opened on an outgoing tide to allow water to reach the sea more quickly. When this occurs the loop of the river that passes through Sandwich is partially by-passed, and water flow through Sandwich is greatly reduced. Sampling took place just to the left of the piling indicated in Figure 2.3.8.



Figure 2.3.8. The Landfill site, photographed from the right bank during low water

Site two – Pfizer Sports Ground TR 3403 6211

Site two (Figure 2.3.9) is adjacent to the Pfizer sports ground. It is the location of the old First World War munitions port of Richborough. The old quay-side runs for approximately a kilometre from Stonar cut down to the modern remains of Richborough Port. Until the last decade this was still an active port, with small tankers bringing fuel for Richborough power station, and pleasure craft being moored

on the banks. More recently, Sandwich Marina has taken its place and the few craft that remain here are in poor repair and seemingly abandoned.

The structure in Figure 2.3.9 is the remains of a railhead that led directly to a ferry berth. The main machinery that held the ferry in place was sold soon after the First World War ended.



Figure 2.3.9. Pfizer Sports Ground site, right hand bank.

Due to the relatively poor transport links to the port following the dismantling of the railway, coupled with development of land between the port and the main road, the port area has declined. Various slipways and berths related to the port and dating back to its heyday can be found as far upriver as the Pfizer compound (site four).



Figure 2.3.10. Common Seals hauled out on a sandbank between site 2 and site 1, viewed from upstream. The town of Ramsgate can be seen to the right of the picture. To the left of the picture are the chalk cliffs of Pegwell Bay, further beyond the cliffs is Manston Airport.

Between site two and site one there are several mud banks. Two in particular seem to be regularly used as seal haul-out sites (Figure 2.3.10).

Site one – Shell Ness TR 3508 6279

The river discharges into the sea at Shell Ness (Figure 2.3.11). The right hand bank comprises Pegwell Bay, which is characterised by muddy sediments. The left hand bank makes up Sandwich Bay where the substratum is more dominated by coarser sandy sediments. Samples were taken between the second and third channel markers on the left hand bank.



Figure 2.3.11. Shell Ness, facing upstream from the right hand bank at low water.

2.4 Methodological problems associated with the biological and physicochemical data-set

Before analysing the dataset several problems needed to be addressed, or at least acknowledged.

2.4.1 The transition from grab sampling to trowel sampling

Between June 1996 and March 2000, two samples were taken at each site using an Eckman grab. During the survey of March 2000, it was felt by the ERG monitoring team that at certain sites, the river bed scoured, and there was no significant soft substratum for the Eckman to sample. As a result of this it was decided to use another method to sample soft substratum. This method involved taking two samples at each site from just below the waterline, using a long handled trowel. This had two advantages over the Eckman; firstly a sample could be guaranteed, and secondly the trowel sample was more standardised than the Eckman. This was because a sample equivalent to a level trowel full of sediment could be consistently

collected, whereas the Eckman could bring up an entire grab full of sediment if it hit an area of soft benthos, or nothing if it hit an area of hard benthos. Furthermore the Eckman grab was prone to misfiring, or jamming half open and losing part of the sample.

The dilemma caused by this was that the two types of sampling took samples of slightly different character from different parts of the river bed, so it would be invalid to treat them as the same type of sample. As no studies had been carried out prior to the change in order to see how these two sample methods related to each other, it was decided not to use grab and trowel samples together.

This had several repercussions. From sites 5 to 1 the only samples were either grab or trowel samples. As the switch from the grab sampling to trowel sampling occurred approximately halfway through the study period this severely curtailed what biotic analysis could be done on these sites. Luckily the principle study organism, *C. curvispinum*, was only found in the upper five sites. These sites had been sampled using a pond net for the entire study period.

It was decided to treat the biological datasets in the following manner. The emphasis during analysis would be on net samples, as this was the most comprehensive sampling technique, and also because these samples covered the stretch of river occupied by the principle study organism. Grab or Trowel samples from sites 10-6 would only be used if a period being investigated was entirely covered by one type of sample. For example, changes in the fauna between June 1996 and March 2000, and between June 2000 and September 2003 could be investigated using the benthic samples. Any investigations that straddled these two time periods would only be analysed using net samples. In order to ensure some degree of consistency, and

also because the principle study organism was never present at these locations, Sites 5-1 would not be analysed in detail.

2.4.2 Lack of replicates

Some univariate statistical analyses, such as the Analysis of Variance (ANOVA) compare the variation within two groups of samples with the variation between two groups of samples. Each 'group' is made up of a number of replicate samples, which between them are assumed to represent the variation found in the population from which the samples have come. The smaller the number of replicates the less likely they are to represent the population. If the number of replicates is low, then it is also more difficult to examine the distribution of group of samples, with a view to transformation, or to decide which statistical tests are most appropriate.

An experiment to examine the number of replicates needed to describe the populations of three species of Corophildae.

It is difficult to estimate how many replicates to take from a sample area in order to get a representation of the biotic populations in that area. A short answer is 'as many as possible'. Elliot (1977) recommended that fifty replicates would provide an adequate representation of a population of a contagiously distributed species. Elliot acknowledged that this was too high a number for practical purposes and suggested another method for calculating how many replicates would be ideal at a given station. A survey was carried out to test whether 2 replicates were enough to accurately describe the number of corophilds at any one site.

Method

On the 15th October 2003, two locations were sampled on the Kentish River Stour. The first location was Plucks Gutter (TR2688 6345), the second location was next to Richborough Castle (TR 3256 6018). On 23rd March 2005 a third location, next to

Richborough Port (TR 3403 6211), was also sampled. Each site was occupied by a different species of corophiid. *C. curvispinum* was found at Plucks Gutter, *C. multisetosum* was found at Richborough Castle and *C. volutator* was found at Richborough Port.

At each site, fifteen samples were taken from the river bed using an Eckman grab (Figure 2.2.2). Corophiids were removed, identified and counted in the lab. The distribution of each species was then quantified to allow estimation of the ideal number of replicates that would have to be collected for each species.

Results

Three different formulae were available for estimating the optimum number of replicates depending on whether the animal in the samples were distributed according to a positive binomial distribution, Poisson distribution or negative binomial distribution. The means and variances for all fifteen grab samples at each of the three sites were as follows: at Plucks Gutter, *C. curvispinum* had a mean of 400 individuals and a variance of 142008, at Richborough Castle *C. multisetosum* had a mean of 29.8 individuals and a variance of 1816, and at Richborough Port *C. volutator* had a mean of 324 individuals and a variance of 87097. As the variance was greater than the mean all three species conformed to a negative binomial distribution. Elliot (1977) recommended allowing for a 20% error with respect to the mean when sampling benthic invertebrates. The formula given by Elliot for working out the optimum number of samples, allowing for a 20% sampling error and assuming a negative binomial distribution was:

$$\mathsf{D}=25\left[\frac{1}{\overline{x}}+\frac{1}{k}\right]$$

Where D = the number of samples needed

x = the mean of the population (in this case the mean of all the samples taken)

1/k = a measure of the degree of clumping of the population in

question

The value for k was given by the formula:

$$\mathsf{K} = \frac{\overline{x}^2}{s^2 - \overline{x}}$$

Based on these calculations, 22 Eckman grab samples would be needed to find a sample mean representative of the population mean at a site containing *C. curvispinum*. For a site containing *C. multisetosum*, 51 Eckman grab samples were required. A site containing *C. volutator* would require 20 Eckman grab samples.

According to the above results the population of any of the corophiid species sampled could not be accurately estimated from the two Eckman grab samples taken at each site.

A more appropriate way of determining the number of replicates needed is to use power analysis (Bausel and Li 2002). Power can be defined as the chance of correctly accepting the alternative hypothesis (Statistical Solutions 2000). If the specified power of a test is 90%, then there is a 90% chance of rejecting the null hypothesis when the alternative hypothesis is true. Power analysis can predict the number of samples needed to achieve a specific effect size for a particular statistical test. A specimen power analysis was carried out with the statistical package nQuery advisor using data from the Stour estuary. This package was used to determine the

number of replicates needed to carry out a t-test with 90% power. The test groups for the t-test were number of invertebrate taxa in net samples at site 10 in June 1996 and September 1996. This analysis concluded that, for 90% power, 37 replicates would be needed. The two replicates taken in each group for the purpose of the Stour Monitoring programme produced 7% power for this t-test. Although this means that there is a substantial chance of a type 2 error with this dataset, it also means that the chance of a type 1 error is reduced, and so any statistically significant patterns found in the data would be more likely to be significant in reality.

One way to increase the number of replicates would be to either group data from different sites on the same survey, or group data from the same site at different times. Although all ten sites were quite far apart, and each site was obviously different, two distinct 'metasites' could be discerned. Sites 10-6 and sites 5-2 were similar in terms of general physical characteristics (such as sediment type and bankside vegetation) and fauna. Site 1 contained similar fauna to sites 5-2, although the substratum was somewhat different. The disadvantage of pooling samples from different sites on the same river is that each site was not statistically independent from other sites, and even if site were several kilometres apart, using them as replicates would be a mild form of pseudo-replication (Hurlbert 1984).

Pooling data from the same site at different times might be more problematic, as different species are more, or less, prominent at different times of the year. Thus different species could lead to heterogenous replicates.

Another possibility is based on work by Wilhm (1970). Wilhm studied the effect of pooling samples from a habitat on the Shannon's diversity index. He found that as samples were pooled the diversity index value at first rose, but then reached an asymptote. This pattern was true in all 13 habitats that he studied. If this pattern

occurs in all habitats, then it would be reasonable to expect that, if different sites are actually effectively the same habitat, then they would follow the same pattern of successively pooled replicates forming an asymptote. Samples from different sites could be progressively pooled and transformed using a diversity index. If they reach an asymptote, then the samples could be considered to come from the same population, and thus could be treated as replicates. If they did not reach an asymptote, then another plan would have to be put into action.

The problem of replication could also be circumvented by using certain multivariate analysis techniques, such as Principal Components Analysis (PCA), Analysis of Similarity (ANOSIM), or non-metric Multi-Dimensional Scaling (MDS) (Clarke and Warwick 1997). When employing these methods, the grouping of samples is to some extent a matter of choice as long as such groupings can be logically justified (Humpheryes 2004, personal communication). This seemed to be the most satisfactory method, and as such would be employed wherever it was appropriate in the thesis.

2.4.3 Taxonomic level

The fauna of the river Stour was not routinely identified to the level of species. Generally taxa were identified to the level of family, although in some cases animals were only identified to the level of class (e.g. the oligochaetes). Low taxonomic resolution would increase the chance of a Type II error when considering this type of analysis (Zar 1999).

2.4.4 Missing values

During the course of several December surveys, no net or grab samples were taken due to either a lack of reedy habitat, or an inability to sample due to flooding. For example, in December 2000, the river had burst its banks at several places, making

sampling of fauna impossible. Any samples that were collected in December had been assigned low priority and had not been analysed. For this reason it was decided that biological data from December surveys would not be used in any analysis.

With the exception of these surveys, the biological dataset was complete. However, the environmental dataset was studded with missing values. Over the eight years of the study, several accidents had occurred which resulted in data loss. For example, probes became water-logged, or ceased to work due to low temperatures, as well as the sort of mistakes that often occur in field sampling such as non-secured kit falling overboard, or knots failing during sampling. During the course of the surveys at least one Casella deep water sampler, a thermometer and an anchor were mislaid in this way. The December samples were particularly affected, probably due to the spoiling effect of the weather. Only December surveys carried out after the year 2000 contained a usable amount of environmental data. For these reasons it was decided not to include December environmental results in the survey. The final portion of data that were omitted in their entirety was the environmental data from the September 1998 survey. Most of the environmental data were unavailable for this survey, and so it was taken out of any analysis.

The remaining data were still partially incomplete. It was decided that for analysis that required all values, such as multivariate analysis, to input missing value data. According to Zar (1999) inputting missing values is only acceptable if less then about 10% of the values are missing. Several environmental parameters exceeded this limit and were removed from the dataset. These were Chlorophyll a, water temperature at depth, pH at depth, and conductivity at depth (μ S). The remaining environmental variables were: total suspended solids, suspended organics, chloride, potassium, sodium, nitrite, nitrate, orthophosphate, surface water temperature, surface pH,

surface conductivity (μ S). Three methods of missing values input were tested to see which one was the most valid: inputting mean values, using multiple regression, and the expectation maximisation technique available in the statistical package SPSS. The data set used for this test comprised all columns of data from 1996-2003 that contained no missing values. The estimated standard deviation values of the expectation maximisation method seemed on the whole to be smallest (Table 2.4.4.1.), so this method was used to input missing values.

Estimation method	SuspendedSolids (mg/l)	Organicsin suspended solids (%)	Chloride (mg/l)	Potassium (ppm)	Sodium (ppm)	Nitrite (mg/l)	Nitrate (mg/l)	Orthophosphate (mg/l)	B.O.D (mg/l)	Water temperature (°C)	Conductivity (uS)	Ha	Dissolved Oxygen (%)	Temperature (°C)
Means	31.5	18.2	51.7	4.4	54.07	.056	2.09	.60	1.55	5.85	174.5	.433	95.1	4.6
Expectation maximisation	31.4	17.9	51.7	4.4	53.23	.056	2.06	.60	1.54	5.82	173.5	.436	94.9	4.5
Regression	34.6	18.8	51.7	4.3	52.95	.058	2.07	.60	1.60	5.81	172.9	.435	84.7	4.7

Table 2.4.4.1. Summary of estimated standard deviations

2.4.5 Scale

The scale at which a physico-chemical parameter affects an organism can also affect the results of any analyses aimed at investigating the relationship between this physico-chemical parameter and the organism. Figure 2.4.5.1 illustrates a hypothetical relationship between an organism (in this case *Corophium sp.*) and a physico-chemical parameter (in this case pH). Curve a represents the relationship between pH and *Corophium* abundance.



Figure 2.4.5.1. A hypothetical relationship between *Corophium sp.* abundance and pH. Curve (a) represents the entire relationship between *Crorphium sp.* abundance and pH. Box (b) represents the proportion of the relationship covered by the pH range of the Stour estuary.

If, however, the range of pH covered in a study of *Corophium* is only that represented in box *b*, then no such relationship will be apparent. The problem of scale could be investigated in the following manner: physico-chemical data for several determinands (such as pH or conductivity) could be obtained from a wide range of other rivers where the corophiid species in question occurs, together with some sort of abundance data. A real version of figure 2.4.5.1 could then be constructed for each determinand.

A possible weakness of this approach, revealed in Chapter One, is the fact that the range of a species could represent its genetic adaptability, rather than the environmental tolerance of an individual corophiid. This possibility could be investigated by exposing the corophilds from the Stour to different physico-chemical

conditions in the lab, such as those conditions found in rivers in other parts of the country, and record their response. The response could be measured either in the form of mortality, or respiratory physiology.

2.4.6 State of the tide

In order to standardise the water chemistry results from surveys and make it in some way comparable, river surveys were always carried out on an outgoing tide. The water chemistry data for an incoming tide are therefore entirely missing. At some point it would be a good idea to sample several sites at low tide, and again at high tide over a spring tide in order to investigate the variation. Although past variation cannot be inferred by such a survey, it could give an indication of how closely survey physico-chemical results represent the results from the estuary across an entire tidal cycle.

2.5 Chapter conclusion

The main dataset that was going to be used to examine invasion by *C. curvispinum* contained missing values or, if the missing values were removed, was much reduced in size. Several aspects of data collection were unsatisfactory, and there were question marks over the suitability of the dataset itself to accurately represent an invasion by Corophiidae. Despite these problems, the dataset had one major positive property: it contained data from before and after the invasion. Before examining this aspect of the dataset in more detail, it would be necessary to chronicle the invasion of the Kentish Stour estuary, in order to provide structure to the analysis. It was known that there could be more than one species of corophiid in the estuary. Therefore, a thorough analysis of the Corophiidae from the dataset was required to establish spatial and temporal distributions of different corophiid species in the estuary.

Chapter Three

The invasion of the Stour Estuary by the Corophiidae

3.1 Overview

Despite the problems described in the last chapter, the data from the Stour monitoring program had several notable strengths that could be exploited. Both the biological and the physico-chemical datasets were quite comprehensive, despite missing values. They also documented conditions in the estuary before, during and after the invasion. This is unusual; in general, most studies take place after an invasion has occurred.

The primary species under study was *C. curvispinum*, but it was known that there were more than one species of Corophiidae present in the estuary. As well as the spatial and temporal distributions of each species, it was also important to establish whether more than one corophiid species had been present in any of the samples, to see if there was any overlap in species distributions.

Apart from *C. curvispinum*, the other species known to occur in the estuary was *C. volutator.* This species had been found in 1992 on the saltmarsh at the mouth of the estuary (Trigwell and Dussart 2001). A third species possibly present in the Stour estuary was *C. multisetosum*. This species is reported as a brackish water species that is tolerant of freshwater (Gledhill *et al.* 1993). It was present in the adjacent catchment of the River Medway (Humpheryes 2001, personal communication), and was suspected to be present in the Stour estuary (Trigwell 2000, personal communication).

Therefore, the aim of this chapter was to chronicle the changes in distribution and abundance of Corophiidae over the course of the study period (June 1996 to September 2003).

3.1.1 Method

Corophilds collected by the ERG from the Stour Estuary had only been identified to the level of family. This was not a problem because all fauna collected during the ten years of the monitoring programme had been stored. Every animal in the Stour Monitoring Program that had been identified as a corophild was removed from storage and identified to the level of species. Finally a sample of each identified species was sent to the British Museum (Natural History) for confirmation of identification. Once the identification had taken place, it was possible to determine when each species had first been detected by the monitoring programme and how many sites they had occupied.

3.1.2 Results

Species identified from samples taken from the Stour estuary

Over the course of the programme three species of corophiid were identified in samples taken from the estuary; *C. volutator*, *C. multisetosum* and *C. curvispinum*.

Changes in distribution of the three species of corophiid in the Stour estuary

The first river survey conducted for which all fauna was analysed had been carried out in June 1996. This survey uncovered a small population of *C. volutator* in the lower part of the estuary, comprising six individuals spread across two sites along the river. The population of *C. volutator* remained low for the following two years, with the maximum abundance during an entire survey being 12 individuals that were found during the March 1997 survey.

The September 1998 survey discovered 14 *C. curvispinum* at site 10. During this survey, 358 *C. volutator* were found across sites 1 to 5. Numbers of both species increased during the following March survey and by June 1999, all sample sites were occupied by a corophild species; *C. curvispinum* was present at site 10 to site 6, and *C. volutator* was found at site 5 to site 1 (Table 3.1.2.1).

	Site									
Date	10	9	8	7	6	5	4	3	2	1
Jun-96							V			V
Sep-96										
Mar-97						V				
Jun-97							V			V
Sep-97						V	V			
Mar-98							V		V	
Jun-98							V	V		
Sep-98	С		v			v	V	v		
Mar-99	С		С			v	V	V		
Jun-99	С	С	С	С	С	v	V	V	V	V
Sep-99	С	С			m	v	v	v	v	v
Mar-00	С	С		С	С		V	V	V	
Jun-00	С	С	С	С	С	m	V	v	v	
Sep-00	С	С		С	С		V	V	V	V
Mar-01	С	С	С	С					V	V
Jun-01	С	С	С	С			V	v	V	v
Sep-01	С	С	С	С	С		V		V	V
Mar-02		С					V	v	V	
Jun-02	С	С	С	С		V	V	V	V	
Sep-02	С	С	С	С	m	V	V	V	V	V
Mar-03	С		С		<mark>c</mark> m	m	V		V	
Jun-03	С	С	С	С	С		V	V	V	
Sep-03	С		С			m	V	V	V	V

Table 3.1.2.1. Changes in distribution of the three species of Corophiidae found in the Stour estuary. c = C. *curvispinum*, m = C. *multisetosum*, v = C. *volutator*.

From June 1999, *C. volutator* continued to expand in population size, reaching a zenith in grab samples in September 1999 with 2763 individuals in total across the survey. After trowel samples replaced grab samples in March 2000, the population of *C. volutator* remained relatively steady, with the lowest numbers in March (2000: 36 individuals, 2002: 15 individuals, 2003: 2 individuals), and the highest numbers in September (1309 in September 2000, and 717 in September 2003) (Figure 3.1.2.1.).

The September 1999 survey yielded the first record (two individuals) of a third species, *C. multisetosum*, at site 6. This species was recovered from samples taken at site 5 in June 2000. No further individuals of this species were found until September 2002, when eight individuals were found at site 6. In June 2003 *C. multisetosum* was found at both site 6 and site 5 (24 animals in total), and in September 2003 it was found at site 5 (17 animals). A summary of where *C. multisetosum* was discovered can be found in Table 3.1.2.1 and Figure 3.1.2.2).



Figure 3.1.2.1. Whole survey abundance of *C. curvispinum* (Cc) and *C. volutator* (Cv) in grab samples and trowel samples.

Abundance of *C. curvispinum* in grab samples reached a peak in June 1999 with 896 individuals found throughout the survey. The following survey showed a slight drop in numbers to 615. The final survey to feature grab samples, March 2000, seemed to show a further drop in the numbers of *C. curvispinum* living in the sediment. This trend appeared to continue after grab sampling was replaced with trowel samples, with only small numbers of *C. curvispinum* being found in trowel samples between
June 2000 and September 2003. The largest number of *C. curvispinum* found in trowel samples was 18 in June 2001. A summary of the abundance of *C. curvispinum* and *C. volutator* can be seen in Figure 3.1.2.1.

The net samples, which were taken from site 10 to site 6, only contained *C. curvispinum* (Figure 3.1.2.3). Abundance of this species in net samples peaked slightly later than it did in grab samples. Only in June 2000 was a sizable population recovered from the littoral margins. This was possibly due to the tidally fluctuating height of the river, even as far from the sea as site 10. In many places in the upper estuary the water level dropped to below the level where reeds could provide shelter or habitat on which to build cases or tubes. At this point it must be noted that the only corophild found in habitat that was ever exposed to air was *C. volutator*. Most *C. curvispinum* caught in net samples came from site ten (Figure 3.1.2.3).



Figure 3.1.2.2. Distribution of the Corophiidae on the Kentish Stour Estuary June 1996-September 2003 grab and trowel samples. Cc = C. *curvispinum* Cm = C. *multisetosum*, Cv = C. *volutator*.

Over the course of the study the following distributions were revealed by grab and trowel samples:

 although *C. curvispinum* was present at the upper six sites, by far the largest population was found at site ten

- *C. volutator* was found relatively evenly distributed across the lower five sites, with the exception of site five, where it was often absent
- *C. multisetosum* was found occasionally in small numbers only at sites 5 and
 6.





Several salient time periods were notable from the above data (Figures 3.1.2.1 and 3.1.2.3). Firstly, there was a period where one species of Corophiidae was present on the river in small numbers (June 1996-June 1998). This period was followed by a rapid expansion in the distribution of one species, coinciding with the introduction and expansion of a second species, followed by a collapse in the numbers of both species (September 1998-approximately September 2000). Finally, a period of relatively stable population size can be discerned for both *C. volutator* and *C. curvispinum*.

Geographically, Site 10 was noticeably the site where the largest population of *C. curvispinum* was consistently found (Figures 3.1.2.2 and 3.1.2.3). Sites 6 and 5 were worthy of note as they appeared to straddle a fluctuating demarcation line between the three species (Figure 3.1.2.3, Table 3.1.2.1). On only one occasion were two species found at one site at the same time: in March 2003 *C. curvispinum* was found

in net samples at site 6, C. multisetosum was recovered from a trowel sample taken from the same site (Table 3.1.2.1).

3.2 A synoptic survey to investigate distribution of Corophiidae between sites 5 and 6

Throughout the survey period, sites 5 and 6 had yielded an occasional sample containing small numbers of *C. multisetosum*. Apart from one occasion, it seemed that when *C. multisetosum* was found at site 6, then *C. curvispinum* was absent. When *C. multisetosum* was found at site 5 then *C. volutator* was absent. In fact sites 7, 6 and 5 had all been colonised at some time by different species of corophiid. At no point had more than one corophiid species been sampled from the same site at the same time. This prompted an investigation to examine the distribution of Corophiidae between sites 5 and 6.

3.2.1 Study area

Ten sites were visited on October 14th 2004. To minimise confusion between this synoptic survey and the main monitoring survey, the sites were labelled A-H, with site A being the site closest to the sea (Figure 3.2.2.1). Site A (TR 34200 58554) was situated 100 metres downstream of site 5, approximately halfway through Sandwich Marina. Site B (TR 3365 5821) was upstream of Sandwich Marina, on a bend in the river. Site C (TR 32971 58609) was approximately 5 metres upstream of the most upstream moorings around Sandwich. Site D (TR 32235 59306) was under the bridge that carried the A256 over the river. Site E (TR 32204 59527) was adjacent to an Environment Agency water sampling device, supported by a wooden gantry. Site F was immediately upstream of a private mooring. Site G (TR 32567 60186) was also next to a private mooring. Site H (TR 32906 60170) was adjacent to some derelict farm buildings. Site I (TR 33120 60528) was 50 m upstream of a 'Danger sudden discharge' sign. Site J (TR 33236 60898) was under a private bridge leading

to an old landfill site. These sites in particular were chosen for ease of access by land, and to ensure relatively even coverage of the stretch of estuary under study.



Figure 3.2.2.1. Sample sites visited during the investigation of corophiid species around and between sites 5 and 6.

3.2.2 Method

The sampling initial sampling was carried out at high tide. The middle of the river was too deep to reach with the grab, so at each site, six grab samples were taken, three from each side of the river. At each side of the river the boat was secured with the boat parallel to the bank. One sample was taken from the bow of the boat, one sample was taken from halfway down the boat, and the final grab sample was taken from the stern. Each grab sample was sieved in the river using a 0.5 mm mesh sieve, then washed into a 4 litre container using 200ml of river water. Back at the lab each sample was preserved by adding 200 ml of 8% buffered formaldehyde solution. Samples were sorted using at x 10 magnification using a Cambridge instruments

Z30E binocular microscope. Corophiids were removed from each sample, identified to species and counted.

According to Attrill (2002) the main factor influencing estuarine organisms is the ability to cope with saline variation, rather then absolute saline tolerance. Therefore, the day after this survey, each site was sampled at low tide, and then again at high tide. Dissolved oxygen, conductivity and pH were measured *in situ*, and a sample of water was taken back to the lab for chloride analysis. These determinands were chosen as they were felt to give a fair representation of the physico-chemical conditions of the Estuary.

The data for each determinand was plotted on graphs (Figures 3.2.3.2-3.3.3.5). Data for each determinand were tested for normality by comparing the size of the mean with the size of the variance. If data did not approximately follow a normal distribution then it was natural log transformed prior to any parametric statistical tests being carried out. Any differences noted in the graphs were tested using a Students t-test, paired or unpaired as appropriate.

Numbers of each species found were analysed with respect to the physico-chemical data using a multiple linear regression. The physico-chemical results were then compared with the total Corophiidae abundance at each site using multiple regression. It had already been established that each of the three Corophiid species present in the estuary followed a negative binomial distribution (Section 2.4.2). Therefore total *C. multisetosum* abundance at each site was natural log transformed before the analysis took place.

3.2.3 Results

The only species found at any of the sites was *C. multisetosum*. There appeared to be a larger number of *C. multisetosum* in sites D, E, and F. The smallest sample of the population came from the four sites furthest from the sea (Figure 3.2.3.1).



Figure 3.2.3.1. Distribution of Corophium multisetosum from sites A-J

Within site pH at low tide was consistently lower than pH at high tide (Figure 3.2.3.2). A t-test showed this difference to be significant (t=5.29, P=0.0002). There was a significantly higher pH at the five sites closer to the sea than the five sites furthest from the sea at high tide (t=-5.51, P=0.0002), but not at low tide (t=-1.7, P=0.07) (Figure 3.2.3.2).



Figure 3.2.3.2. pH at high and low water for sites A-J.

Conductivity data were not normally distributed, and so conductivity data were natural log transformed before statistical tests were carried out. Conductivity was significantly higher at the five sites closest to the sea than at the five sites closest to the freshwater (Figure 3.2.3.3). This was the case for both samples taken at high water (t= -3.5, P= 0.004) and low water (t= -5.04, P=0.001). Conductivity at high water was significantly higher than at low water (t=7.46, P=0.011).

Chloride (Figure 3.2.3.4) was significantly higher at the five sites closest to the sea, both at low water (t= -3.07, P= 0.013), and high water (t= -3.602, P = 0.0043).



Figure 3.2.3.3 Conductivity at high and low water sites A-J



Figure 3.2.3.4. Chloride concentration at high and low water for sites A-J.

Oxygen (Figure 3.2.3.5) was significantly higher at low water than at high water (t= - 4.43, P=0.0008). Apart from this there was no significant difference between sites (F=1.89, P=0.166).



Figure 3.2.3.5. Dissolved oxygen concentration at high and low water for sites A-J.

3.2.4 Results of regression analysis

The predictor variables used for this analysis were: pH at low water, conductivity at low water, percentage dissolved oxygen at low water and chloride concentration at low water. The predictor variables at low water did not, as a group, significantly predict for *C. multisetosum* abundance (F = 1.57, P = 0.313, *df*=9). None of the predictors showed a significant relationship with *C. multisetosum* abundance (Table 3.2.4.1a).

The second multiple regression was carried out on dissolved oxygen at high water, pH at high water, conductivity at high water, temperature at high water and chloride concentration at high water. Overall these physico-chemical variables taken at high water did not significantly predict *C. multisetosum* abundance (F=1.03, P =0.474, *df*=9)). No significant relationship was found between any single variable and *C. multisetosum* abundance (Table 3.2.4.1b).

Table 3.2.4.1. t and P values for variables used to predict log *C. multisetosum* abundance using multiple regression. a) low water values as predictor variables, b) high water values as predictor variables c) range between high and low values as predictor variables. ** = significant at P=0.01 level, * = significant at the P=0.05 level, NS = not significant

a)

Predictor	t	Significance
pН	-0.39	NS
Conductivity	1.98	NS
Temperature	0.08	NS
Dissolved oxygen	1.60	NS
Chloride concentration	-1.26	NS

b)

Predictor	t	Significance
рН	-0.17	NS
Conductivity	-0.57	NS
Temperature	-0.73	NS
Dissolved oxygen	-0.56	NS
Chloride concentration	1.24	NS

c)

Predictor	t	Significance
рН	0.28	NS
Conductivity	-0.82	NS
Temperature	0.6	NS
Dissolved oxygen	0.63	NS
Chloride concentration	2.45	NS

A final round of multiple regression was carried out to investigate whether the ranges of each physico-chemical variable related to *C. multisetosum* abundance. The range was taken to be the difference between the high water value and the low water value for each environmental variable measured. The predictor variables used for this regression were: chloride range, pH range, temperature range and dissolved oxygen (%) range. This regression did not show a statistically significant relationship between the combined ranges of the physico-chemical predictors and *C. multisetosum* abundance (F= 0.82, P= 0.563, *df*=9). None of the individual variables significant predicted *C. multisetosum* abundance (Table 3.2.4.1c), although chloride range was close to being significant (t= 2.45, P=0.071)

3.2.5 Discussion of October 2004 survey

None of the regression analyses showed any significant relationship between *C. multisetosum* abundance and any of the physico-chemical variables sampled. However, the natural variability of the estuary, the relatively limited amount of physico-chemical data collected for this experiment, and the fact that some *P* values were relatively close to the *P*=0.05 level make the results worthy of further investigation. Natural variability would include the contagious distribution of the animal being studied. It was established in section 2.4.2 that approximately 51 Eckman samples of *C. multisetosum* were needed to accurately estimate the population at any one site, and only six samples were actually taken at each site. Also, the limit of sea water penetration will vary from day to day according to factors such as tidal height, river discharge, air pressure. The essentially random nature of anthropogenic discharges into the river could also affect physico-chemical results. Therefore any analysis should have been carried out on data taken on several different days, preferably in different seasons.

The final multiple regression identified chloride range as a possibly important variable in determining the distribution of *C. multisetosum*. This would support the observations of Attril (2002) who suggested that the range of a variable at a site is more important then its absolute highest or lowest value when determining the distribution of fauna in an estuary. The results of regressing high water only, and low water only, values against *C. multisetosum* abundance did not suggest that the absolute high or low values could be important factors in themselves.

A more detailed study could look at river physico-chemistry at several sites over a tidal cycle, and compare the results with data for the distribution of corophiidae.

During the October 2004 survey, water samples were taken from the surface, whereas the animals were retrieved from the bed of the river. If there is a difference in water chemistry between the top and the bottom of the water column, taking water samples from the bottom of the water column might improve the accuracy of the model.

Because a number of t-tests were carried out on the same data-set, there was an increased chance of a type 1 error (Abdi 2007). This could be corrected for using the Bonferonni correction:

$$\alpha[p_i] = \frac{\alpha[p]}{C}$$

Where:

 $\alpha[p_i]$ = the critical value of *p* adjusted for multiple independent tests on the same

dataset

 $\alpha[p]$ = the critical value of p for a single test

C = the number of tests being carried out on the data

In the case of the t-tests carried out on high versus low water data, eleven different independent tests were carried out. The Bonferonni correction would result in a critical *P* value for each of these tests being adjusted from 0.05 to 0.0045. Even if the Bonferonni adjustment is accepted, most of these tests are still statistically significant, with the exception of conductivity at high water versus conductivity at low water, and chloride concentration at the upper five sites versus the lower five sites.

3.2 Further work

Plucks Gutter was site furthest from the sea, and also the site where the greatest density of *C. curvispinum* occurred. In 2000, a small population of *C. curvispinum* was found further inland, at Grove Ferry. A scoping survey failed to find *C. curvispinum* any further upstream of this point, but it is possible that further populations of *C. curvispinum* might be found, both upstream of Grove Ferry, and between Grove Ferry and Plucks Gutter.

3.3 Chapter epilogue

The findings of this chapter allow the Stour physico-chemical and biological datasets to be examined with respect to different episodes in the invasion. At this point it is possible to start addressing the main aims of this thesis, such as, was there any relation between *C. curvispinum* and the physico-chemistry of the Stour?

Chapter Four

Changes in water physico-chemistry in the Kentish Stour Estuary at the time of the Corophiidae invasion

4.1 Overview

This chapter explores changes in the physico-chemistry of the Stour Estuary in the context of the invasion of the Stour Estuary by *C. curvispinum*. At the heart of this chapter is the question of whether changes in physico-chemical parameters influenced the invasion. There are three kinds of intrinsic change in ecosystems according to Hellawell (1977); cyclical, stochastic and successional. Stochastic, or random changes are those which, by their nature are hard to predict and assess. Cyclical changes can be examined with a dataset of an appropriate length. For example, temperature will drop in the winter and then rise in the summer. However, it can sometimes be difficult to distinguish a trend from a long term cycle, or indeed a series of stochastic events. In Lake Mikotajskie, in Poland, the population of *D. polymorpha* displayed two abundance peaks, 16 years apart (Lewandowski 1982, in Ramcharan *et al.* 1991), which could have been caused by any one of these three mechanisms of change.

Changes in the physico-chemistry of a river can be responsible for changes in the biotic community of a river. These changes, often measured in terms of change in water quality rather then specific physico-chemical parameters, can be measured by the use of biotic indices. The rationale behind biotic indices is that the fauna of an ecosystem reflects to some extent the physico-chemical milieu in which the fauna are found. Certain taxa have wide environmental tolerance, whereas others indicate specific conditions. For example, an invertebrate family such as the non-biting midges (Chironomidae) are found in a range of water qualities, from clean water

through to highly polluted. Stoneflies (Plecoptera), on the other hand, are only found in relatively clean, dystrophic water, with high oxygen content. Therefore, the appearance of taxa such as stoneflies in a waterbody can reveal information about the physico-chemistry of the waterbody (Wright *et al.* 2000).

This rationale can be applied to an invader such as *C. curvispinum*. If, as a species, it has specific environmental requirements then, as is the case with stoneflies, its appearance, along with other species with similar requirements, could indicate a change in the physico-chemical parameters of that system.

The possibility that a *C. curvispinum* invasion implies a specific change in the ecosystem prior to its successful colonisation could be investigated by examining the score given to Corophiidae within a specific biotic index. The Biological Monitoring Working Party Score (BMWP), for example, is a biotic index that expresses the sensitivity of various invertebrate families to organic pollution as a score between one and ten (Hawkes 1997). The score for invertebrate families that are less affected by organic pollution is low: the Chironomidae family is given a score of 2. Most stonefly families score 10. The Corophiidae score 6. This score implies that corophiids are not particularly sensitive to organic pollution, nor are they highly pollution tolerant.

The literature appears to show relatively few references to the physico-chemical requirements of *C. curvispinum*. Taylor and Harris (1986) found that it could only maintain its sodium balance in freshwater that was relatively rich in sodium. According to Bayliss and Harris (1988), low chloride concentrations could be a factor limiting the distribution of *C. curvispinum* in British freshwaters. Den Hartog *et al.* (1992) found that relatively high temperatures, brackish water, and an abundance of phytoplankton were some of the factors linked with the successful invasion of the lower river Rhine by *C. curvispinum*. Furthermore, den Hartog *et al.* (1992) reported finding the highest densities of *C. curvispinum* in association with hard substratum, in

water depths exceeding 1 metre. Musko et al. (1995) inferred from measurements of metabolic rate that *C. curvispinum* was less likely to experience anoxic conditions than *C. volutator*. Bayliss and Harris (1988) found *C. curvispinum* in the following determinand ranges: chloride - 21.3 mg/l to 99.3 mg/l, pH – 6.8 to 8.2, and sodium – 11.5 to 71.3 mg/l. According to van den Brink *et al.* (1993), *C. curvispinum* density at the micro-habitat level was positively correlated with current speed, within the range of current speeds they measured on the Rhine, which ranged from 0 to 60 cm s⁻¹.

Based on all this information, in a freshwater environment, rises in sodium, temperature, chlorophyll a, channel depth, chloride and by inference dissolved oxygen, might positively benefit an invasion by *C. curvispinum*.

4.1.1 Changes in physico-chemical variables caused by invaders

Once an invader has successfully established itself, it might start to exert a measurable effect on its new environment, for example, *Dreisenna polymorpha* in the North American Great Lakes (Rayl 1999). With a filter feeder like *C. curvispinum*, the main possible direct effects could be a decrease in suspended solids and turbidity, and an increase in benthic accretion, as suspended solids are filtered out of the water column by the animal and then reintroduced to the environment as faeces. This both decreases the suspended solid load of the water column and potentially alters the character of the benthic substrata. Indirect effects could include an increase in chlorophyll a as more light penetrates the water column, encouraging phytoplankton, and a drop in concentration of nutrients such as nitrates and phosphates as they are harnessed by the phytoplankton (Gilbert and McCarthy 1984, Moll 1997).

4.1.2 Post-invasion effects of the environment on the invader

Once an exotic has invaded and established itself in a habitat, it is subject to the influence of its environment. A change in the environment could have an effect on an

organism, for example a flood could wash away animals living attached to a hard substratum.

A statistical relationship between *C. curvispinum* abundance and any physicochemical change does not prove a causal relationship between the two. Nevertheless, one way to see if there is any evidence that any of the physicochemical factors explained any of the variation in *C. curvispinum* abundance would be to carry out multiple regression analyses with *C. curvispinum* abundance as the dependent variable and the various physico-chemical factors as independent variables. Studies relating aquatic organisms to the physico-chemistry of their environment have been carried out by Dussart (1973), Dussart (1979), and Attrill (2002).

4.1.3 The danger of the Just-so story

Kipling (1908) took characteristics of animals and used them as the base for a series of fantastic tales, none of which bear any resemblance to reality. Another example of over-interpretation of data is provided by Lowell (1895, at http://www.wanderer.org /references/lowell/Mars/), who stated categorically that there were artificial canals on Mars. These canals were later found to be optical illusions caused by factors such as craters that appeared from a distance to form straight lines (www.answers.com). When trying to infer relationships from an archive dataset it is possible to find patterns that although statistically valid, have no bearing on reality. This danger can be avoided by manipulating data as impartially as possible, deciding what patterns are expected before looking at the data, and treating results that are unsupported by findings of other research with suspicion.

4.1.4 Aims of the chapter

The main aim of this chapter is to investigate possible evidence of relationships between *C. curvispinum* and its physico-chemical environment. Four main questions are explored:

- 1. Were there any changes in physico-chemical parameters of the Stour before the invasion that could have had an effect on the success of the invasion?
- 2. Was there any relationship between any of the physico-chemical parameters and *C. curvispinum* abundance ?
- 3. Could any changes that occurred during the invasion have been caused by an increased abundance of filter feeders in the river ?
- 4. Were there any implied relationships between changes in physico-chemical parameters and *C. curvispinum* abundance before, during and after the invasion?

4.1.5 A description of the sources used to investigate the physico-chemical parameters of the Stour Estuary

Three sources of information were used to examine physico-chemical changes in the river over the course of the invasion. These were; (1) a descriptive account of changes in anthropogenic river use; (2) a dataset containing physico-chemical readings provided by ERG Stour surveys; (3) weather data provided by the Meteorological (Met) office.

An examination of general changes of anthropogenic influence could reveal two things. Firstly, they could suggest a reason for any patterns found in the Stour monitoring data, or secondly, they could highlight gaps in the Stour monitoring dataset. A hypothetical example of the second possibility might be the development of a new marina on a stretch of the river. Increased boat traffic might increase the disturbance of the banks. However physical disturbance is a determinand that is not measured by the monitoring survey.

The Stour monitoring dataset comprised results of almost eight years of monitoring, three times a year, of a suite of physico-chemical variables. The strengths of this dataset lay in its coverage of such a long time period. One weakness of this dataset was that it was weighted more towards chemical factors in the river, such as chloride and pH. Relatively few physical factors, such as flow speed or discharge, were measured. Another weakness lay in its brevity of detail. Physico-chemical parameters can change from one second to the next, and the monitoring survey collected a single example of each determinand at each point on the estuary once every three months. Thus, anything that happened to the river in the time between samples would not be recorded in the dataset. It would be preferable to have some sort of dataset that could 'fill in the blanks', at least for some of the physico-chemical determinands. The Met office had just such a database.

The weather dataset, provided by the Met office, comprised data from Manston Airport, the weather station closest to the Stour Estuary, from 1995 to 2000. These were not raw data, but contained a summary of data, either in the form of means or highest value for the month, collected at several points over the course of every day for the entire time period covered. Although these data were less relevant to the biology of the river than data collected directly from the estuary itself, they had the advantage of high sample resolution.

4.2 Changes in anthropogenic impacts in the Stour region

There were several changes in anthropogenic impacts on the estuary. In response to the 1991 EU bathing waters directive, in 1994 sewage outflows discharging into the sea from Ramsgate were closed and the sewage redirected to a new effluent treatment works (ETW) at Wetherlees Hill. This treatment works discharges into the Stour estuary between sample sites seven and eight (Figure 2.1.1.), and came online in 1994 (Rees-Jones 1998). Richborough power station (sample site 7), which used Stour water as coolant, was closed in 1995. Another new effluent treatment works was commissioned by Pfizer to treat all their domestic sewage. This plant came fully online in May 1997.



Figure 4.2.1. Drought orders issued by the Environment Agency in Southern region 1976-2002





Episodes of drought in the region were inferred by examining drought orders issued by the Environment Agency. There were two main periods of drought in the 1990s in the southern region of the Environment Agency, one lasting from 1989 to 1992, and a less serious drought from 1995-1997 (Figure 4.2.1). Flow data were also provided by the Environment Agency from their monitoring station at Horton, which is approximately 4 kilometres upstream of Canterbury. These data showed that the lowest average daily flow occurred 1996 (Figure 4.2.2). The highest daily flows occurred in 2001.

4.3 Changes in weather data from Manston Weather Station

4.3.1 Overview

The dataset comprised thirty one determinands representing different aspects of the weather. Some of these determinands had no relevance to the study, for example the day of the month on which there was the lowest temperature.

The following determinands were chosen from the weather dataset:

• average monthly maximum and minimum temperature (an average of the entire month's lowest temperatures and an average of the entire months highest temperatures)

- absolute monthly maximum and minimum temperature (the lowest and highest temperatures recorded in that month)
- number of days when temperature dropped below freezing
- rainfall
- number of hours of bright sunshine.

These determinands were chosen for the following reasons:

1. Temperature has been suggested as a factor in the success of *C. curvispinum* invasions in Europe (den Hartog *et al.* 1992, Conlan 1994). The temperature data from the Stour dataset were only collected once every three months and therefore might not represent the general climate over the course of the study. Temperature is an important indicator of direct factors affecting organisms on the river, such as metabolic activity, and indirect factors, such as availability of food. Average monthly maximum and minimum temperatures were used, as any long term changes in these could affect the viability of animal populations exposed to them. Absolute minimum and maximum temperature values were used in order to quantify the range of temperatures that an exposed population would experience on a month by month basis. The number of days when minimum air temperature was below freezing was also used, as none of the other measures gave any information about the persistence of cold weather. Persistent cold weather can have an adverse effect on invertebrate survival (Ponsonby 2003, *personal communication*, Smithers 2004, *personal communication*).

2. Rainfall is a possible factor as it would give some indication of the discharge of the river.

3. Number of hours of bright sunshine, was included in the analysis because it could correlate, together with temperature, with photosynthetic activity, and thus metabolism of organisms such as planktonic diatoms and green algae in the water column.

The dataset only extended to December 2000, and therefore could only inform the periods Before and During the invasion.

4.3.2 Average Maximum and minimum monthly temperatures

As would be expected, there was a strong seasonal component to both maximum and minimum average monthly temperature. Neither, however, showed a particular upward or downward trend (Figures 4.3.2.1 and 4.3.2.2).



Figure 4.3.2.1 Average maximum monthly temperature 1995-2000



Figure 4.3.2.2 Minimum average monthly temperature 1995-2000

Average maximum and minimum temperatures were both lowest in 1996. There was a slight year on year increase in average minimum temperature between 1996 and 1999 (Figure 4.3.2.3).



Figure 4.3.2.3 Average maximum and minimum temperatures 1995-2000

4.3.3 Temperature range

Temperature range varied the most between months in 1996 (S.D. = 3.31). After this peak in 1996, the standard deviation of temperature range dropped year on year until 1999, when it reached a low point of 1.45. The month in which the temperature range was greatest varied from year to year (Figure 4.3.3.1).



Figure 4.3.3.1 Average monthly temperature range 1995-2000

As with the standard deviation, average temperature range for the year was highest in 1996 and dropped successively to its lowest in 1999 (4.3.3.2).



Figure 4.3.3.2. Average annual temperature range 1995-2000

There was evidence of weak seasonality in the data (Fig. 4.3.3.3) with temperature range generally being higher towards the end of summer, with secondary peaks around November and February. An exceptional year was 1999, in which temperature range was lowest in late summer, with additional peaks in June and December.



Figure 4.3.3.3 Time series plot of temperature range 1995-2000

4.3.4 Number of days when temperature dropped below freezing

The greatest number of days below freezing in any one month occurred in February 1996. Overall, throughout the time period covered by the dataset, temperature dipped below freezing at least once during the month for an average of six months of the year. Only 1997, with days below freezing occurring during eight months of the year, was above this average. The years 1999 and 2000 were both below this average (Figure 4.3.4.1).



Figure 4.3.4.1. Number of days when temperature dropped below freezing

Overall temperature dropped below freezing for the greatest number of days in 1996, followed by 1997 and 1995. After 1996 the number of days where the temperature dropped below freezing fell steadily to its lowest number in 1999, before rising slightly in 2000 (Figure 4.3.4.2).



Figure 4.3.4.2. Number of days per year when temperature dropped below freezing

4.3.5 Rainfall

The highest overall rainfall occurred in October 2000. The lowest rainfall occurred in September 1997 (Figure 4.3.5.1).



Figure 4.3.5.1. Annual monthly rainfall over 1995-2000

There was no obvious seasonal component to rainfall (Fig. 4.3.5.2).



Figure 4.3.5.2 Time series plot of rainfall

Annual total rainfall dropped slightly in 1996, and then generally rose, although the rainfall in 1999 was generally slightly lower than 1998. Rainfall was at its highest in 2000 (Figure 4.3.5.3).



Figure 4.3.5.3. Total annual rainfall

4.3.6 Number of hours of bright sunshine

There was a strong seasonal component to the number of hours of bright sunshine, with generally more hours in summer and less in winter. The actual month with the most hours of bright sunshine varied each year from May to August (Figure 4.3.6.1).



Figure 4.3.6.1. Hours of bright sunshine 1995-2000

There was no particular trend in the number of hours of bright sunshine during the five years covered by the dataset. The least bright sunshine was in 2000. The most bright sunshine occurred in 1999 (Figure 4.3.6.2).



Figure 4.3.6.2. Total annual number of hours of bright sunshine

4.4 Physico-chemical changes highlighted by the Stour monitoring programme

4.4.1 Overview

Examination of corophiid abundance in Chapter Three identified three time periods pertinent to the invasion of the Stour Estuary by *C. curvispinum*.

- The first time period was from June 1996-June 1998, which represented the time period before *C. curvispinum* was found in the Estuary. This period was designated the 'Before' period.
- The second period, from September 1998 to September 2000, represented the initial invasion and the time when *C. curvispinum* was found in the greatest abundance. This period was designated the 'During' period.
- The final period, from March 2001 to September 2003, represented a time when *C. curvispinum* numbers crashed. Following this crash *C. curvispinum* was only found in relatively small numbers at the sample sites (Figure 3.1.2.3). This period was designated the 'After' period.

Changes in the physico-chemical parameters within and between the three time periods were documented. The classification of the dataset into the three periods mentioned above can only be justified as a convenient device to enable comparisons to take place. At no point can these divisions be taken to represent three boxes into which the dataset neatly fits. With this proviso in mind, I examined each parameter for trends stretching across the three time periods, and for changes within each time period.

Examination of within-period change was most important for the Before time period, as changes during this period could give clues to any change in conditions that could have enhanced the success of the ensuing invasion. Did any of the parameters increase, decrease, or become more or less variable? Changes in the physico-chemical parameters during the initial explosive period of the invasion (the During time period) could provide a basis for a future investigation into what causes populations of *C. curvispinum* to crash. Finally, examination of changes in physico-chemical parameters after the initial invasion (the After time period) could be used in concert with the findings from the other two time periods to predict what might happen to the *C. curvispinum* population in the Stour after September 2003.

4.4.2 Developing a methodology for examining changes in physico-chemical variables within and between the three time periods

The results used for this investigation comprised data from June 1996 to September 2003, from sites 10 to 6. Sites 10 to 6 were chosen as they represented the eventual range of *C. curvispinum* on the river. For the reasons given in section 2.4.4, the physico-chemical parameters examined were: pH, conductivity, chloride, dissolved oxygen, biochemical oxygen demand, nitrites, nitrates, phosphates, suspended solids, suspended organics and water temperature.

Initially, multivariate discriminant function analyses were carried out using the statistical package SPSS (Version 13) to assess whether there had been an overall change in the physico-chemical parameters included in the dataset. The two questions that were addressed by this analysis were: was there a change in overall physico-chemistry between the three time periods, and was there any change in the overall physico-chemistry in the period prior to the *C. curvispinum* invasion?

Box and whisker plots and Kruskal-Wallis tests were carried out using SPSS to test whether there were any differences in individual physico-chemical parameters between the three time periods Before, During and After the invasion. The box and whisker plots were used to give an overall picture of change between the time periods, and used all the data for each time period together. For the Kruskal-Wallis tests, the data were further split into separate months, in order to minimise the effect of seasonal variation. All March data from Before the invasion were then tested against all March data from During the invasion, and all March data from After the invasion. The same was carried out for data from June and September. Results were not split by site, since it was felt that splitting the data further into individual sites would unacceptably weaken the statistical analysis. Thus, temporal variation and spatial autocorrelation were possible confounding factors.

Runs up-and-down tests were carried out using SPSS to examine whether there were any trends in each determinand over the entire period covered by the dataset. For this analysis the mean of each determinand for sites 10 to 6 was taken for each survey, then samples were further separated by month before the analysis was carried out. For example, a runs up and down test was carried out on site-averaged pH data for June 1996, June 1997, June 1998 *etc.* to June 2003.

4.4.3 Results: Changes in overall water chemistry

Two multivariate discriminant function analyses were carried out using SPSS to see if there were any changes in overall water chemistry.

The first discriminant function analysis was carried out between data from sites 10-6, split into the years 1997 and 1998. This analysis found that there was a distinct difference between the physico-chemical parameters of these two years. Because the co-variance matrices of physico-chemical variables for the two groups didn't match, it was necessary to use a quadratic distance rather then a linear distance function. This meant that it was not possible to isolate which physico-chemical parameters accounted for the difference.

The second discriminant function analysis was carried out to see if the time periods of Before, During and After the invasion, as classified in Chapter Three, were reflected in the physico-chemical dataset.

Once again it was decided, after examining the covariance matrices of the three groups, to use a quadratic distance function. This analysis revealed that each sample statistically "belonged" to the groups Before, During and After. There was a single datum which didn't fit in the group into which it was assigned. This was a single datum from the group 'After' the invasion; the discriminant analysis found it shared greater similarity with group designated 'During' the invasion. Again, due to the distance function used, it was not possible to state which of the physico-chemical variables contributed most to the groupings.

To see if there had been any particularly strong contribution by any one parameter to this difference, a Principal Components Analysis was carried out using the PRIMER statistical package (version 5). Prior to this, all determinands, with the exception of organics in suspended solids, were log transformed to approximate multivariate normality. The first two principle components explained 51.2% of the total variation (Table 4.4.3.1).

Principal Component	Eigenvalue	% Variation	Cumulative % Variation
1	3.74	34	34
2	17.3	17.3	51.2
3	12.6	12.6	63.8
4	9.2	9.2	73
5	7.3	7.3	80.2

Table 4.4.3.1 Percentage variation explained by each of the first five principal components

Examination of the eigenvectors for the first two principle components revealed no particularly strong contributions to variation by any one parameter, although chloride

and phosphate contributed the highest eigenvector values to the first principle component, with eigenvector values of 0.464 and 0.424 respectively. Temperature and nitrates contributed the highest eigenvector values to the second principle component (Table 4.4.3.2). The highest eigenvector value was 0.824. This was found for nitrite, as part of principle component number four.

The ordination of the first two principle components seemed to show a relatively clear distinction of the three groups, which agreed with the findings of the functional discriminant analysis (Figure 4.4.3.3).

Variable	PC1	PC2	PC3	PC4	PC5
Suspended					
solids	-0.290	-0.377	0.407	-0.01	0.164
Suspended					
Organics	0.305	0.294	-0.039	-0.250	-0.353
Chloride	-0.464	0.205	-0.085	-0.062	0.016
Potassium	-0.233	0.351	-0.312	0.061	0.395
Sodium	-0.371	0.224	-0.405	-0.010	-0.241
Nitrite	-0.048	-0.235	-0.251	0.824	-0.061
Nitrate	-0.267	-0.397	0.026	-0.278	-0.383
Phosphate	-0.424	-0.132	0.157	0.075	-0.247
Temperature	-0.121	0.427	0.301	0.248	-0.549
Conductivity	0383	0.186	0.141	-0.194	0.304
рН	0.031	0.331	0.605	0.262	0.170

Table 4.4.3.2 Eigenvectors produced by the principle components analysis of Stour

 physico-chemical data



Figure 4.4.3.3 Ordination of the first two principle components with data from the three time periods highlighted

Finally an Analysis of Similarity was carried out using PRIMER to see if the difference in overall physico-chemical parameters between the three groups was statistically significant. This test revealed a highly significant difference between the three groups (Global R = 0.303, P<0.01). The biggest difference was between the Before and After groups (Global R = 0.475, P<0.01), followed by the Before and During groups (Global R= 0.278, P<0.01) and the During and After groups (Global R= 0.131, P<0.01).

4.4.4 Results: Changes in individual parameters

4.4.4.1 pH

There seemed to be no particular trend over time with pH at the upper six sites of the Stour Estuary over the two years prior to the invasion (Figure 4.4.4.1.1). June and September 1997 showed the highest overall pH.

The pH at site ten was consistently lower than at other sites. On some surveys there was a trend of increasing pH as the survey moved downstream, but this was not a universal trend.



Figure 4.4.4.1.1. pH levels at sites 10-6 before the C. curvispinum invasion

Between the three periods there was an apparent difference in pH. From the period Before to the period During, there was a decrease in the median pH from 7.7 to 7.6 and an increase in the inter-quartile range of pH from 0.38 to 0.68. After the invasion, the median pH increased to 8.0. The inter-quartile range of pH After the invasion was 0.195 (Figure 4.4.4.1.2).

A Kruskal Wallis test found that for March surveys there was a significant difference between the three time periods (H=14.36, P<0.01). Mann-Whitney U tests revealed there was a significant drop in pH between Before and During (W= 147, P<0.01). There was a significant rise in pH between the periods During and After (W=73, P<0.01) and Before and After the invasion (W=90.5, P<0.05).

A Kruskal-Wallis test found that, for the June surveys, there was a significant difference in pH between the three time periods (H=20.6, P<0.01). Mann Whitney U testing found no significant difference between the periods before and during the invasion (W=185.5, P= 0.6176), however there were significant rises in pH between

the periods During and After (W= 64.5, P < 0.001) and Before and After (W= 136, P < 0.001).

A Kruskal-Wallis test found that for the September surveys there was a significant difference between the three time periods (H=7.8, P<0.05). Mann Whitney U tests found that there was no significant difference between either Before and During (W= 135, P> 0.05) or Before and After the invasion (W= 124, P>0.05). There was a significant rise in pH between During and After (W = 87, P<0.0184).





Overall there was not a significant upward or downward trend in pH over the course of the invasion (expected number of runs=11.636, observed number of runs = 10, runs above and below K = 7.783 P > 0.05)
4.4.4.2 Conductivity

Conductivity generally showed a decreasing trend. There was a significant drop in conductivity between 1997 and 1998, for both March (W=39, P<0.05) and June (W=40, P<0.05). There was also a drop between June 1996 and June 1997, although this was not significant (W=35, P>0.05). Conductivity did not universally drop, however, since the September 1997 conductivity was significantly higher than September 1996 (W=16, P<0.05) (Figure 4.4.4.2.1).



Figure 4.4.4.2.1. Conductivity for sites 10-6 Before the C. curvispinum invasion

There appeared to be a gradual decrease in both median and upper and lower quartiles between the three groups. Median conductivity during the invasion (867 μ S) was lower then conductivity before the invasion (882.7 μ S). Conductivity after the invasion was 811 μ S (Figure 4.4.4.2.2).



Figure 4.4.4.2.2. Conductivity at sites 10-6 Before, During and After the invasion of the Stour Estuary by *C. curvispinum*

A Kruskall-Wallace test found for March surveys there was no significant difference in conductivity between the three sites (H=5.87, *P*>0.05).

A Kruskall-Wallace test found for June surveys there was no significant difference in conductivity between the three sites (H=3.64, *P*>0.05).

For September surveys there were not enough data to analyse the period during the invasion. However a Mann-Whitney U test found a significant drop in conductivity between the two time periods of Before and After the invasion (W=168, P<0.05). Overall there was not a significant upward or downward trend in conductivity over the study period (Observed number of runs=12, expected number of runs = 9.84, P>0.05).

4.4.4.3 Chloride

Before the invasion there was a steady drop in chloride in the June samples. Although June 1996 was not significantly higher than June 1997, (W= 37, P>0.05), and June 1997 was significantly higher than June 1998 (W= 40, P<0.05). Chloride in September 1997 was overall higher than September 1996, although this was not universally true at all sites. Chloride increased from Site 10 to Site 7 on most surveys (Figure 4.4.4.3.1).



Figure 4.4.4.3.1. Chloride concentrations for sites 10-6 Before the C. curvispinum invasion

Median chloride concentration dropped from each period to the next. Before the invasion the median chloride concentration was 108 mg/l. During the invasion the median chloride was 86.5 mg/l. After the invasion, median chloride was 62 mg/l. The interquartile range increased with each successive group, from 51 Before the invasion, to 52.3 During the invasion and 55.25 After the invasion (Figure 4.4.4.3.2).



Figure 4.4.4.3.2. Chloride concentration at sites 10-6 Before, During and After the invasion of the Stour Estuary by *C. curvispinum*

A Kruskall-Wallis test found that, for March surveys, there was a significant drop in chloride concentration between the three time periods (H=10.98, P<0.01). Mann-Whitney U tests found no significant difference in chloride concentration between the periods Before the invasion and After the invasion (W=114.5, P>0.05). There was a significant drop in chloride concr\entration between the periods Before and After the invasion (W=174, P< 0.05) and the periods During and After (W= 182, P<0.01).

A Kruskall-Wallis test found that for June surveys there was no significant difference in chloride between the three time periods (H=0.69, P>0.05).

A Kruskall-Wallis test found that for the September surveys there was a significant difference in chloride between the three time periods (H=11.61, P<0.01). Mann-

Whitney U testing found no significant difference between the periods Before and After the invasion (W=108, P>0.05). There were significant differences between both the periods During and After the invasion (W=174.5, P<0.05) and the periods Before and After the invasion (W=187, P<0.001. There was no significant trend, either up or down, over the entire study period (observed number of runs=8, expected number of runs=11.636, P>0.05).

4.4.4. Dissolved oxygen

Dissolved oxygen before the invasion showed no particular trend (Figure 4.4.4.1). June samples were similar in 1996 and 1997, and then dropped slightly in 1998. The highest dissolved oxygen concentration was in September 1997, and the lowest dissolved oxygen concentration was in June 1998.



Figure 4.4.4.4.1. Dissolved oxygen levels at sites 10-6 before the C. curvispinum invasion

Median dissolved oxygen Before the invasion was higher than After the invasion (97.4% and 92% respectively). There was also a greater interquartile range of

dissolved oxygen Before the invasion (34.13) than After the invasion (11.6) (Figure 4.4.4.2).

A Mann-Whitney U test found that for March surveys, there was a significant drop in oxygen After the invasion, compared with Before the invasion (W=171, P<0.05). For June surveys there was not a significant difference (W= 216, P>0.05).



Figure 4.4.4.2. Dissolved oxygen at sites 10-6 Before and After the invasion of the Stour Estuary by *C. curvispinum*. There was not enough data for a During group.

Overall there was no significant upward or downward trend in dissolved oxygen concentration during the survey (observed number of runs=8, expected number of runs=9.23, *P*>0.05).

4.4.4.5 BOD

Biochemical oxygen demand (BOD) generally dropped in the period before the invasion. If surveys were examined by season, then BOD fell as the time of the initial invasion approached. June surveys were a slight exception to this pattern. BOD in June 1997 was lower than in June 1996, with the exception of site 6 which was slightly higher in 1997. There was then a rise in BOD in June 1998, again with the exception of site 6, where there was a drop in BOD compared with the previous year. The highest BOD was in September 1996. The lowest BOD was recorded in June 1997 (Figure 4.4.4.5.1).



Figure 4.4.4.5.1. B.O.D₅ results for sites 10-6 before the C. curvispinum invasion

Before the invasion, the median BOD was 2.9 mg/l, with an interquartile range of 3.4. During the invasion, the median BOD dropped slightly to 2.7 mg/l and inter-quartile range fell to 1.98. The inter-quartile range of BOD After the invasion was 1.68. The median BOD was 1.83 After the invasion (Figure 4.4.4.5.2).



Figure 4.4.4.5.2. Biochemical oxygen demand at sites 10-6 Before, During and After the invasion of the Stour Estuary by *C. curvispinum*

For March and June and September surveys, there were insufficient data to form a During group for the analysis. Mann-Whitney U tests found there was no significant difference in BOD for March between the time Before the invasion and the time After the invasion (W=114, P>0.05). There was also no significant difference in June for BOD Before and After the invasion (W=186, P>0.05).

A Kruskall-Wallis test found that for September surveys there was a significant difference in BOD between the three time periods (H=10.14, P<0.01). Mann-Whitney U testing found that for September surveys there was a significant drop in BOD between the time period Before the invasion and the time period During the invasion (W=105, P<0.01). There was a significant difference between Before and After the invasion (W= 176, P<0.05).

There was no significant upward or downward trend in BOD over the course of the study period (observed number of runs=10, expected number of runs=10, *P*>0.05).

4.4.4.6 Nitrite

Nitrite concentration was highest at the beginning and the end of this period. Nitrite concentrations fluctuated over the course of time. There was an overall rise in Nitrite from September 1996 to June 1997. Nitrites dropped slightly in September 1997, but the results for March 1998 were the highest for the entire period. There were no results for June 1998 (Figure 4.4.4.6.1).



Figure 4.4.4.6.1. Nitrite levels at sites 10-6 Before the C. curvispinum invasion

Median nitrite concentration dropped with each successive time period, from 0.1 mg/l Before the invasion to 0.06 mg/l During the invasion and 0.037 mg/l After the invasion. Inter-quartile range also dropped successively, from 0.083 Before the invasion, to 0.05 During the invasion, and 0.027 After the invasion (Figure 4.4.4.6.2).



Figure 4.4.4.6.2 Boxplots of the nitrite concentrations over the three time periods

A Kruskall-Wallis test found that for March surveys there was no significant difference in nitrite concentrations between the three time periods (H=5.47, *P*>0.05).

A Kruskall-Wallis test found there was a significant difference in nitrite concentrations between the three time periods. Mann-Whitney U tests found that for June surveys there was a significant drop in nitrite concentration between the time periods Before and During the invasion (W=155, P<0.01), During and After (W=189, P<0.01), and Before and After the invasion (W=205, P<0.01).

A Kruskall-Wallis test found that for September surveys there was no significant differences in nitrite concentration between the time periods (H=2.70, *P*>0.05).

A runs test found no significant trends over the course of the invasion (Observed number of runs = 8, expected number of runs = 9.72, P>0.05).

4.4.4.7 Nitrate

The nitrate readings for June 1998 were missing. Nitrate concentrations between June 1996 and March 1997 appeared to remain stable, and there was little evidence of seasonal variation. Nitrate then appeared to decrease in June 1997, and remain relatively stable at this lower concentration. The highest nitrate concentration was found in September 1996, and the lowest concentration of nitrate was found in March 1998 (Figure 4.4.4.7.1).



Figure 4.4.4.7.1. Nitrate concentrations at sites 10-6 before the C. curvispinum invasion

As with the phosphate results, each successive period saw an overall drop in median nitrate concentration, from 7.6 mg/l before the invasion, to 6.31 mg/l during the invasion and 3.73 mg/l after the invasion. Before the invasion, the interquartile range of nitrate was 2.43. Nitrate concentration showed the smallest interquartile range, 1.605, during the invasion. After the invasion nitrate concentrations were at their most variable, with an interquartile range of 2.681 (Figure 4.4.4.7.2).



Figure 4.4.4.7.2. Nitrate concentration at sites 10-6 over the three time periods

A Kruskall-Wallis test found that for March surveys, there were significant differences in nitrate concentrations between the three time periods (H=13.95, P<0.01). Mann Whitney U testing found that there was a significant drop in nitrate concentration between the time periods Before and During the invasion (W=110.5, P>0.05). There were also significant drops between the time periods During and After the invasion (W=189, P<0.01) and the time periods Before and After the invasion (W=183, P<0.01).

A Kruskall-Wallis test found that for June surveys, there was a significant difference in nitrate concentrations between the three time periods. Mann Whitney U tests found that for the June surveys there were significant drops in nitrate concentration between the time periods Before and During the invasion (W=135, P<0.05), During and After the invasion (W=171, P<0.05), and Before and After the invasion (W=192, P<0.01).

A Kruskal-Wallis test found that for September surveys there was a significant difference between the time periods Before and During the invasion (H=20.84, P<0.01). Mann Whitney U tests found that there were significant differences Before and During the invasion (W = 138, P<0.05), During and After the invasion (W=185, P<0.01), and Before and After the invasion (W=201, P<0.01).

There was a significant downward trend over the course of the invasion (Observed number of runs=4, expected number of runs = 11.909, *P*<0.05).

4.4.4.8 Phosphate

There was an overall drop, year on year for phosphate in the period before the invasion. Phosphate concentrations in June 1998 were lower than either June 1997 or June 1996. March 1998 phosphate concentrations were lower than March 1997. The September 1997 phosphate concentration was lower than the September 1996 phosphate concentration. The highest phosphate concentration was recorded in September 1996. The lowest phosphate concentration was recorded in March 1998 (Figure 4.4.4.8.1).





Phosphate concentrations in the period Before the invasion had both the highest median value and largest interquartile range of the three time periods (Figure 4.4.4.8.2). Median phosphate During the invasion was 1.46 mg/l and the interquartile range was 0.84. During the invasion, the median phosphate and interquartile range dropped to 0.78 mg/l and 0.7320 respectively. After the invasion the median phosphate was 0.24 mg/l and inter-quartile range was 0.24.



Figure 4.4.4.8.2. Boxplots of phosphate concentrations during the three time periods

A Kruskall-Wallis test established that for March surveys there was a significant difference between the three time periods (H=22.69, P<0.01). Mann-Whitney U tests found significant drops in the concentration of phosphate between the periods Before and During the invasion (W=155, P<0.01), During and After the invasion (W=186, P<0.01), and Before and After the invasion (W=197, P<0.01).

A Kruskall-Wallis test found that for June surveys, there was a significant difference in phosphate concentration between the three time periods (H=32.12, P<0.01). Mann Whitney U tests found significant drops in phosphate concentration between the periods Before and During the invasion (W=258, P<0.01), During and After the invasion (W=204, P<0.01), and Before and After the invasion (W=345, P<0.01).

A Kruskall-Wallis test found that for September surveys, there was a significant difference in phosphate concentration between the three time periods (H=29.24, P<0.01). Mann-Whitney U tests found that for September surveys, there was a significant drop in phosphate between the period Before and During the invasion (W=193, P<0.01), During and After the invasion (W=331, P<0.01) and Before and After the invasion (W=205, P<0.01).

A runs test revealed a significant downward trend in phosphate over the period of the study (observed number of runs = 6, expected number of runs = 12.48 P < 0.01)

4.4.4.9 Suspended solids

In the period Before the invasion, the concentration of suspended solids in the water column peaked in March 1997, particularly at site seven. After this period suspended solid concentrations decreased. The lowest concentration for suspended solids was recorded in March 1998 (Figure 4.4.4.9.1).



Figure 4.4.4.9.1. Suspended solids concentrations at sites 10-6 before the *C. curvispinum* invasion

There was a steady drop over time in median suspended solid concentration from one period to the next, from 51 mg/l Before the invasion to 18.7 mg/l During the invasion and 9.2 mg/l After the invasion. The inter-quartile range dropped similarly from 39.4 Before the invasion to 10.75 During the invasion and 6.9 After the invasion (Figure 4.4.4.9.2).

For March surveys there were not enough data to form a 'During' group. There was a significant drop in suspended solids between the time periods Before and After the invasion (W=198.5, P<0.01).



Figure 4.4.4.9.2. Boxplots showing suspended solids concentrations during the three time periods

A Kruskall-Wallis test found that for June surveys, there was a significant difference in suspended solids between the three time periods (H=26.29, P<0.01). Mann-Whitney U tests found that for June surveys there were significant drops in suspended solids concentrations between the time periods Before and During the invasion (W=258, P<0.01), During and After the invasion (W=174, P<0.05) and Before and After the invasion (W=342, P<0.01).

A Kruskal-wallis test found that for September surveys, there was a difference in suspended solids between the three time periods (H=24.72, P<0.05). Mann-Whitney U tests found that for September surveys, there were significant differences between the time periods Before and During the invasion (W=205, P<0.01), During and After

the invasion (W=261.5, P<0.05) and Before and During the invasion (W=175, P<0.01).

A runs test found a significant downward trend over the entire study period (observed number of runs = 2, expected number of runs = 10.55, *P*<0.001)

4.4.4.10 Suspended Organics

In the period Before the invasion, there was a slight overall increase in percentage organics in suspended solids over time. The lowest percentage organics in suspended solids was recorded in March 1997. The highest percentage of organics in suspended solids occurred in June 1998 (Figure 4.4.4.10.1).



Figure 4.4.4.10.1. Organics in suspended solids concentrations at sites 10-6 before the *C. curvispinum* invasion

There was a successive increase in median percentage organics in suspended solids with each time period, from 23% Before the invasion, to 38.38% During the invasion, and 46.51% After the invasion. There was a similar increase in inter-quartile range with each time period from 8.6 Before the invasion, to 13.34 During the invasion and 38.78 After the invasion (Figure 4.4.4.10.2).



Figure 4.4.4.10.2. Boxplots showing percentage organics in suspended solids over the three time periods

For March surveys there were insufficient data to analyse the period During the invasion. There was a significant difference in percentage organics between the time periods Before and After the invasion (W=78, P<0.05).

A Kruskall-Wallace test found that for June samples, there was a significant difference between the three time periods. Mann-Whitney U testing revealed that for June, there was a significant increase in percentage organics between the time periods Before and During the invasion (W=135, P<0.01). There was no significant difference between the time periods During and After the invasion (W=118, P>0.05). There was a significant increase between the time periods Before and After the invasion (W=173, P<0.05).

A Kruskall-Wallace test found that for September samples there was a significant difference between the three time periods (H=10.66, P<0.01). Mann Whitney U testing revealed that for September surveys, there was a significant increase in percentage organics between the time periods Before and During the invasion (W=76, P<0.01). There was no significant difference between the time periods During and After the invasion (W=174, P>0.05). There was a significant increase between the time periods Before and After the invasion (W=79.5, P>0.05).

A runs test found that overall there was a significant upward trend in the data (observed number of runs = 6, expected number of runs = 11.29, *P*<0.05)

4.4.4.11 Sodium

Before the invasion there were no particular trends. The lowest concentration of sodium was recorded in March 1997, although there is a distinct possibility that this low reading is an artefact of the analysis (see section 4.4.4.12). The highest salinity was recorded in September 1997 (Figure 4.4.4.11.1).



Figure 4.4.4.11.1. Sodium concentration at sites 10-6 before the C. curvispinum invasion

The median sodium fell during each successive period, from 70.5 mg/l Before the invasion, to 68 mg/l During the invasion, and 51.25 After the invasion. The interquartile range also dropped successively, from 45.05 Before the invasion, to 36.9 During the invasion, and 34.46 After the invasion (Figure 4.4.4.11.2).



Figure 4.4.4.11.2. Boxplots showing sodium concentrations during the three time periods

For March surveys there were not enough data to analyse the period of time During the invasion. There was no significant difference between Before and After the invasion (W=116, P>0.05).

A Kruskal-Wallace test found no significant difference in sodium concentration in June samples between the three time periods (H=1.99, P>0.05).

A Kruskall-Wallace test found that for September surveys, there was a difference between the three time periods (H=8.1, P<005). Mann-Whitney U tests found no significant difference in sodium concentration between either the periods Before and During the invasion (W=147.5, P>0.05), or the periods During and After the invasion

(W=257, P>0.05). There was a significant decrease in sodium concentration between the time periods Before and After the invasion (W=191, P<0.01).

A runs test failed to show any significant trend over the length of the study period (observed number of runs=10, expected number of runs=11.18, *P*>0.05).

4.4.4.12 Potassium

There were no particular trends in potassium concentration Before the invasion. The lowest potassium concentration for this period was recorded in March 1997. This finding was consistent with the results for sodium (section 4.4.4.11). The highest potassium concentration was recorded in September 1997 (Figure 4.4.4.12.1).



Figure 4.4.4.12.1. Potassium at sites 10-6 before the C. curvispinum invasion

The median potassium concentration was 6.5 mg/l Before the invasion. It increased to 8.65 mg/l During the invasion, then dropped to its lowest concentration After the invasion. Inter-quartile range was 2.48 for the period Before the invasion, 2.56 for the period During the invasion, and 3.06 for the period After the invasion (Figure 4.4.4.12.2). One salient feature is the low results for March 1997, these results mirror the results for sodium (Figure 4.4.4.11.1), but not chloride (Figure 4.4.4.3.1).

During all other years these three graphs are closely matched. Because sodium and potassium are analysed using the same machine, it is possible that the low values during March 1997 are a sampling artefact, and not indicative of the actual state of the estuary at that time.



Figure 4.4.4.12.2. Boxplots showing potassium concentrations during the three time periods

For the March surveys there were not enough data to analyse the period During the invasion. There was no significant difference in potassium concentration between the periods Before and After the invasion.

A Kruskall-Wallis test found that for the June surveys, there was no significant difference between the three time periods (H=3.36, *P*>0.05).

A Kruskall-Wallis test found that for the September surveys there was no significant difference between the three time periods (H=4.30, *P*>0.05).

A runs test found no significant upward or downward trend for potassium over the length of the study (observed number of runs = 10, expected number of runs = 11.909, *P*>0.05).

4.4.4.13 Temperature

During the time period Before the invasion there was no obvious overall trend in the data. There was a general seasonal pattern. Temperature was lowest in March, higher in September, and highest in June. The highest temperature was in June 1997, the lowest temperature was in March 1998 (Figure 4.4.4.13.1).



Figure 4.4.4.13.1. Temperature at the sites 10-6 before the C. curvispinum invasion

Median temperature rose between the period Before and the period During the invasion. It then fell in the period After the invasion. Inter-quartile range increased with each successive time period (Figure 4.4.4.13.2).



Figure 4.4.4.13.2 Boxplots showing temperature values over the three time periods

A Kruskall-Wallis test found that for March surveys there was a significant difference between the three time periods. A Mann-Whitney U test found no significant difference between the period Before the invasion and the period After the invasion (W=130, P>0.05). There were significant decreases in temperature between the periods During and After the invasion (W=180, P<0.05), and Before and After the invasion (W=200, P<0.01).

A Kruskall-Wallis test found that for June surveys, there was a significant difference between the three time periods. A Mann-Whitney U test found no significant difference between the periods Before and During the invasion (W=197.5, P>0.05). There was a significant decrease in temperature between the periods During and After the invasion (W=55, P<0.01). There was also a significant decrease between the periods Before and After the invasion (W= 170, P<0.05). A Kruskall-Wallis test found that for the September surveys there was a significant difference in temperature between the three time periods (H=9.36, P=0.01). A Mann-Whitney U test found a significant decrease in temperature between the periods Before and During the invasion (W=55, P<0.01). There was no significant difference between the periods During and After the invasion (W=63, P>0.05). There was a significant drop in temperature between the periods Before and After the invasion (W=91, P<0.05).

A runs test found no significant trends over the course of the invasion (observed number of runs = 15, expected number of runs = 11.28, P>0.05)

4.5 Investigation of the relationship between physico-chemical parameters and C. curvispinum abundance using multiple regression

4.5.1 Overview

For all multiple regression analyses, the predictor variables were checked to ensure linearity with the response variable, non-additivity and the absence of perfect colinearity between variables. Residuals of each equation line were checked graphically to ensure that they approximated to a normal distribution, and the predictor variable was transformed until it satisfied the assumptions of normality, according to an Anderson-Darling test. For *C. curvispinum* abundance, the transformation required was double ln (X+1).

Only samples that contained at least one corophiid were used in this analysis. This was because *C. curvispinum* sample could be absent from a sample for a number of reasons, for example a lack of opportunity to colonise the area being sampled, Whereas if at least one corophiid was found then there was no question of opportunity to colonise being a confounding factor.

After samples that didn't contain any *C. curvispinum* were removed, the number of data points available for this analysis was forty nine. This figure could be further split into nineteen data points for the period during the invasion and thirty data points for the period after the invasion. In order to preserve the power of the multiple regression, there should ideally be at least ten times as many data as variables in the equation (Zar 1999). Therefore a maximum of two variables during the invasion, three variables after the invasion, or five variables overall were used as predictor variables.

4.5.2 Examining the possibility of a direct relationship between C. curvispinum abundance and physico-chemical parameters

Three best-subsets multiple regression analyses were carried out to investigate whether there was a direct relationship between *C. curvispinum* abundance and physico-chemical parameters. The first multiple regression covered the entire period when *C. curvispinum* was present in the study area, the second regression only used data from the period During the invasion, and the final regression only used data from the period After the invasion.

Overall, the best predictors of log *C. curvispinum* abundance over the two periods During and After the invasion were provided by the four variables: suspended solids, suspended organics, potassium and water temperature. There was a significant relationship between these variables and *C. curvispinum* abundance (F= 4.18, P=0.006), and the four parameters accounted for 22.1% of the variation in log *C. curvispinum* abundance. Individually none of the variables showed a statistically significant relationship with *C. curvispinum* abundance (Table 4.5.2.1.)

Table 4.5.2.1. T and P values for the regression carried out on data from During and After the invasion

Predictor variable	T value	<i>P</i> value
Suspended solids	-1.08	0.287
Suspended organics	1.46	0.125
Potassium	-1.85	0.072
Water temperature	1.11	0.272

For comparison with the period During the invasion, a best subsets multiple regression model to find the two best predictors of *C. curvispinum* abundance was calculated. The two best predictors of *C. curvispinum* over the entire time period that *C. curvispinum* was found were suspended organics and potassium, which between them explained 21.9% of the variation in log *C. curvispinum* abundance

The two best predictor variables for *C. curvispinum* abundance During the invasion were chloride and nitrate, which between them accounted for 50.6% of the variation in log *C. curvispinum* abundance. Overall there was a significant relationship between these two variables and *C. curvispinum* abundance (F=9.21, *P*=0.003). Nitrate was the most significant variable of the two (T=-2.94, P=0.011), followed by chloride (T=-2.35, *P*=0.034).

The three best predictor variables for *C. curvispinum* abundance After the invasion were suspended solids, pH and potassium. These parameters accounted for 32.4% of the variation in log *C. curvispinum* abundance. Overall these values showed a significant statistical relationship with *C. curvispinum* abundance (F=5.47, P=0.005). Potassium concentration was the only individual variable to exhibit a significant relationship with *C. curvispinum* abundance (Table 4.5.2.2). The best *two* predictor variables (for comparison with the regression carried out on the During data) were pH and potassium concentration, which explained 30.1% of the variation of log *C. curvispinum* abundance.

Table 4.5.2.2. T values and P values for the multiple linear regression carried out on data from the period After the invasion

Predictor variable	T value	<i>P</i> value
Suspended solids	-1.37	0.184
рН	2.02	0.054
Potassium	2.43	0.023

4.5.3 Investigating the possibility that changes in physico-chemical parameters before the invasion aided the establishment of C. curvispinum

Multiple regression was also used to indirectly examine the effect that changing physico-chemical parameters before the invasion may have on the success of the invasion. It is difficult to prove that a change in any particular physico-chemical parameter did or did not facilitate the establishment of an invader. As a scoping exercise two multiple regression analyses were carried out, using double logged *C. curvispinum* abundance as the dependent variable. The first analysis was carried out using as x variables the three factors that had changed the most between the before and during periods. These variables were suspended solids, suspended organics, and nitrite. The second analysis was carried out using the variables that had changed the least between the two periods Before and During the invasion; these were sodium, potassium and biochemical oxygen demand.

Suspended solids, suspended organics and nitrite together explained 28% of the variability of log *C. curvispinum* abundance. None of these variables were found to significantly relate to *C. curvispinum* abundance (Table 4.5.3.1).

Table 4.5.3.1. T values and P values for the	ne multiple regression carried out on data from the
time periods During and After the invasion.	Variables used were those that changed the most
Before the invasion.	

Predictor variable	T value	<i>P</i> value
Suspended solids	0.524	0.609
Suspended organics	1.769	0.1
Nitrite	0.744	0.47

The three variables that changed the least in the period before the invasion were water temperature, potassium and chloride. These variables accounted for 15.6% of the overall variation in double logged *C. curvispinum* abundance. There was a significant relationship between these variables and *C. curvispinum* abundance

(F=3.8, P=0.017). The only individual variable that showed a significant relationship

with C. curvispinum abundance was potassium (Table 4.5.3.1).

Table 4.5.3.1. T values and *P* values for the multiple regression carried out on data from the time periods During and After the invasion. Variables used were those that changed the *least* Before the invasion.

Predictor variable	T value	<i>P</i> value
Water temperature	1.71	0.094
Potassium	-2.36	0.023
Chloride	-0.94	0.351

4.6 Discussion

4.6.1 Before the invasion

4.6.1.1 Descriptive account of changes in anthropogenic river use and river flow

The most notable changes occurring in the river in the period before the invasion was the change in the pattern of sewage discharge in to the estuary. However it was difficult to see a direct trend in sewage treatment, in relation to the estuary, before the invasion. This was because increasing impacts, for example the opening of a new sewage works discharging sewage from Wetherlees Hill, were balanced by decreasing impacts, for example the improvement in water quality of sewage discharged from the Pfizer compound.

The three years of drought in the EA southern region, prior to the invasion (Figure 4.2.1) may have affected the success of the invasion, either directly, by slowing flow of the river to the point where *C. curvispinum* could colonise, or indirectly, by adversely affecting competing fauna. Flow measurements were taken on the Stour Estuary during the June 2004 monitoring survey. These found flow speeds of between 12 cm s⁻¹ and 167 cm s⁻¹ when the water in the estuary was ebbing, and between 6 cm s⁻¹ and 103 cm s⁻¹ when the water in the estuary was in flood. As mentioned previously, Den Hartog (1992) found a positive correlation between

current speed and population density of *C. curvispinum*. However, the current speed is in excess of that reported by Den Hartog *et al.* (1992) on the Rhine and it is possible that the positive correlation that he reported does not extend to the current speeds found in the Stour estuary.

4.6.1.2 Weather data from Manston

Over the period leading up to the invasion there were several changes in the climate. Weather reached several extremes in 1996, when there was the lowest maximum and minimum temperature, highest and most variable temperature range, greatest number of days when the temperature dropped below freezing and lowest annual rainfall. After this period the following variables changed steadily until 1999: maximum and minimum temperatures rose, average annual temperature range became smaller and varied less from one month to the next, temperature dropped below freezing with less regularity, and the amount of rainfall increased.

Whether these changes had any effect on the invasion is impossible to say, although raised temperature was one of the factors linked to the success of the *C. curvispinum* invasion of the Rhine (Den Hartog *et al.* 1992). Atrill (1992) suggested that the range of salinity in an estuary could be a more important factor than absolute salinity when determining biodiversity. It might be possible that certain taxa could be stressed by the range in other physico-chemical factors, such as temperature. Reduction in temperature range could reduce the amount of physiological stress to an organism living in the estuary, which in turn could increase its fitness and chance of success in the estuary.

4.6.1.3 The Stour physico-chemical dataset

The Stour monitoring dataset spanned only one continuous year and two partial years before the invasion. This made any trends before the invasion difficult to detect. However even a short term change might have affected the potential for *C. curvispinum* to successfully invade.

There were several changes in physico-chemical parameters in the period before the invasion: there was an increase in suspended organics, a drop in chloride concentration, nitrate, orthophosphate, BOD and conductivity. The drops in nitrate, phosphate and BOD would seem to indicate a possible increase in water quality in the Stour estuary. Also the increase in percentage organics of suspended solids in the water column could have made filter feeding a more profitable niche, as more filtrate was likely to contain material of nutritional value.

Indeed it is possible to postulate a mechanism by which most of these changes could have come about. With a reduction in suspended solids, more light could have penetrated the water column, leading to an increase in photosynthetic plants. This hypothetical situation could partially explain both the reduction in nutrients, as they were taken up by phytoplankton, and the proportional increase in suspended organics, as the increased productivity would have increased the proportion of organic matter in the water column.

Without chlorophyll a results, the evidence for such a mechanism is weak and it can be only be given the credence due to a plausible hypothesis. Also BOD would be expected to rise if productivity in the water column increased, and this did not appear to happen.

4.6.2 During and After the invasion

The demarcation between the periods During and After the invasion was marked by an apparent crash in the population of *C. curvispinum* in the Estuary. Several changes in physico-chemical parameters over the period During and After the invasion might have affected the *C. curvispinum* population in the Stour Estuary. In particular suspended solids, nitrate, phosphate and dissolved oxygen all dropped, and the percentage organics in suspended solids also rose. All these patterns, with the exception of the drop in oxygen, also occurred Before the invasion. This raises

three possibilities in relation to *C. curvispinum*: the first possibility is that *C. curvispinum* is not associated with anoxic conditions, and the drop in oxygen concentrations contributed to the population crash. Evidence for this might come from finding a relationship between dissolved oxygen and *C. curvispinum* abundance. None of the multiple regression analyses carried out in this chapter found dissolved oxygen to be a significant factor contributing to *C. curvispinum* abundance. Therefore there is no evidence to support this possibility.

The second possibility is that the changing factors passed through the range for which they conferred optimal fitness for *C. curvispinum* and into a range where they did not. This could be the case if the relationship between *C. curvispinum* and any of the variables was non-linear (see Figure 2.4.5.1a for a hypothetical example). Evidence for this possibility would come from finding a non-linear relationship between *C. curvispinum* and any of the variables in question. As a preliminary step before carrying out the linear regressions in section 4.5.2 and 4.5.3, all the variables were tested for linearity with respect to *C. curvispinum* abundance, and no strong evidence for a non-linear relationship was found. Therefore there is no evidence to support this possibility either.

The final possibility, is that these physico-chemical changes had nothing to do with the population crash, and some other, unmeasured variable, caused the crash in numbers of *C. curvispinum*. Given that the best multiple regression result only accounted for 50.6% of the variation in *C. curvispinum* abundance, there is plenty of un-accounted for variation. This variation could be found either in the form of factors that could not be used in the multiple regression, such as the commissioning of sewage outflows, or as factors that weren't measured at the time the Stour Dataset was being compiled, such as prevalence of hard substratum at a site, flow speed, or chlorophyll a. There could also be factors that have not been considered with respect to *C. curvispinum* such as introduction of a parasite or pathogen.

4.6.3 Changes that could have been caused by an increase in the number of filter feeders in the Estuary

A reduction in suspended solids and a decrease in nutrients were postulated effects of introducing a filter feeder into an aquatic ecosystem. Both these patterns were seen on the Stour Estuary over the course of the invasion. If there were enough C. *curvispinum* in the water column they could have filtered the suspended solids in the river out of the water column in a way similar to the filtering of the North American Great Lakes by D. polymorpha. In order to achieve this effect on the river there would have to be a huge population of C. curvispinum in the river. Sampling has found the largest population of C. curvispinum attached to hard substratum under the bridge at Plucks Gutter. The average density was found to be 400 individuals per grab. The maximum density found was 1125 individuals in a grab. Availability of hard substratum is known to be a factor affecting the density of C. curvispinum (Den Hartog 1992, and personal observations). The prevalence of hard substratum at Plucks Gutter is possibly unique in the upper estuary as a whole. For the rest of the estuary, hard substratum is confined to reeds and other littoral vegetation, the occasional concrete outfall, and any debris that found its way into the river and sank. Thus it can be predicted that Plucks Gutter has the greatest population density of the entire Estuary.

Further experimentation is necessary in order to discover whether there is a direct link between *C. curvispinum* population density and any particular environmental parameters. A possible future experiment to test whether *C. curvispinum* had a measurable effect on water physico-chemistry would be to carry out a flume experiment, with at least three flumes populated by a set number *C. curvispinum* and three flumes without *C. curvispinum*. A set amount of water of known physico-chemical properties could be introduced to the six flumes and then the water could be measured for these properties after a period of time and the effect of *C.*

curvispinum on the water column. This experiment would give a quantitative measure of the effect of a known number of *C. curvispinum* on a known volume of water. These data could then be used, together with the measure of the density of the *C. curvispinum* population in the Stour, and the discharge of the Stour, to ascertain whether *C. curvispinum* had a measurable effect on these parameters.

The assumption made for the regression models in this chapter were that *C. curvispinum* was the dependent variable and the physico-chemical parameters were all independent variables. If *C. curvispinum* had a measurable effect on any of the physico-chemical parameters then this assumption would be violated. Faced with a lack of definitive evidence of the effect of *C. curvispinum* on the physico-chemical parameters of the Kentish Stour, it was decided for the purpose of the multiple regression analysis to assume that *C. curvispinum* was the sole dependent variable.

4.6.4 Changes over the entire course of the Study Period

Over the course of the study period there were overall downward trends in suspended solids, nitrates, and phosphates. There were also successive drops in median concentration for chloride and sodium. There was an overall upward trend in suspended organics.

The drop in median chloride concentration over the three successive periods would seem to make the estuary less ideal for a successful invasion, as low chloride is supposed to be a limiting factor in *C. curvispinum* distribution (Bayliss and Harris 1988). Also chloride was one of the two variables that explained 50.6% of the variation in *C. curvispinum* abundance during the invasion. However, a best subsets multiple regression carried out over the periods During and After the invasion did not feature chloride as a significantly contributing variable when explaining *C. curvispinum* variation. Furthermore the range of chloride concentration in which Bayliss and Harris (1988) found *C. curvispinum* was from 21.3 mg/l to 99.3 mg/l.

Before the invasion median chloride concentration on the Stour was 108 mg/l. During the invasion it was 86.5 mg/l. Even at its lowest concentration after the invasion, median chloride was 62 mg/l, well above the lower limit suggested by the data of Bayliss and Harris (1988). This would seem to suggest that the falling chloride concentration was not a major factor influencing invasion success in this case.

4.6.5 Changes and implied relationships between C. curvispinum and physicochemical parameters.

A multiple regression carried out on the *C. curvispinum* population in the Stour Estuary both During and After the invasion found 22.1% of the variation could be explained by suspended solids, suspended organics, potassium and water temperature. However, none of these, variables were found to be statistically significant. This also left 77.9% of the variation in *C. curvispinum* abundance unexplained.

Two further multivariate analyse were carried out, firstly to investigate the relationship between *C. curvispinum* and its environment during the invasion and secondly to investigate this relationship after the invasion. The analysis carried out on the period During the invasion found that the two variables nitrate and chloride, which between them accounted for 50.6% of the variation in *C. curvispinum* abundance, were both significant. After the invasion the best three variables were suspended solids, potassium and pH. Together these accounted for 32.4% of the variation, although none of them were statistically significant. The fact that the three analyses emphasised different variables and the fact that the r^2 value for the 'During' group was greater then the r^2 value for the analysis of both periods (During and After) together could indicate that the relationship between *C. curvispinum* and its environment changed between the two periods. This could be due to an additional
stressor not included in the analysis, such as a pathogen or parasite of *C. curvispinum.*

The speculative multiple regression carried out on physico-chemical parameters that changed most before the invasion produced an r² value of 28%. This compared with an r² value of 15.6% for parameters that changed the least over the period before the invasion. Whilst not providing much evidence either way, this does give a weak indication that some of the changes that occurred to the physico-chemical parameters prior to the invasion may have had some influence on the invasion's success.

4.6.6 Further work

It would be useful to examine the behaviour of *C. curvispinum* different at flow speeds, using a flume in the laboratory and examining parameters such as speed of tube construction. It would also be useful to see how they orientate their tube with respect to the current, as this might have implications for the success of *C. curvispinum* in the flow speeds found in the Stour estuary. Finally an experiment to investigate the effect that manipulation of the parameters highlighted by the multiple regression analyses has on *C. curvispinum* success could help to determine whether these parameters had a real effect on *C. curvispinum* or whether the relationships were just a Type One error.

Chapter 5

Changes in the native fauna of the Stour Estuary

5.1 Overview

There would appear to be three interacting components to consider when examining an invasion. These are: the invader, the habitat into which it invades, and the native fauna of that habitat. Each of the three elements can be regarded as the point of a triangle, with the sides made up of two way interactions between each component (refer back to Figure 1.1.6). The invader, in this case *C. curvispinum*, was considered in Chapter 3. The physico-chemical environment into which *C. curvispinum* invaded was examined in Chapter 4. The current chapter examines the third compartment, namely state of fauna other than *C. curvispinum* in the Kentish Stour Estuary over the course of the study period. This examination is carried out in the context of the invasion of the Kentish Stour by *C. curvispinum*.

Several topics in invasion biology are concerned with the relationship between the native fauna and the process of an invasion. These are diversity and invasion resistance; impacts of the invasion on the native fauna; post invasion changes in the fauna; and the relationship between *C. curvispinum* and the native fauna.

5.1.1 Diversity and invasion resistance

Elton (1958) suggested a possible link between the biodiversity of an ecosystem and the resistance of that ecosystem to invasion. This phenomenon was termed the invasion resistance. Some researchers report a positive relationship between diversity and invasion resistance (e.g. Stachowitz *et al.* 2001). Others report no link

(e.g Moyle and Light 1996b), or in fact a negative association between the two phenomena (e.g. Stohlgren *et al.* 1999, Palmer and Maurer 1997).

There is little evidence in the literature that some aspect of diversity has influenced past invasions by *C. curvispinum*. Some circumstantial evidence was provided by Den Hartog *et al.* (1992) who noted that one of the contributory factors to the successful invasion of the Rhine by *C. curvispinum* might have been the 1986 Sandoz incident, which resulted in the fauna of the river being severely impoverished by toxic waste for almost the entire length of the river.

5.1.2 Impacts of the invasion on the native fauna

As well as the native community having an effect on the invader it is possible to envisage situations where the invader has an impact on the native fauna.

During an invasion there can sometimes be an invasion spike, where the abundance of an invading species reaches high levels; the abundance can then drop (Nalepa *et al.*1999). This phenomenon appears to have occurred in the invasion of the river Stour by *C. curvispinum* between September 1998 and September 2000. It would be reasonable to hypothesise that during the phase of highest population, the effect of the invader on the invaded community will be at its greatest.

C. curvispinum could affect a native community and their associated habitat in several ways. As a filter feeder it removes particles from the water column and concentrates them. By building mud tubes on available hard substratum, *C. curvispinum* denies hard substrata to other organisms. *C. curvispinum* is capable of building tubes on virtually any hard surface. The population in the Stour has been found encrusting metal, wood, plastic (including shopping bags), as well as stones bricks and both living and dead *Anodonta* shells. This last point has implications for

sessile bivalves. Thus, in the river Meuse in Belgium, *C. curvispinum* was reported to build tubes on top of the zebra mussel *Dreisenna polymorpha* (Bachman *et al.* 2001), which was one possible reason given for the success of *C. curvispinum* in places that had once been *Dreisenna* strongholds. Therefore, *C. curvispinum* might be expected to have a particular effect on bivalve filter feeders, both in terms of direct competition for food and by smothering the filter feeders themselves.

5.1.3 Post invasion changes in the fauna

Changes in the fauna after an invasion could be examined for two reasons. Firstly, after the numbers of an invader subside, any impact on the fauna might have changed.

Secondly, an invasion may not be due to a weakness in the fauna, but an improvement in the river, which may be reflected in the state of the fauna. Since the late 1970s, successive governments together with the European Union have produced legislation that has required that river water quality be improved. Recent examples of this legislation include the protection of water against agricultural nitrate pollution 1996, the water framework directive 2000, and the Water Supply (Water Quality) Regulations 2001 (Netregs 2006). As this legislation is enacted and water quality improves, the opportunities for less pollution tolerant species increases. It is possible that an invader, such as *C. curvispinum*, is just one of these potential colonists.

5.1.4 Possible patterns of change in the native fauna concurrent with the invasion by C. curvispinum

In Figure 5.1.4.1, I suggest that there might be four possible patterns of change in the invertebrate community of the Kentish Stour that could be considered. These models are hypothetical, but based on the literature discussed in chapter 1. In the first pattern (1 in Figure 5.1.4.1.), which could almost be thought of as the 'null' relationship, there is no change in the fauna either Before, During or After the invasion. This scenario would be indicated by a lack of significant differences between any of the three time periods. This model is based on the effect of invasions such as *A. dorrieni*, where an invader seems to have no impact on the community it invades (Smithers 2003, personal communication)..



Figure 5.1.4.1. Four hypothetical models of invertebrate community change over time in relation to an invasion. 1. Cyclical or seasonal changes only, with no signs of impact before during or after invasion. 2. Weakening of the fauna just prior to invasion. 3. Trend of continuous improvement, reflecting improvement in water quality. 4. Native community impacted at the same time as invasion, with the native community recovering as the invading population collapses

The second pattern 2 in Figure 5.1.4.1. describes a weakening of the fauna before the invasion. This could indirectly suggest some degree of invasion resistance on the part of the native fauna in the invaded habitat. Evidence for this model would be found in the period preceding the invasion, if the biological quality of the native community dropped, followed by either improvement or no change in the native invertebrate community during and after the invasion. This model examines the theoretical link between diversity and invasion resistance (Elton 1958, Stachowitz 2002).

The third model reflects a general improvement in the health of the invertebrate community of the invaded habitat over the period of time covered by the study. Evidence for this model would be provided by a trend of continuous increase in measures of native community health over the entire study period. This model considers the invasion as part of a succession of colonisers, as a result of ongoing improvements in water quality in Europe.

The fourth model described an impact on the fauna at the same time as an invader invades, with the impact becoming greater as the population of the invader increases, but then lessening as the population of the invader crashes. Evidence for this model could come from a lower biological quality in the period during the invasion than in the periods before and after the invasion. This model is based on evidence from invasions where the invader has a definite impact on the native fauna, for example *D. villosus* in Europe (Dick *et al.* 2002).

5.1.5 Aims of the chapter

The aim of this chapter is to address three questions:

- did the data from the Kentish Stour Estuary provide evidence for any of the four models?
- are there any trends in the fauna over the course of the entire study period?
- was there any correlation between native benthic invertebrate diversity and
 C. curvispinum abundance?

5.2 Methods

5.2.1 Spatial differences in the fauna and methodological issues

The fauna from sites 10 to 6 was quite distinct from the fauna of sites 5 to 1. Insects and molluscs dominated sites ten to six, and crustaceans and polychaetes dominated sites five to one. *C. curvispinum* was only found in sites ten to six. Also the change from grab to trowel sampling in the March 2000 survey meant that there was no method that had been used continuously throughout the study at sites five to one. In the light of all these factors, it was decided to only analyse changes from sites ten to six.

With the exception of the regression analysis, all the following analysis was carried out on datasets with *C. curvispinum* data removed. This was to minimise the chance of the tautology of examining a dataset for changes during an invasion, with the invader included in the dataset.

Having determined what aspects of the fauna to examine over this period it was necessary to determine the best way to measure these aspects. There are several types of metric for examining changes in fauna and assigning some sort of value to these changes. A full review of methods was carried out.

5.2.2 A review and critique of analytical tools

The four models described in section 5.1.4 all require some sort of measure of change in the invertebrate community of the invaded habitat. Furthermore, these measures should ideally allow a value judgement as to whether the 'health' or 'biological quality' of the habitat had changed. Five approaches for measuring change in a biotic community were considered for use in this study. These approaches included diversity indices, taxonomic distinctiveness, biotic indices,

multivariate analyses provided by the PRIMER statistical package, and univariate analysis carried out using SPSS and Minitab.

5.2.2.1 Diversity indices

The term diversity refers to two different phenomena comprising: species richness, which is the number of species in a sample, and relative abundance, which is the differences in abundance of species in the sample (Magurran 1988). The authors of different diversity indices assign varying importance to these two elements, for example, Margalef's index (Margalef 1958, in Peet 1975) emphasises species richness, whereas both Simpson's index (Simpson 1949) and Shannon-Weiner's index (Shannon 1948, in Zar 1999) virtually ignore species richness, and put more emphasis on the evenness of samples. As a curious by-product of this approach different indices can be used to examine changes in different parts of the fauna. An index that examines just species richness confers the same importance to rare fauna as it does to abundant fauna, so examines change within the entire community. An index that examines only species evenness tends to emphasise the dominant species in a sample. Simpson's index, for instance, almost entirely disregards rare taxa and so, by default, can be used to track changes in the dominant faunal elements. One caveat is that none of these indices 'track' individual taxa. If a taxon completely disappeared between one sample and the next, but was replaced by the same number of a taxon novel to the sample site, then this change would not be picked up by either evenness or species richness based indices. A diversity index broadly follows the premise that a loss of diversity in a community can indicate the presence of some sort of impact, and that an increase in diversity can herald an 'improvement' to that community.

The main criticism of diversity indices is that although they can illustrate change, this change often does not show a linear relationship with the underlying impact being

measured. More specifically, an increase in diversity does not necessarily show an improvement in an ecosystem. Thus, in some cases, pollution and disturbance can lead to counter-intuitive changes in diversity. For example, a small amount of organic pollution into a dystrophic stream running through Cwm Idwal in North Wales resulted in an increase in diversity (personal observation, 2006). A community, for instance a coppiced wood, can respond to that disturbance with an increase in diversity. These examples illustrate that there is no definitive linear relationship between diversity and environmental impact.

A way to make diversity relevant to a specific ecological phenomenon is to observe the effect of that phenomenon on diversity in different communities. Regarding the effect that diversity of the native fauna has on invasibility of a community, Elton (1958) suggested that diverse communities could resist invasion better then simple communities. Some studies seem to show that a diverse native fauna will resist invasion, usually due to the fact that more niche space is taken up by animals already present (Case 1991, Shea and Chesson 2002). Conversely Moyle and Light (1996a), and Burke and Grime (1996) find either no link, or in fact a positive correlation between the native diversity and habitat invasibility. Here, the arguments are that physico-chemical parameters are of more importance to the long term success of an invasive species than biotic resistance.

There is one final note of caution with regard to diversity indices. Diversity indices are used because it is believed that diversity or evenness reveal some fundamental underlying quality of an eco-system. However, some authors maintain that diversity of organisms doesn't really reveal anything about the function of an underlying ecosystem (e.g. Hurlbert 1971). There are anecdotal examples where diversity and species evenness can usefully sum up the health of an ecosystem; for example, on the river Axios in northern Greece, an abbatoir was observed discharging blood and

offal directly into a side channel of the river (personal observation, 2000). The effect of this discharge could be seen in the fauna, which was composed exclusively of large numbers of red chironomid larvae. Upstream of this discharge, the fauna showed much greater diversity, both in terms of species richness and relative abundance. In less obvious examples, however, it can be difficult to link diversity to ecosystem function. Hurlbert (1971) examined Shannon and Weavers index (H') and stated that a species importance in the community was not necessarily reflected by its H' value. An illustrative example is provided by the leaf-litter dwelling, land amphipod *Arcitalitrus dorrieni*, which despite invading woods around Plymouth in huge numbers, seems to have had no effect on the native leaf litter fauna (Smithers 2003, personal communication). In this case, an evenness index could show a decline in the leaf litter community, as a numerically dominant species arrived. By contrast, a species richness based index could show a slight improvement, and neither index would lead to any practical enlightenment as to what was actually happening in the wood.

In order to mitigate this problem, it is necessary to use other factors to imply ecosystem function. In the case of *A. dorrieni*, a good way might be to examine the parts of the ecosystem that would be most likely to be affected by it, namely the leaf litter that the amphipod lives in and feeds on, or potential predators that prey on it.

In the case of the Stour dataset, data on the plankton and benthic diatoms, on which *C. curvispinum* feeds, was largely unavailable. Fish data were also not collected, so it was not possible to see if the *C. curvispinum* invasion had affected the upper parts of the food chain. Therefore other methods were needed.

One possible method involves looking at specific portion of the dataset. For example, a specific taxonomic group, such as the crustaceans, could be examined.

Another data sub-set could consist of competitors for the same resource as the invader. In the case of *C. curvispinum* this could be native filter feeders such as *Anodonta sp.*

In some cases positive associations have been found between native diversity and the invasibility of a habitat when a single taxonomic group from the total invertebrate fauna was examined. Stachowitz *et al.* (2002) found a positive correlation between native ascidian (sea squirt) diversity and invasion resistance. In examining only one part of the fauna, they were able to sensibly test, find a result, and suggest an ecological mechanism by which that result might be explained. The mechanism that Stachowitz proposed was as follows: hard substratum on which to settle was the limiting resource, and different native ascidians dominated the substratum spatially at different times of year. Thus, the more native species present, the greater the coverage of hard substratum all year round and the less chance invasive species would be able to settle. Examination of all parts of the fauna might not reveal changes in diversity in such smaller portions of the fauna.

5.2.2.2 Taxonomic distinctiveness

Another possible way to examine change in native fauna is by examining changes in how taxonomically distinctive the taxa in a community are from each other. The examination of changes in the taxonomic level was suggested by Clarke and Warwick (1994) as a way of assessing an impact on an invertebrate community. They stated that impacted communities are made up of taxa that are not only simpler in terms of species but are also more closely related than a community that has not suffered from any adverse impacts (such as pollution, or disturbance). This idea cannot be applied to the Stour biological dataset because of the different taxonomic levels to which the fauna have been identified.

5.2.2.3 Biotic indices

An alternative to diversity indices is provided by biotic indices, such as the Trent Biotic Index (Woodiwiss 1964), or the Biological Monitoring Working Party Score (Chesters 1980). Rather than taking a mathematical theory and seeking to apply it to biological communities, biotic indices utilise the opinions of expert biologists, which are then turned into a score.

An example is the Biological Monitoring Working Party score (BMWP), which was designed for use "in national river pollution surveys" (CIES 2003). Invertebrate taxa are assigned a number between 1 and 10 dependent on whether they are indicative of organic pollution. A high scoring taxon, such as the family Siphlonuridae, which scores 10, is highly sensitive to pollution, and oligochaetes, which as a taxon are pollution tolerant, scores 1. Taxa found in a sample are given a score, then all the scores are summed. A BMWP score sheet from the Environment Agency can be found in Appendix 1. As the score sheet shows, not all taxa are given a BMWP score, and non-scoring taxa play no part in the final calculation. Later in the development of the BMWP, it was decided that sampling effort could be a confounding factor, as the number of taxa could be correlated with the sampling effort. To compensate for sample effort, the BMWP score was divided by the number of taxa in the sample (Hawkes 1997). This calculation was called the Average Score Per Taxon (ASPT), and was used in concert with the BMWP score from 1979 onwards.

One of the problems in producing an index to measure the effect of a specific impact is that a great range of conditions are needed in order to demonstrate a causal link between the various taxa and the impact to be measured. When the BMWP was being developed, biologists from across the United Kingdom were involved in calibrating it (Chesters 1980). It also has to be updated according to local conditions,

or as knowledge improves. The BMWP score itself has been adapted for use in other countries, for instance Spain (Alba-Tercedor and Sanchez-Ortega 1988). The BMWP score in the UK has recently been updated to reflect greater knowledge and the availability of computer aided analysis (Walley and Hawkes 1997). Unfortunately the specificity of biotic indices could also be their downfall, as they are mostly designed to detect pollution, and not other kinds of disturbance. To date, as far as I know, there is no specific 'invasion impact' biotic index, and there would be little point in producing one, since the impacts of different invaders vary widely.

5.2.2.4 Multivariate analysis

All the methods mentioned so far in this chapter condense the data into a single metric, which is then analysed. This leads to a loss of information. Multivariate analysis, however, has the advantage that it doesn't initially attempt to condense the data from one sample into a single number, but instead tries to examine all variables at once. There are many computer based multivariate packages available that contain several different analyses. The only multivariate analyses that were considered here are those found in the Plymouth Laboratories package, (PRIMER). Those tools relevant to this analysis comprise; analysis of similarity (ANOSIM), non-metric multi-dimensional scaling and the species contributions to similarity analysis (SIMPER).

The Analysis of Similarity bears some resemblance to the univariate analysis of variance. However, instead of comparing the variation within a sample to the variation between samples for a single variable, the ANOSIM compares the similarity within two or more groups of samples and compares this to similarity between the groups. The result is expressed as a Global R value of between -1 and 1. A value of 1 shows that the groups are completely different from each other, a value of 0 shows that the samples are completely homogenous. A value of less than one shows that

the relationship between samples in *different* groups is stronger than the relationships between samples in the same group. However, a low R value can still represent a significant difference between groups, if all samples (both within and between groups) are quite similar to each other. To distinguish between approximately similar groups, the ANOSIM programme shuffles the sample labels around to create a distribution of all possible Global R results within the context of data provided. The initial Global R result is then compared with this result and the chance of the result occurring by chance is expressed as a percentage, with the lower the percentage the greater probability that there is a difference between groups. The advantage of this method over a diversity index is that the ANOSIM gives a statistical result, whereas once a diversity index has been calculated, a statistical test still has to be carried out. However, like the diversity indices, an ANOSIM can illustrate a change, but doesn't necessarily highlight what the change is. Therefore ANOSIM needs to be accompanied by other methods to assist with interpreting its results. One such method is non-metric multi-dimensional scaling.

Multi-dimensional scaling examines the similarity of samples, and then plots them on a two dimensional ordination. Individual variables such as the abundance of a single animal can then be superimposed onto this ordination. Additionally, data that were not present in the original ordination, such as season, site and year can also be superimposed and the resulting ordination visually interpreted. In 'squashing' a multidimensional analysis into two dimensions there is some loss of information. This loss of information is summarised as a number called 'stress'. Ordinations with stress levels of above 0.3 should be rejected, and ordinations with stress levels above 0.2 should be treated with scepticism (Clarke and Warwick 1994).

A philosophical problem with multivariate analysis is that it can take the place of logical reasoning. For example, a cluster analysis could split a dataset into a number

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of groups, an ANOSIM could confirm the strength of the relationship and a nonmetric MDS or a SIMPER analysis could reveal several factors that distinguish the groups. The results can then be justified *a posteriori*. This increases the chance of inferring some sort of causality to numbers that could be entirely spurious, and also analysis then becomes led by an analysis method, and not by the researcher. As many researchers (the author included) fail to fully understand all multivariate methods, this is possibly a dangerous direction in which to go. In order to avoid this trap it is necessary to have hypotheses in mind before using a multivariate analysis. The method can then confirm or refute these hypotheses. It is obviously still possible that the results will reflect differences between samples that have been taken rather than actual differences in the population under study.

5.2.3 Summary: Choosing suitable methods of analysis

The above review of methodologies can be summarised with the following conclusions. There is still no completely satisfactory 'off the shelf' measure of the health of a community. Each invader will affect the different components of the fauna to differing degrees. Diversity indices might measure a change, but due to their somewhat abstract nature, it is difficult to infer causality, or in many cases relevance to that change unless the change is of a certain magnitude

Biotic indices can illustrate change in communities, but again they do not necessarily illustrate change that is relevant to an invasion, as invasions are not what they were designed to detect, especially in the lowland part of a river where pollution tolerant taxa are more likely to be found anyway due to the prevailing conditions.

As the biological dataset from the Stour monitoring program has at least the first two taxonomic levels (that of genera and species) removed, and several taxa that are not identified to family level (for example oligochaetes), using a ready-made taxonomic

distinctiveness index might not prove fruitful, although an examination of number of taxa in major taxonomic groups could give clues as to both change in the community before the invasion, and the impact of *C. curvispinum* on the community after the invasion. Also, consideration of how *C. curvispinum* might impact on a community, and formulation of *a priori* hypotheses that could then be tested using the dataset, could provide evidence of its impact on benthic invertebrates in the Stour estuary.

It was decided to use three different measures of diversity in the analysis: number of taxa, Margalef's index and Simpson's index. Changes in the number of taxa can denote an impact in the rare fauna. Margalef's index gives a good indicator of what is happening to the diversity of the fauna in general, but because there is no mathematical 'link' between abundances and taxa, Margalef's index doesn't provide any information about the evenness, or relative abundance of the community. Simpson's index gives a good indicator of the evenness, but species richness is not considered. Between these three metrics, the two aspects of diversity, species richness and relative abundance, were quite comprehensively covered.

These three metrics can be complemented by a multivariate ANOSIM. Severely transforming data before carrying out an ANOSIM will emphasise changes in the rare fauna, and leaving the data in an un-transformed state prior to ANOSIM will emphasise changes in the dominant fauna. Thus, transformation of data prior to multivariate analysis can examine different parts of the fauna in the same way that different diversity indices do (Clarke and Warwick 1994). Accordingly, before the ANOSIM was carried out on data, the latter were transformed in one of three ways: a presence/absence transformation, to emphasise changes in the rare taxa and measure changes in species richness; a log transformation, to emphasise the changes in the taxa overall; and no transformation, in order to emphasise the changes in

dominant fauna. In addition to ANOSIMs, the results were ordinated on a non-metric multi-dimensional scaling plot.

Finally, selected subsets of the dataset were examined in isolation. The two subsets chosen were filter feeders and crustacean taxa. Because of the often low abundance and diversity of these groups when considered separately from the rest of the dataset, it was decided to analyse these data from a descriptive point of view only, because using high powered statistical testing on such flimsy data might lend disproportionate weight to any findings.

5.3 Results

5.3.0. Results: preliminary ANOSIM

A preliminary ANOSIM of the whole dataset showed that there was a significant difference between seasons (Global R = 0.196, P = 0.1%). Therefore, it was decided to analyse surveys carried out in different months separately. A further ANOSIM was carried out on all the net data which revealed a significant difference between sites (Global R = 0.066, P = 0.1%). This meant that it would be preferable to analyse samples separately by site as well as season. Because reducing the number of replicates to such a small number would unacceptably compromise the power of the analysis, ANOSIM was carried out with sites 10 to 6 treated as replicates. The fact that these sites were treated as replicates can be justified on the basis that any variation between sites would increase the chance of a Type I error. The noise created by the low number of replicates overall, and the error inducing method of net sampling would ensure that any changes in community structure would have to be extremely statistically significant in order to produce a significant ANOSIM result.

5.3.1 Results: Changes in multivariate aspects of the fauna at sites between June 1996 and June 1998

Change in the fauna before 1998 were examined mainly to assess whether there was any evidence of a change in the fauna before the invasion. An ANOSIM analysis was used to see if there was any statistically significant change in the fauna and was then followed by an examination of trend lines for evidence of whether any of these changes denoted a rise or fall in the biological quality of the invertebrate community in the Stour Estuary.

A multidimensional scaling plot of the net samples taken from sites 10 to 6 seemed to show a slight but unconvincing clustering of data into years (Figure 5.3.1.1).



Figure 5.3.1.1. MDS plot of net samples for the three years prior to the invasion of the Stour Estuary by *C. curvispinum*

A series of one way ANOSIM tests, split into season, showed that in all but three cases there was a significant difference between fauna from 1997 net samples and fauna from 1998 net samples (Table 5.3.1.2). The three transformations allowed more detailed scrutiny of different aspects of the fauna to be carried out. For March samples, there was a significant difference overall; the most significant differences

were in taxon richness (presence absence transform), and also in the non-dominant fauna (Ln transformation). Conversely, in June samples, the only significant differences between the two years occurred in the dominant fauna. In September samples, there was no significant difference in the numerically dominant fauna but significant differences in both non-dominant fauna and taxon richness.

 Table 5.3.1.2.
 Results of ANOSIM 1997 against 1998 Net samples

N.B. ** - significant at the 1% level, * - significant at the 5% level,

Transformation	March	June	September
Pres/abs	**	NS	*
Ln	**	NS	*
No transform	*	*	NS

NS – not significant at the 5% level.

An MDS of grab samples appeared to show little distinction of samples from different years (Figure 5.3.1.3). ANOSIM analysis confirmed that there was no significant difference between the years for any component of the fauna during any season (Table 5.3.1.4).



Figure 5.3.1.3. MDS plot of grab samples the three years prior to the invasion of the Stour Estuary by *C. curvispinum*

Table 5.3.1.4. Results of ANOSIM 1997 against 1998 Grab samples N.B. ** - significant at the 1% level, * - significant at the 5% level, NS – not significant at the 5% level.

Transformation	March	June	September
Pres/abs	NS	NS	NS
Ln	NS	NS	NS
No transform	NS	NS	NS

The main differences between the univariate and equivalent multivariate methods was that the multivariate methods tracked changes in individual taxa, whereas the univariate methods did not. Also the univariate methods could imply a direction to any change, which the multivariate analyses could not. Therefore the results were examined in more detail using appropriate univariate metrics.

5.3.2 Results: Changes in univariate aspects of the fauna at sites between June 1996 and June 1998, prior to the C. curvispinum invasion

Three univariate methods were used to complement the results of the MDS and ANOSIM procedures. Number of taxa was examined, as this metric was felt to be equivalent to an ANOSIM with data presence/absence transformed. Margalef's index was used to examine the results in an equivalent way to the Ln transformed ANOSIMs. Simpson's index was used to assess changes in the dominant fauna, paralleling the ANOSIMs carried out on untransformed data.

Number of taxa

The ANOSIM results had suggested a change in the fauna taken from net samples between 1997 and 1998 for March and September samples. The number of taxa in grab samples appeared to stay relatively constant in the period leading up to

September 1998. Trend lines seemed to show no particularly striking overall upward or downward trend (Figure 5.3.2.1).



Figure 5.3.2.1. Number of taxa in net samples June 1996-June 1998

There was a slight downward trend in number of taxa in grab samples at most sites, with the exception of site eight, where a small overall rise was recorded (Figure 5.3.2.2). The shallow angle of all the trend lines corroborated the findings of the ANOSIM analysis, i.e. that there were no significant changes in numbers of families in grab samples in the period preceding September 1998.



Figure 5.3.2.2. Number of taxa in Grab samples June 1996-June 1998

Margalef's index

The ANOSIM performed on natural log-transformed results found a difference between 1997 and 1998 for both March and September samples. There appeared to be an overall drop in Margalef's index at sites ten, nine, and seven. A slight rise was recorded at sites eight and six.



Figure 5.3.2.3. Margalef's index in net samples from sites 10-6 from June 1996-June 1998

With the exception of site eight, there was a drop in Margalef's index in grab samples at all sites between June 1996 and June 1998 (Figure 5.3.2.4). Site ten consistently attained a higher Margalef's score then the other sites.



Figure 5.3.2.4. Margalef's index in grab samples at sites 10-6 from June 1996-June 1998

Simpson's index

There was very little overall change in the dominant fauna in net samples, as described by Simpson's index, between June 1996 and June 1998. The only site that did seem to show any trend in Simpson's was site seven, where there was a decreasing trend (Figure 5.3.2.5).



Figure 5.3.2.5. Simpsons index from net samples at sites 10-6 from June 1996-June 1998

The grab samples showed a different pattern to the net samples, in terms of Simpson's index (Figure 5.3.2.6). There was a drop in Simpson's index at sites 10, 9 and 7. There was a rise in Simpson's index at sites 8 and 6.



Figure 5.3.2.6. Simpsons index from grab samples at sites 10-6 from June 1996-June 1998



Figure 5.3.2.7. Abundance of filter feeders in net samples June 1996-June 1998

Filter feeders

There appeared to be a drop both in numbers of taxa and within taxa abundance of filter feeders in net samples between sites 10 and 6 in the two years leading up to September 1998 (Figure 5.3.2.7).



Figure 5.3.2.8. Abundance of filter feeders in grab samples at sites 10-6 from June 1996-June 1998.

There was no particular trend in abundance of filter feeders from grab samples between 1996 and 1998 (Figure 5.3.2.8). The highest abundance for filter feeders occurred in 1997.

Crustacea

The only taxon that was present in net samples through the entire period 1996-1998 was the family Gammaridae (Figure 5.3.2.9). There was no particular linear trend for any crustacean taxa, although there was a greater abundance of gammarids in March and June 1998 than in March and June 1997.



Figure 5.3.2.9. Crustacea in net samples at sites 10-6 from June 1996-June 1998

Gammarids were the only crustacean taxon to be present in grab samples during all surveys between June 1996 and June 1998. There was no obvious discernable trend in either individual taxon abundance or number of crustacean taxa present in each survey. There was evidence of a possible invasion by *Crangonyx pseudo-gracilis* in 1997.

5.4 Results: Overall differences in the fauna before, during and after the invasion

The main aim of this section is to examine evidence for an impact on the native fauna at the time *C. curvispinum* invaded. Therefore, *C. curvispinum* was removed from the dataset prior to the analysis taking place.

For the March 2000 survey, grab sampling was superseded by trowel sampling. Therefore, the following analysis was carried out on net samples only. Also in the period after the invasion, it was sometimes impossible on occasion to take net samples at site six. Therefore, the following analyses were only carried out on sites ten to seven inclusive. Table 5.4.1.1. ANOSIM of differences between the time periods Before and After theinvasion. NS – not significant, * - significant at the 5% level of probability, ** - significantat the 1% level of probability

Transformation	March	June	September
Pres/abs	**	**	**
Ln	**	**	**
No transform	**	*	**

The two time periods Before and After the invasion were compared (Table 5.4.1.1.). For these two time periods there were significant differences in the fauna at all three levels of transformation for all three months. The un-transformed data from the June survey showed a difference between the periods Before and After the invasion, which was significant at the 1% level of probability. All other results were significant at the 5% level of probability.

There was no significant difference between the time periods Before and During the invasion for September, presence/absence transformed data. For all other seasons and transformations, there was a significant difference between the time periods Before and During the invasion (Table 5.4.1.2.).

Table 5.4.1.2. ANOSIM of differences between the time periods Before and During theinvasion. NS – not significant, * - significant at the 5% level of probability, ** - significantat the 1% level of probability

Transformation	March	June	September
Pres/abs	*	**	NS
Ln	**	**	**
No transform	**	**	**

ANOSIM analysis of the periods During and After the invasion found significant differences at the 5% level of probability for all sample months except March. For March there was no significant difference in fauna for presence/absence transformed data and log transformed data. There was a significant difference at the 1% level of probability for the untransformed data (table 5.4.1.3.).

Table 5.4.1.3. ANOSIM of differences between the time periods During and After theinvasion. NS – not significant, * - significant at the 5% level of probability, ** - significantat the 1% level of probability

Transformation	March	June	September
Pres/abs	NS	**	**
Ln	NS	**	**
No transform	*	**	**

The univariate analysis was carried out as follows: the raw data from the entire study period for net samples from sites ten to seven were converted into Simpson's index, Margalef's index and number of taxa. Each of these three measures was then separated into data from March, June and September. Each month's data were then further broken down by the time periods Before, During and After the invasion. Finally the three time periods, separated by month, were compared using a Kruskal-Wallis ANOVA by ranks.



Figure 5.4.1.4. Boxplot of Simpson's index over the three time periods

The boxplot of all data showed no obvious difference in Simpson's index between the three time periods (Figure 5.4.1.4.). Kruskal-Wallis testing found no significant difference in Simpsons index between the three time periods for March (H= 1.94, P>0.05), June (H = 4.43, P>0.05), or September (H=0.57, P>0.05).

The boxplot of Margalef's index showed a slight drop between the time periods Before and During the invasion, with a slight rise in the period After the invasion (Figure 5.4.1.5.). Kruskal-Wallis tests found that there was no difference in Margalef's index between the three time periods for March (H=0.89, P>0.05), June (H=2.22, P>0.05), or September (H=2.06, P>0.05).



Figure 5.4.1.5. Boxplot of Margalef's index over the three time periods

The boxplot of number of taxa showed a slight decrease in median number of taxa between the periods Before and During the invasion followed by an increase in interquartile range between the time periods During and After the invasion (Figure 5.4.1.6).



Figure 5.4.1.6. Boxplot of number of taxa over the three time periods

Kruskal-Wallis testing showed there was no significant difference in number of taxa between the three time periods for March (H= 0.44, P>0.05), June (H=0.84, P>0.05) or September (H=1.74, P>0.05).

Crustacea

There was an apparent clustering of similarity amongst the crustacean communities in the During and After groups, although not in the Before group (Figure 5.4.1.7.).





Figure 5.4.1.7. Native crustaceans in the river over the three time periods

The most prevalent crustaceans throughout the study period were the gammarids. These occurred in 91% of the surveys. The second most prevalent crustacean taxa were the cladocerans, which appeared in 34% of surveys, followed by the Asellidae, which occurred in 26% of surveys (Figure 5.4.1.8)



Figure 5.4.1.8. Abundance of crustacean taxa at site 10 in net samples from June 1996 to September 2003

Filter feeders

The following taxa were identified from net samples as filter feeders over the entire course of the study: Sphaeridae, Pisididae, Polycentropodidae, Cladocera, and Hydropsychidae. This list differs from that of section 5.3.2.8 because some filter feeders, such as the polycentropodidae, only appeared in the estuary after the invasion, and some filter feeders, for example unionids, were not found in net samples.



Figure 5.4.1.9. Native filter feeders in net samples during the three time periods

This MDS seemed to show no particular demarcation in similarity between the three groups (Figure 5.4.1.9). During the MDS process, many of the samples had to be removed from the data matrix, because the sample contained no filter feeders and the MDS could not be performed on empty columns. These samples, from sites ten to seven, that contained no filter feeders were later quantified: 47% of samples taken before the invasion contained no native filter feeders, 61% of samples taken during the invasion contained no native filter feeders, and 53% of samples taken after the invasion contained no native filter feeders.

The highest abundance of individual filter feeder taxa was during the period after the invasion (Figure 5.4.1.10). There was no notable difference in number of filter feeding taxa between the three time periods.



Figure 5.4.1.10. Change in abundance and occurrence filter feeders other than C. curvispinum over the course of the study.

5.5 Results: Linking C. curvispinum to patterns in the fauna

Spearmans Rank Correlation analysis was carried out between the abundance of *C. curvispinum* and Simpson's index, Margalef's index and number of taxa for the native fauna. This was to test whether there was a localised relationship between *C. curvispinum* abundance and the diversity of the native fauna.

The grab sample correlation analysis was based on 36 observations. In grab samples between September 1998 and March 2000 (within the period During the invasion) there was no significant correlation between *C. curvispinum* abundance and Simpson's index (Rho=0.309, *P*>0.05). There was a significant correlation between *C. curvispinum* abundance and Margalef's index (Rho=0.452, *P*<0.01). There was also a significant correlation between *C. curvispinum* abundance and number of taxa (Rho=0.473, *P*<0.01).

The net sample correlation analysis was carried out on 148 observations. In net samples taken During and After the invasion, there was a significant correlation between *C. curvispinum* abundance and Simpson's index (rho=0.183, P<0.05). There were significant correlations between *C. curvispinum* and Margalef's index (rho=0.397, P>0.01) and between *C. curvispinum* abundance and the number of taxa (rho=0.425, P>0.01).

5.6 Chapter Discussion and Conclusions

5.6.1 A note on the use of 1996 as a baseline

The weather analysis carried out in Chapter Four showed that in terms of weather patterns, 1996 was an unusual year. As 1996 was the first year included in the dataset, then any effect of the weather on the fauna would have been missed, because there were no earlier data on which to judge whether the invertebrate community from 1996 was impacted in any way. This could mean that using 1996 as a base year could mask a possible earlier impact on the fauna. The Ecology Research Group has stored samples from surveys from the period before 1996, and it might be profitable at some point to examine these, to see if there was any change in the fauna.

5.6.2 Evidence for models

Figure 5.1.4.1. illustrated four hypothetical models of change in invertebrate community 'health' over the study period. The results are now considered in the light of these models to see of there was any evidence to substantiate any individual model.

Model 1

This "null" model described a situation where there were fluctuations of some description within the benthic invertebrate community of the estuary, but no particular
differences between each time period, or overall trends over the entire time period. When comparing the three time periods, there are significant changes in multivariate similarity as shown in Tables 5.4.1.1-5.4.1.3. There were however, no significant differences between the three time periods for the three diversity indices, according to Kruskal-Wallis testing (section 5.4.1).

Therefore, it can be concluded that there is evidence to support a model of no significant changes in the invertebrate fauna over the study period. However, the multivariate analysis suggested changes occurred in the structure of the native benthic invertebrate community which were not picked up by the univariate metrics that were used.

Model 2

Model 2 described a situation in which a weakening of the fauna might have heralded or even caused the invasion of the Kentish Stour by *C. curvispinum*. Multivariate analysis of similarity found there were differences in most components parts of the fauna in net samples (Table 5.3.1.2), although there were no significant differences between 1997 and 1998 in any aspect of the fauna in grab samples (Table 5.3.1.4).

The univariate analysis was ambiguous with respect to a change in the fauna. Thus, in net samples there were no pronounced changes in the number of taxa (Figure 5.3.2.1.). There was a fall in Margalef's index over the period preceding the invasion at some sites, but a rise at other sites. At site seven there was a drop in Simpson's index over the period preceding the invasion, but not at any of the other sites.

The results for the grab samples were similar to the net sample results. There were no particular trends in number of taxa at any site. There were downward trends in

Margalef's index at some sites, but upward trends at other sites. There were also downward trends at three sites in Simpson's index, and a rise in two sites.

There did seem to be a reduction in both numbers of species and abundance of filter feeders in net samples in the period Before the invasion (Figure 5.3.2.7). There was no corresponding decline in the filter feeders collected in grab samples, so it would be difficult to argue that filter feeders were affected by some impact in the period before the invasion.

There was no particular increase or decrease in native crustaceans, either in terms of relative abundance or species richness, in the period leading up to the invasion.

When all this evidence is taken into account it must be concluded that there was no evidence of a 'weakening' of the fauna before the invasion of the Stour. This finding would seem to agree with the position of workers such as Moyle and Light (1996b) who also found no link between diversity and invasion resistance.

Model 3

The third model described a situation where there was a continuous trend of improvement in the health of the biotic community over the entire study period. ANOSIM found that in most cases there was a significant difference between the three time periods (Tables 5.4.1.1-5.4.1.3). However, the ANOSIM could not assign a direction to this change. Boxplots of net samples showed no increase from one period to the next for Simpsons index, Margalef's index, or number of taxa (Figures 5.4.1.4-5.4.1.6). Kruskall-Wallis tests, split into season, failed to find any significant differences between the three time periods for Simpson's index, Margalef's index or number of taxa. As discussed in section 5.2.2.1., diversity indices can be insensitive

to small changes in an invertebrate community, therefore it is conceded that this analysis would only have detected a major change in the invertebrate community.

However, it must be concluded that there is no evidence to support the theory of continual improvement in invertebrate community health, expressed as diversity, over the course of the study period.

Model 4

The final model described a situation where there was a definite impact on the native fauna concurrent with the invasion of the estuary by *C. curvispinum*. Multivariate analysis showed differences in the community structure between the three time periods for the majority of seasons and transformations (Tables 5.4.1.1-5.4.1.3).

Examination of the boxplots of the period (Figures 5.4.1.4-5.4.1.7) showed no particular decrease in any of the measures in invertebrate community health between the periods Before and During the invasion. Even when data were split into season there was no significant difference, between periods, for any of the metrics used (section 5.4.1).

Therefore it can be concluded that there was no evidence for an impact on the native fauna concurrent with the invasion. Bachman *et al.* (2001) implicated *C. curvispinum* invasion, in addition to other issues, as one of the factors in the decline of the zebra mussel (*D. polymorpha*) population in the river Meuse. The findings from the Stour imply that *C. curvispinum* requires particular conditions to impact on the native fauna. Alternatively the invasion of the Meuse by *C. curvispinum* could have been a spurious event that was unconnected with the population of *D. polymorpha*. The apparent lack of impact by *C. curvispinum* could also be due to the relatively small population of *C. curvispinum* present in the Stour estuary.

Although there was some evidence to support the 'null' model (model 1). This evidence was limited to a single metric that was not supported by the other metrics that were used. Therefore, on balance, none of the four hypothetical models could be applied to the data.

5.6.3 Relationship between other fauna and C. curvispinum abundance

In all but one case (grabs and Simpson's index) there seemed to be a locally significant positive correlation between *C. curvispinum* and the native fauna, in terms of Simpson's index, Margalef's index, and number of taxa. It is difficult to find a reason for this. It may be the case that *C. curvispinum* modifies its immediate environment in such a way that is beneficial to native fauna, or it may be that micro-habitats that are ideally suited to colonisation by *C. curvispinum* are incidentally sites of high native diversity. The fact that *C. curvispinum* seems to be associated with higher diversity would seem to challenge the theory of greater native diversity necessarily conferring resistance to invasion. These findings are consistent with work by Stohlgren *et al.* (1999) and Palmer and Maurer (1997).

5.6.4 Chapter conclusions

Overall there was little evidence to support any of the four models suggested at the beginning of this chapter. In most cases evidence was contradictory across the supposedly complimentary metrics. There does seem to have been a change in community structure between the three time periods. What this change was, and whether the change represented deterioration or improvement in the invertebrate community, was not revealed by the metrics that were used.

It can therefore be concluded that there is no evidence that the invasion of the Stour Estuary by *C. curvispinum* was influenced by the native fauna. Furthermore, there is no evidence that *C. curvispinum* had a significant impact on the native fauna.

These last two chapters have used the medium of a case study to investigate the act of invasion by *C. curvispinum*. The shortcoming of an investigation of this type is that it is difficult to assess whether a change in the invaded habitat is connected in any way to the invader. Also, with a relatively small dataset it is more likely that spurious statistical relationships will emerge. An example of this sort of problem is the problem of scale touched on in section 2.4.2. A change of physico-chemistry or native fauna that appears statistically significant on the river Stour could be biologically insignificant to *C. curvispinum* as a species.

One way to address these issues is to examine the case study in the context of a wider national dataset, in order to differentiate between genuine ecological relationships and quirks of sampling and analysis emerging from the Stour Estuary Monitoring Programme.

Chapter 6

Changes in the national distribution of *C. curvispinum* 1985-2003

6.1 Overview

Examination of an invasion within a single estuary is useful from a case study point of view. However, in order to see that case study in a wider context, an examination on a greater scale is required. In the case of *C. curvispinum*, a look beyond the Stour invasion to changes in the distribution of *C. curvispinum* nationwide could reveal whether its appearance in the Kentish Stour estuary was part of a more widespread expansion of the species, or just an isolated chance event. Furthermore, if physico-chemical data could also be obtained for the habitats in which this species occurred, then it might be possible to learn more about the niche of the species and how its habitat nationwide compares with its habitat in the Kentish Stour.

6.1.1 Extraction and manipulation of Environment Agency datasets

In order to investigate the distribution of *C. curvispinum* in Britain, two data sets were obtained from the Environment Agency. The first dataset contained all records of *C. curvispinum* from 1985 to 2003, expressed as abundance of animals found. This dataset also included both the name of the site where the *C. curvispinum* was found, and the water body that the site was part of. Henceforth this dataset will be referred to as the national abundance dataset. The second dataset contained physico-chemical data from the thirty sites where *C. curvispinum* was found most frequently. Henceforth this dataset will be called the national environmental dataset.

Although the national abundance dataset contained records of all the *C. curvispinum* found by the Environment Agency, the method by which each of these records was obtained was not provided. Therefore, although the abundance figures have been used in several graphs and analyses, the conclusions drawn from these data must be treated with caution. An additional *caveat* is that up to 2000 the Environment Agency gave priority to some sites but not to others (Humpheryes 2005, Environment Agency, personal communication), therefore finding several records in one site could indicate that either the site was more suitable for *C. curvispinum*, or that the site was visited by EA biologists more often than other sites where *C. curvispinum* was found less often.

The national environmental data set did not exactly match the national abundance dataset. In some cases certain sites and also certain dates did not match up. The national environmental dataset also contained much more data then the national abundance dataset. The national abundance dataset contained 743 data, the national environmental dataset contained 402 889 data.

After the dataset had been obtained, the Environment Agency were contacted to try to quantify aspects of how the data had been collected. The response was as follows (Buffam 2007, personal communication): there is a chance that sampling of waterbodies was more comprehensive in 1990, 1995 and 2000. Furthermore some EA regions regularly record at species level (Anglian, Northern and North-West regions), whereas others generally identify invertebrates to family level, and only go to species level with some 'notable' species. Therefore, it would be wise to treat any conclusions coming from this data as evidence to elicit further study, not as substantial evidence in its own right (Buffam 2007, Personal communication).

6.2 Distribution maps

In addition to variation in within-site abundance and number of water bodies occupied by *C. curvispinum*, there was also variation in the physico-chemical properties of water bodies in which *C. curvispinum* was found. The changes in distribution are summarised by Figures 6.2.1-6.2.19.

In 1985 the principle water body in which *C. curvispinum* was found was the Grand Union Canal. This canal yielded six records of *C. curvispinum* for the year. Second to the Grand Union Canal in 1985 was the Trent and Mersey Canal (Figure 6.2.1). The same pairing occurred for the following two years. In 1988 The Trent and Mersey Canal and the Chesterfield Canal were the principle water bodies occupied by *C. curvispinum* (Figure 6.2.4). In 1991, *C. curvispinum* was found most often in the River Severn. With the exception of 1994, the River Severn remained one of the strongholds of the species until 1999. Over this time it was, at worst, the second most productive waterbody for *C. curvispinum* records.

In 1996, *C. curvispinum* was found for the first time in the Thames (Figure 6.2.12). The following year the species appeared to be in retreat, with the exception of the River Derwent, where *C. curvispinum* was recorded 21 times (Fig. 6.2.13). In 1998, the year when *C. curvispinum* was first discovered in the Kentish Stour, it was found predominantly in the Severn and the Derwent. In 1999, when the species was at its lowest point both in terms of average abundance and distribution, it was found only in the rivers Severn and Thames (Fig. 6.2.16). The subsequent increase in abundance and number of reports in 2000 can mostly be attributed to a burgeoning population in the River Thames. For the final three years the two main waterbodies in which *C. curvispinum* appeared were the Thames and the Derwent.







































6.3 Changes in distribution of C. curvispinum over time

6.3.1 Number of records of C. curvispinum found over time

According to the national abundance dataset, between 1985 and 2003, *C. curvispinum* appeared in EA samples 743 times. These records were spread across 206 sites and 84 distinct waterbodies nationwide. The change in number of records of *C. curvispinum* over time is illustrated in Figure 6.3.1. On a year by year basis, these records appear to show a short steady phase between 1985 and 1989 when there was little increase or decrease in the number of reports. This was followed by two peaks in the number of records, and then a substantial drop in records which then seems to slowly increase again, broadly mirroring the pattern at the start of the dataset, between 1986 and 1989.

C. curvispinum was recorded 20 times in 1985 and 17 times in 1986. The number of times *C. curvispinum* was found nationally then steadily rose, with the exception of a dip in 1991. By 1996, the number of records of the animal had reached a high of 93. After this point the number of records dropped considerably, first to 75 in 1997, then 17 in 1998. The lowest annual figure for the whole dataset came from 1999, where *C. curvispinum* was only recorded seven times nationally. From this point the number of sites tentatively increased again until 2003 when 21 records of *C. curvispinum* were made.



Figure 6.3.1. Number of sites in Great Britain occupied by C. curvispinum 1985-2003

6.3.2 Number of water bodies where C. curvispinum was found over time

Knowledge of the number of water bodies where *C. curvispinum* was found could indicate whether this species was extending its range within a water body, or moving between water bodies. It must be noted that simplistically considering water bodies as discrete units ignores the possibility of connectivity between some water bodies. A good example of connectivity is provided by the Trent and Mersey canal, which is listed by the EA as a separate water body from the River Trent, even though they connect near Shardlow (Canal Junction 2005a). Another example is the Grand Union Canal which connects to the Trent and Mersey Canal and the Thames (Canal Junction 2005b). There is thus a connection between three great rivers, all of which are considered by the EA to be separate water bodies. Nevertheless, the study of the movement of an invasive species across water bodies is still useful in providing a picture of gross movements of that invader over a period of time.

A similar pattern occurred with the number of water bodies as with the number of records. In 1985, *C. curvispinum* was recovered from 9 water bodies. This number dipped the following year, but remained largely stable until 1990 when the number of water bodies occupied by *C. curvispinum* rose to 23. After a dip in 1991, which mirrors the dip in numbers of sites occupied, the number of occupied water bodies rose steadily until 1996. In this year, *C. curvispinum* occurred in 40 water bodies nationally. This peak was followed by a sharp drop in the number of waterbodies occupied. This drop was sharper than the drop in number of records, suggesting that *C. curvispinum* held on better in some waterbodies than in others. The pattern after 1998 shows a steady, low state in the number of waterbodies occupied by *C. curvispinum*.



Figure 6.3.2. Number of waterbodies found to contain C. curvispinum 1985-2003

6.4 Changes in the national abundance of C. curvispinum over time

The changes in national abundance cannot be interpreted as absolute numbers, because there is no detail on how the Environment Agency gathered each sample. However, there are dips and troughs in overall abundance which are worthy of note. Also, it might be possible to look for evidence of certain patterns or trends in the data, such as evidence of life history plasticity. If these patterns are found then it might be possible to investigate them at a future date using a more robust dataset.

Ricciardi *et al.* (2004) observed that most invasions of freshwater ecosystems occur by passive dispersal of propagules, via natural or anthropogenic vectors, from one site to another. Assuming that there was no increase in the role of these vectors, then the following theory could be suggested, and tested empirically. It would seem reasonable to hypothesise that a <u>large</u> population of *C. curvispinum* at a particular source site would be more likely to generate the number of propagules necessary to invade a new site than a source site with a <u>small</u> number of propagules. This hypothesis could be expanded to the national level, and it could be suggested that a maximum within-sample abundance of *C. curvispinum* at sites across the country could be a precursor to an increase in range. Accepting previous *caveats* about sampling methodology, Figure 6.4.1 describes the sum of all *C. curvispinum* found by the EA each year between 1985 and 2003.



Figure 6.4.1. Changes in overall abundance of C. curvispinum 1985-2003

Both the greatest overall abundance (Fig. 6.4.1) and the greatest mean abundance (Fig. 6.4.2) occurred in 1990. This coincides with the first increase in distribution of this animal 195

(Fig. 6.3.1). However, in 1991 the distribution of the *C. curvispinum* dropped (Fig. 6.3.1). This would seem to cast doubt on any idea that an overall increase of abundance nationally within a species is a necessary precursor to that species invading.

An inverse hypothesis could be put forward that a low average within-site abundance could presage a national crash in distribution. At the height of the nationwide distribution of *C. curvispinum* in 1996 (Fig. 6.2.12), the average number of individuals at a site was lower than in previous years (Fig. 6.4.2). Comparison of these figures shows that 1996 was followed by a prolonged drop in the number of reports of *C. curvispinum*. This instance, however, is not part of a larger pattern. In several other years, like 1992, 1993 and 1998, a drop in mean number of *C. curvispinum* (Figure 6.4.2) did not predict a crash in distribution (Figure 6.3.1). Therefore it must be concluded that, for this dataset at least, national mean abundance of *C. curvispinum* is not a good predictor of subsequent changes in national distribution.



Figure 6.4.2. Mean number of C. curvispinum in records 1985-2003

6.5 Within-site changes in abundance nationwide

According to Parker *et al.* (1999), there are several different patterns of invasion. During some invasions, the invasive organism slowly increases in population size until it reaches an equilibrium. For other invasions, the invader explosively increases in abundance initially in a new habitat, then the population crashes (Simberloff 2004). The classic example given by Parker *et al.* (1999) of *both* these life histories is the zebra mussel, *Dreisenna polymorpha*, over sixty years of monitoring in Eastern Europe. In some lakes, *Dreisenna* populations would 'boom' and then 'bust' in a cyclical fashion, whereas in other lakes the population showed a slow, steady increase.

Ten sites nationwide were examined to see if there had been any *in-situ* changes in abundance. None of the sites showed a steady increase of population. However, there did seem to be an indication of a 'boom and bust' invasion at Loftsome Bridge, Barmby Barrage, and Misterton. For each of these sites, one year there would be large numbers of corophilds and in the following years there were fewer (Fig. 6.5.1). It was also possible to see a broadly steady state at Walkeringham and Stourport.



Figure 6.5.1. Within-site abundance of C. curvispinum at ten sites nationwide from 1985 to 2003

6.6 Persistence of C. curvispinum at sites nationwide

From the EA records, it seems that the success of *C. curvispinum* varied considerably from site to site. This was investigated further by examining the total duration (i.e. persistence) of *C. curvispinum* at each site. A complication for some sites was that *C. curvispinum* was not found for several years, but then appeared again. It was decided to ignore such sporadic occurrences and simply use the time between the first and last record of *C. curvispinum* as the measure of persistence. It is entirely possible, and accepted as a possible confounding factor, that records appearing at a site year after year could represent waves of successive invasion rather then a continuous population being present and successfully maintaining itself at a site.

C. curvispinum was found at 204 sites nationwide. At many of these sites, *C. curvispinum* was recorded by the EA only once. At 52% of sites, *C. curvispinum* persisted for less than a year (Figure 6.6.1). At 10% of sites, *C. curvispinum* was found for one year. At 2.4% of sites, *C. curvispinum* persisted for 11 years.



Figure 6.6.1. The number of years that C. curvispinum persisted at various sites

6.7 Changes in nationwide physico-chemistry

6.7.1 Sourcing the national physico-chemical data with relevance to C. curvispinum

In order to further investigate the relationship between *C. curvispinum* and water chemistry, it was necessary to obtain physico-chemical data from sites where *C. curvispinum* had been found, other than the Stour estuary. Accordingly, the EA was sent grid references for *C. curvispinum* records that they had originally provided and asked to provide as much physico-

chemical data as possible, between 1985 and 2003, for the following variables: pH, nitrates, phosphates, BOD₅, suspended solids and suspended organics. The dataset compiled by the EA was particularly wide-ranging. As well as the sites requested, it also contained data from sites close to locations where *C. curvispinum* had been found. For some sites, there were bimonthly records for the entire 18 year period that had been requested. By contrast, some locations provided only a small amount of data, and in many cases this did not coincide in any way with reports of *C. curvispinum*.

6.7.2 Formulating an extraction criteria

Initially these data were sorted by date and time. Dates and locations of *C. curvispinum* reports were then matched. Upon first sorting the data, the number of physico-chemical data that exactly matched both time and location of *C. curvispinum* reports was small. In order to maximise both the size and integrity of the dataset the following compromise was used: physico-chemical data were included, if they were from the same location, and at most three days preceding a record of *C. curvispinum*. The reason for the specific time frame was that coincidentally in many cases, physico-chemical sampling seemed to be carried out three days prior to a *C. curvispinum* record. Physico-chemical results obtained from the right location, but at a later date than *C. curvispinum* records were not included, simply because *C. curvispinum* could not be linked to water chemistry that occurred after they had been removed from the river.

6.7.3 Results of the data extraction process for physico-chemical and C. curvispinum data

Overall, 22 sites provided both physico-chemical and *C. curvispinum* data. As mentioned in section 6.1.1, 402 889 items of data had been provided by the Environment Agency. The variables yielded the following data items once the above extraction criteria were applied; pH – 36 data, phosphate – 24 data, suspended solids - 34 data, $BOD_5 - 6$ data, $DO_2\% - 32$ data, 200
nitrates -2 data. Due to lack of data it was decided not to analyse either national BOD₅ or national nitrates. The remaining dataset was called the national *C. curvispinum* physico-chemistry dataset.

6.7.4 Some physico-chemical parameters under which C. curvispinum is found in Great Britain

C. curvispinum is found in some quite wide ranging physico-chemical conditions in Britain. It occurs in the pH range of between 7.5 and 8.8, (mean - 7.97 S.D - 0.308). It was found in the phosphate range of 0.05 to 6.5 mg/l (mean -1.26 S.D. - 1.49). It occurred in waterbodies with suspended solid loads of between 3 and 173 mg/l (mean, 23.02 S.D. 33.037), although two thirds of suspended solid loads where *C. curvispinum* was found were below 20 mg/l.

Finally *C. curvispinum* was found nationally in waters ranging in dissolved oxygen concentration from 68.8 to 108% saturation (mean 89.46, S.D. 8.97).

6.7.5 Comparisons between local and national datasets

The national results for pH, orthophosphate, suspended solids, and dissolved oxygen that had been provided allowed the physico-chemical results from the Stour estuary to be put into some sort of context. Physico-chemical parameters from the most upstream six sites on the River Stour Before and After the invasion were compared with results from the national dataset using box plots. This comparisons could serve two functions. Firstly, it could address the questions of scale that arose from the Stour data-set as considered in Chapter 2 (section 2.4.6). If, for a given physico-chemical parameter, the range at which it was found nationally at sites occupied by *C. curvispinum* was much greater than the range at which it was found on the Stour at sites occupied by *C. curvispinum* on the Stour would be harder to detect. Secondly, it

might be possible to detect shifts in physico-chemical parameters on the Stour to bring them within the limits at which *C. curvispinum* was found nationally.

6.7.5.1 Orthophosphate

Before the invasion, dissolved phosphate concentrations in the Stour were around the centre of the range of distributions nationally. After the invasion phosphate concentrations were lower on the Stour than before. However, this change occurred within the overall range of phosphate in which *C. curvispinum* has been found nationally.



Figure 6.7.5.1. Orthophosphate Before and After the invasion of the Stour Estuary, compared with Nationwide orthophosphate from locations where *C. curvispinum* is found

Mann-Whitney U tests were carried out on the phosphate data. They found that concentrations of phosphate before the invasion were significantly different from phosphate levels after the invasion (W=3205, P<0.05). Phosphate levels before the invasion were

significantly different from phosphate levels nationally where *C. curvispinum* was found (W=1199.5, P<0.05). Phosphate levels After the invasion were also significantly different from phosphate levels nationally (W = 3810.5, P<0.05).

6.7.5.2 pH

Mann-Whitney U testing found there was no significant difference in pH between the Stour Estuary Before the invasion and After the invasion (W=1616, P>0.05). There was a significant difference in pH between before the invasion and the national dataset (W=1054.5, P<0.05). There was no significant difference in pH between after the invasion and the national dataset (W=3670, P>0.05).



Figure 6.7.5.2. pH Before and After the invasion of the Stour Estuary, compared with Nationwide pH from locations where *C. curvispinum* is found

6.7.5.3 Suspended Solids

Mann-Whitney U tests also showed a significant difference between suspended solid concentrations Before and After the invasion (W=3104.5 P<0.05). Suspended solids Before the invasion was significantly different from suspended solids nationally (W =1653, P<0.05). There was no significant difference between suspended solids After the invasion and suspended solids nationally (W=4249.5, P=0.42).



Figure 6.7.5.3. Suspended solids Before and After the invasion of the Stour Estuary, compared with Nationwide suspended solids from locations where *C. curvispinum* is found

6.7.5.4 Dissolved oxygen



Figure 6.7.5.4. Dissolved oxygen before and after the invasion of the Stour Estuary, compared with Nationwide dissolved oxygen from locations where *C. curvispinum* is found

According to Mann-Whitney U tests there was no significant difference in dissolved oxygen in the Stour before and after the invasion (W= 1493, P= 0.0625). There was a significant difference between the dissolved oxygen results from before the Stour invasion, and the national dissolved oxygen results (W= 1120, P<0.05). There was no significant difference, however, between national dissolved oxygen and dissolved oxygen on the Stour after the invasion (W=2565.5, P=0.202).

6.8 Chapter Summary

There are pronounced uncertainties with the datasets used in this chapter. These uncertainties include; the possibility of different sample effort by individual Environment Agency sampling staff, regional differences in sampling collection or sorting method, and any possible changes in resources or sampling protocol over the period covered by the datasets in this chapter. These problems have effectively been ignored, with the proviso that any conclusions drawn from these data should be made with caution, and should be investigated further, at a later date, with a more robust dataset.

Number of records of C. curvispinum found over time

Within the eighteen year EA dataset it is possible to discern a clear rise in the number of *C. curvispinum* and a distinct crash, in both within-site abundance and distribution. These changes could be a result of some particular change in national environmental conditions, or they could be the result of a cyclical pattern that is not picked up by an eighteen year dataset. It would be interesting to look at the next eighteen years and see if there is some sort of a national boom and bust cycle, or whether the population and distribution spike of the mid nineties was part of some sort of succession that followed the clean-up of the country's river systems.

Number of water bodies where C. curvispinum was found over time

Most of the waterbodies that *C. curvispinum* was reported from were connected via canal systems. Obviously, *C. curvispinum* is capable of being transported between waterbodies without a direct connection, as evidenced by its presence both in Britain as a whole and also more specifically in the Kentish Stour, which is not directly linked to other waterways. It is therefore difficult to conclude whether transportation by boats or passive dispersal was the main agent in the species expansion of range in the mid 1990s.

Changes in the national abundance of C. curvispinum over time

The idea that mean abundance of C. curvispinum at a site could determine invasive potential from that site assumes that within-site population size is the most important determinand of invasive potential. This assumption is perhaps a little naïve. It is probably true that certain sites have greater invasive potential then others, possibly for reasons other then the sheer number of *C. curvispinum* that they contain. For example, sites with moorings would give the species more chance to colonise stationary boats than sites where boat traffic just passes through. Also, there could be fitness factors within sub-populations across the country, and C. curvispinum from one site could be more invasive than other sub-populations. These possibilities invite further investigation in the future, but for the time being are beyond the scope of this study. It is therefore not surprising that increases in national C. curvispinum abundance were a poor predictor of subsequent range expansion. In order to be able to predict invasive potential of a site, both average residence time of boats, as well as volume of boat traffic to other sites would both be useful in constructing a model of invasiveness. This model might be used to give a weighting that could be combined with abundance of C. curvispinum to produce some sort of score for invasive potential from each site. This could then be combined with scores from other sites nationally, and a prediction for the following years distribution of *C. curvispinum* could be made.

Persistence of C. curvispinum at sites nationwide

Although *C. curvispinum* was unsuccessful at persisting for any length of time some sites, there were several sites where it persisted for several years. At one site, Stourport on the River Severn, the Environment Agency recorded *C. curvispinum* from 1989 to 1998. There are also specimens in the British Natural History Museum from this site that were collected in 1962, and a record from the Monks Wood National Biological Records Centre, dated 1979. It is possible then that this site could represent the ideal site for this species in England. This

site could be used as a reference condition when assessing vulnerability to *C. curvispinum* invasion of sites where *C. curvispinum* is not yet found.

It is difficult to draw meaningful conclusions from the sites where *C. curvispinum* was present for less then a year. The failure to successfully invade could be due to the environment being unsuitable, but equally the invader could have been introduced in numbers too few to establish successfully. An experiment to carry out in the future might be to find out how many *C. curvispinum* it would take to establish a viable population. It might be possible to put differing numbers *C. curvispinum* in fine mesh bags into a river, then see how many individuals (and how many of each sex) were needed to form a viable population. This would be likely to change according to the characteristics of the river and so it might be a good idea to carry out this experiment in several rivers. The experiment would have to be carried out in an area where *C. curvispinum* was already established, in order to prevent the risk of spreading this alien species. Also the mesh would have to be fine enough to prevent interaction between the captive and wild populations of *C. curvispinum* in the river.

Distribution maps

Most of the sites where *C. curvispinum* was found were canals. This was consistent with findings on the Stour Estuary, where the largest and most persistent population was found at Plucks Gutter, a place where an abundance of benthic hard substratum was found. Incidentally, Plucks Gutter is also a 'navigation intensive' site. The maps show a clear shift in population centre over the eighteen year period, from the Grand Union Canal in the north in 1985 to the Thames in 2003. This brought populations of *C. curvispinum* closer to the Stour Estuary, and it is possible that this geographical proximity was the sole factor responsible for the invasion of the Stour in 1998. The fact that the national distribution of *C. curvispinum* was in decline at the time of its first record in the Stour is curious, although it is obviously not known for how long *C. curvispinum* was present in the Stour before it was detected.

Comparisons between local and national datasets

The first point to note is that the four determinands that were used in this comparison were chosen because they were all that was available, and not necessarily for their biological relevance to *C. curvispinum*. However, it is possible to suggest that low oxygen levels and high phosphate could be an indicator of eutrophication, high suspended solids could choke the feeding mechanism of a filter feeder like *C. curvispinum*, and most animals have an optimum pH range at which they operate. However this is *a postiori* reasoning.

Before the invasion, suspended solid concentrations on the Stour were much higher than those found nationally in *C. curvispinum* occupied sites, whereas after the invasion the suspended solid concentrations on the Stour closely tallies with national suspended solids concentrations. It is difficult to unravel whether this shows that suspended solid levels in the Stour dropped and made the river more amenable to filter feeding amphipods, or whether the filter feeding amphipods reduced the suspended solids in the river by their own feeding action. The fact that suspended solid levels were dropping steadily prior to the invasion on the Stour (as detailed in Chapter Four, section 4.4.4.9.), suggests that the environment may have been changing before the invasion. Whether this made the estuary a more suitable habitat for *C. curvispinum* would need to be investigated further, possibly by placing *C. curvispinum* in several containers of water with various suspended solid concentrations and measuring stress levels with either a respirometer, or by examining stress hormones.

The recordings for pH in the Stour after the invasion were generally lower than the national pH levels. This could indicate that the Stour pH covers a large portion of the pH range of *C*. *curvispinum*. If this is the case then it would be more likely that a relationship between *C*. *curvispinum* abundance and pH could be successfully investigated, once the initial invasion spike in abundance had subsided.

This chapter has attempted to put the findings of the Stour Monitoring Programme into a national context. This has generated further questions, for example; 'what traits does *C*. *curvispinum* have that have made it a successful invader in recent times?' In order to investigate these questions it is necessary to examine some aspects of *C. curvispinum* experimentally.

Chapter Seven

Experimental work to investigate the *C. curvispinum* population in the Kentish Stour Estuary

7.1 Overview

C. curvispinum is a recent addition to the freshwater fauna. According to Harris and Bayliss (1990) its osmoregulatory mechanisms provide evidence that it is a relatively recent introduction to the freshwater fauna, having at some time in the past been a brackish water organism. It is a rapid breeder, and can quickly come to dominate a habitat (Den Hartog *et al.* 1992). It is therefore possible that *C.curvispinum* could be both a rapidly colonising r-selected organism, *and* eclectic for certain traits, such as saline tolerance. This positive relationship between r-selection, eclectic requirements and global distribution has been suggested both for *Gammarus spp.* (Gaston and Spicer 2001) and *D. polymorpha* (Sykes 2003).

Chapters Four and Five speculated on possible relationships between physicochemical conditions or native fauna and the distribution of *C. curvispinum*. There was no effective way to use these data, gathered in the Stour monitoring programme, to investigate issues such as:

- A) Why did the distribution of *C. curvispinum* not extend below site 6?
- B) What was the likely vector that brought *C. curvispinum* to the Stour Estuary?
- C) Did the pattern of colonisation revealed by the monitoring programme reflect the true progress of the invasion, or was the apparent progress of the invasion a mirage caused by sampling artefacts?

In addition to these three questions there were two further issues, which were difficult to address using archive datasets, such as those used previously in the thesis. The first issue related to the eclectic nature of invaders *per se* and *C. curvispinum* in particular. This issue was inspired by Sykes (2003) who studied *D. polymorpha* populations in Northern Ireland. She found that *D. polymorpha* could adapt its physiological tolerances to local conditions. A question which arose from her thesis was: are successful invaders eclectic in a somatic sense, or does a wide tolerance of a physico-chemical determinand (for example pH) at a national level actually mask local adaptation by species with a high turnover of generations? Therefore, a second aim of the chapter was to investigate the possible factors contributing to niche breadth in *C. curvispinum*.

Without physically seeing how an invader arrived in an estuary, the vector for an invasion is difficult to identify. The literature on *C. curvispinum* variously attribute *C. curvispinum* invasion to a consequence of either connectivity of watersheds due to canalisation (Jadzewski 1980), or activity by boats (Pygott and Douglas 1989). As the Stour is not connected to any other watershed, an obvious invasion route could involve *C. curvispinum* attaching to the hull of a boat, which then travelled to the Stour. The route taken by such a boat, either on a trailer overland or via the sea, was something that could be investigated experimentally. The third aim was therefore to simulate possible vectors of *C. curvispinum* in order to establish the most likely route by which it invaded the Stour.

Three pilot studies were carried out to explore these aims.

7.2 Experiment 1: Colonisation rate of C. curvispinum at Plucks Gutter

The colonisation ability of an aquatic invader is difficult to ascertain in the lab, because it would take a long time to get results, and during this time the environment created in the lab would start to depart in character from the environment of the river. One way around the problems inherent in a lab-based experiment is to introduce a

new substratum into the habitat that has been invaded and assess the rate at which the invader colonises it. I have observed that *C. curvispinum* can colonise a whole range of hard substrata, for example stone, glass, metal and plastic.

The objective of this experiment was therefore twofold: firstly to measure the colonisation rate of *C. curvispinum* when a hard substratum was placed directly adjacent to a dense *C. curvispinum* population. Secondly to investigate the effect of distance on the colonisation ability of *C. curvispinum*.

7.2.1 Method

On the 9th of August 2005, sixteen clay fired bricks, each measuring 10cm x 6 cm x 21cm, were placed in the river at Plucks Gutter. Eight were placed directly under the bridge, where most of the exposed hard substratum for the site was found, and from previous observations it was known that the population of *C. curvispinum* was at its most dense. The bricks were placed in a single line running parallel to the wooden pilings under the bridge. The bricks were placed a metre apart parallel to the river bank. Each brick was attached to the wooden pilings under the bridge using plastic coated wire.

The remaining eight bricks were placed approximately two hundred metres downstream of the bridge, in a part of the river where there was no hard substratum, which was taken to be evidence of a lower density of *C. curvispinum*. The bricks were again placed approximately a metre apart. A wooden stake was hammered into the bank and each brick was attached to the stake using plastic coated wire.

No replicates were taken, as this was intended to be an initial pilot for future work which has yet to be carried out. Plucks Gutter was the only suitable site identified for this experiment, and so any replicates would, by necessity, be pseudo-replicates.

The choice of bricks as the hard substratum was made because: a) they were readily available, and b) bricks removed previously from Plucks Gutter during other sampling were generally found to support large populations of *C. curvispinum*.



Figure 7.2.1.1. Sketch map of Plucks Gutter showing the location of the two colonization sites. ______ = portion of river where bricks were placed



Figure 7.2.1.2. Photograph of Plucks Gutter showing the two experimental sites

After a week, one brick was removed at random from each site. The brick was washed in 70% industrial methylated spirit, and individual *C. curvispinum* that had colonised the brick were removed and counted. This continued for seven weeks. The final two bricks were removed after fourteen weeks had elapsed.

7.2.2 Results

After the first week, thirty *C. curvispinum* had colonised the brick retrieved from under the bridge at Plucks Gutter. However, no corophilds had colonised the brick retrieved from the bank downstream of the bridge. By the third week, when the first *C. curvispinum* was found at the bank site, 66 *C. curvispinum* had colonised the third brick from the site under the bridge. The bricks retrieved from the bank site were colonised by only few *C. curvispinum* (Figure 7.2.2.1). Thus the largest population found at the latter site was three individuals after the brick concerned had been in the water for fourteen weeks. The number of *C. curvispinum* retrieved from bricks retrieved from the bridge site increased steadily, reaching its highest level of 1617 individuals the last brick, which was removed from the river on the fourteenth and last week of the experiment. The results are summarised in Figure 7.2.2.1.



Figure 7.2.2.1. Colonisation of artificial substratum by *C. curvispinum*. Each observation is a separate brick, removed from the river.

7.2.3 Discussion

The choice of bricks as the hard substratum was made because of their ready availability at the lab. An earlier idea was to paint marine ply with the kind of paint found on boats in order to simulate a boat's hull, which seemed like the most likely vector. Weights would be attached to the ply, which would then be sunk in the river and examined at a later date for evidence of colonisation by *C. curvispinum*. This idea was abandoned for logistical reasons, though it would be a useful experiment to carry out in the future.

Given that the bricks placed under the bridge were placed on a substratum which was already supporting a large population of *C. curvispinum*, the migration of individuals onto the novel substrata seemed to be relatively slow. From these results it can be predicted that the vector of *C. curvispinum* would have to be situated for some time in an area where there was a *C. curvispinum* population. For example, if the vector was a boat, then it would have to be moored in an area containing a *C. curvispinum* population for a period of weeks rather than days, in order to pick up a population of *C. curvispinum*. The same might be true in reverse, and a vector might have to remain in a new habitat for some time in order to transfer enough individual *C. curvispinum* into the habitat to initiate a successful invasion.

The results of this experiment, if coupled with a study of how many individuals were needed to form a viable *C. curvispinum* population, could be used to identify possible invasion corridors, and identify sites most at risk from colonisation by *C. curvispinum*.

Based on the results from the bank site, it would seem that *C. curvispinum* is a poor coloniser over a distance of greater then a few metres. This shows that the chance of a vector being colonised by *C. curvispinum* rapidly decreases with distance from a dense population. In a future experiment, it would be useful to put a site closer to the

large population than the distance between the Bank site and the Bridge site, and try to establish whether there was a critical distance over which colonisation of the substratum by *C. curvispinum* was most likely to take place.

There is a distinct possibility that *C. curvispinum* was present in the river for some time before it was discovered by the monitoring programme. Furthermore, the slow colonisation over distance casts doubt on the pattern of colonisation of the upper sites by *C. curvispinum*, as revealed by the data in Chapter 3 (section 3.1.2).

Anecdotal evidence from the river would seem to suggest that hard substratum that is present in the river will eventually be colonised by *C. curvispinum*. For the past four years, whenever hard substratum has been retrieved from the river bed accidentally by the anchor during surveys anywhere between Plucks Gutter and the Haffenden's outfall, it has been encrusted with *C. curvispinum* tubes.

In future experiments, it would be useful to use full replication. It would also be useful to investigate colonisation ability at different times of the year and see if there are seasons when *C. curvispinum* is particularly invasive. Based on the spatial effect of population size seen in this experiment, it would seem to be reasonable to predict that these invasive periods will be during times of high population, e.g. mid-summer to mid-autumn.

If a straight line was drawn though the first seven collection dates at the bridge site, and then extrapolated to week fourteen, it would be much lower than the result obtained by the brick that was actually collected from this site at this time (Figure 7.2.3.1).



Figure 7.2.3.1. Projected and actual colonisation of bricks at the Bridge site

There might be a point between week seven and week fourteen when the trajectory of the line of population increase becomes steeper, or it could be a relatively smooth exponential curve. The only way to investigate this further would be to run the experiment again, but put enough bricks into the river to be able to sample on weeks 8 to 13 in addition to those weeks covered in the initial experiment. Finally a station, or number of stations, between the bridge and bank sites might give an indication of the effective colonisation range of *C. curvispinum*. Such an experiment could even be used to examine interactions between 'islands' of hard substratum.

7.3 Experiment 2: Is C. curvispinum eclectic in its ecological requirements?

7.3.1 Overview

If *C. curvispinum* is not a particularly rapid initial coloniser of hard substratum then perhaps one reason it is such a successful invader is due to a wide tolerance for the environmental conditions found in the lower course of a river.

The true environmental range of *C. curvispinum* in the estuary was unknown. Although the distribution was documented, the bulk of the physico-chemical data available was only from one point in the tide, so the full range of physico-chemical parameters at sites colonised by *C. curvispinum* was not recorded. The role of competition from other species in the river was also unknown.

Preliminary considerations

The primary aim of this experiment was to test the premise that invasive organisms were more eclectic in their requirements then native organisms. A suitable native test organism was needed with which to compare the requirements of *C. curvispinum*. Two other species of the family Corophiidae were also present in the estuary, *C. multisetosum* and *C. volutator*. These species were therefore used in the analysis.

7.3.2 Method

The rationale for this experiment followed that of a study by Finlay and Wilkinson (1963), who measured the yield of different varieties of barley in different growing conditions. They then calculated the mean yield for all varieties in each growing condition and ranked each growing condition according to mean yield. The yields of each different variety were then plotted against the mean yield for all varieties in each growing condition.

This idea was adapted for use as a method to assess how eclectic each corophiid species in the Stour Estuary was when compared to the average Instead of using yield, the metric by which adaptability was measured was mortality. This is illustrated in Figure 7.3.2.1.



Mean mortality for all species (ranked)

Figure 7.3.2.1. Regression lines for mean mortality of all species at a site versus mean mortality of individual species at a site (adapted from Finlay and Wilkinson 1963).

Line a in Figure 7.3.2.1. represents a regression line of mean mortality for all species regressed against itself. This line provides a standard by which individual species regression lines can then be compared.

Line b in Figure 7.3.2.1 represents a regression line for an individual species. Mortality for the species represented by line b is consistently lower than the average mortality for all species (line a). This line would, therefore, represent a species that had a better then average mortality for all conditions than average, or in other words, a species that was more eclectic in its environmental requirements than average. Line c in Figure 7.3.2.1 represents a regression line for an individual species that is more suited to environments that are, on average, less suitable for the majority of the species in the analysis. Mortality in the environments that are more lethal to the 'average' species are less lethal to a species depicted by line c. This species would be a harsh environment specialist. In conditions that are more benign for the group of species under study, a species displaying this sort of regression line would perform below the group average.

Line d in Figure 7.3.2.1 represents a regression line for an individual species that is more suited to conditions typical for a species that specialises in the type of environment typical for the group of species being studied. In habitats where the mean mortality is low, mortality for a species displaying this regression line is particularly low. As conditions become less ideal, a species displaying this kind of regression line suffers more mortality than the all species average.

As a secondary objective, evidence for a competition effect between the three species was also sought by examining how each species survived in conditions normally occupied by the other two species. If, for example, *C. curvispinum* could survive in conditions normally occupied by *C. multisetosum* then this could suggest that *C. curvispinum* wasn't found in habitats occupied by *C. multisetosum* because *C. multisetosum* was the superior competitor.

The reason for including the two native species *C. multisetosum* and *C. volutator* in this experiment was to use them as a 'yardstick' to measure the response of *C. curvispinum*. The obvious difficulty with such a straight comparison is that *C. curvispinum*, *C. multisetosum* and *C. volutator* are all from different habitats with different environmental requirements.

The first step in the experiment was to determine the physico-chemical requirements of *C. volutator* and *C. multisetosum*. The requirements of *C. curvispinum* could then be measured against the response of the other two species: *C. volutator* is an intertidal species, living primarily in the muddy, brackish lower reaches of estuaries. Within the Stour estuary, isolated individuals have been found as far up the Stour estuary as the British Rail Bridge (site eight). However, there is no evidence of it successfully colonising the freshwater, either by direct observation or in literature, and so its requirements could not be specifically described as eclectic.

C. multisetosum is a brackish water specialist. It inhabits waters that range in salinity from 0 to 30 ‰ (Quieroga 1990). Therefore, it might be expected to show the widest environmental range of the three species. The final species is the species under investigation, *C. curvispinum*. It is without doubt a successful invader.

If this success is due to *C. curvispinum* having eclectic environmental requirements, then it would be likely to respond to being placed in a novel estuarine environment in a similar way to *C. multisetosum*. If it is not so eclectic in its environmental requirements, then it might be expected to behave in a similar way to *C. volutator*. A final possibility is that *C. curvispinum* could be even more eclectic in its environmental requirements then *C. multisetosum*.

Experimental design

Three sites on the river were selected to represent different habitats in the Stour estuary. Each of these three sites was occupied by a different species of corophiid. The first site was Richborough Port, which is about two miles from the mouth of the river. The substratum at Richborough Port has a lower clay content than further up the river. This site was occupied by *C. volutator*. The second site was next to Richborough Castle, a Roman fort situated about half a mile downstream of Stour Monitoring site six (Haffendens Sewage Discharge). This site was occupied by

Corophium multisetosum. The third site was Plucks Gutter, which formed Stour monitoring site ten. This site was occupied by *C. curvispinum*.

Sediment was collected from the bed of the river at each site and passed through a sieve with a mesh size of 0.3 mm to exclude macrofauna. Twenty litres of water was also collected from each site, ten litres at high water and ten litres at low water. The water was also passed through a 0.3 mm sieve to remove gross particulates and and pelagic macrofauna. The sediment from Plucks Gutter contained a large amount of gravel, which was retained by the sieve. This gravel was brought back to the laboratory, acid washed and scrubbed in distilled water to remove macro-fauna, then mixed in with the rest of the sediment from Plucks Gutter.

Approximately one hundred adult corophiids were also collected at low water from each site and placed in clean 4 litre square plastic tubs that contained two litres of water from the site. *C. volutator* was collected from Richborough Port, *C. multisetosum* from Richborough Castle and *C. curvispinum* from Plucks Gutter.

At Plucks Gutter, a dredge was used to collect substratum from the river bed. Corophiids were then collected as they emerged from their tubes, which were attached to the substratum. At Richborough Castle the dredge was also employed. The resulting soft substratum was sieved, causing it to separate into lumps about the size of a golf ball. Each lump was then broken by hand, and occasionally would break along the line of a *C. multisetosum* burrow. The occupant of the burrow was then collected using a soft piece of foliage (n.b. in future a paint brush would be a preferable tool). At Richborough Port, sediment was collected from the intertidal zone using a spade, and then sieved. The sediment at this site had a much lower silt content. Thus, after a short period of gentle sieving, only the animals were retained

by the sieve. These were then washed into the collection vessel. The collection vessel was placed into a cool box for the journey back to the lab.

Back at the lab, the corophiids were left in four litre tubs along with two litres of water. The tubs were then aerated using standard aquarium air pumps.

Sediment and water was added to eighteen 2 litre beakers in the following manner (Figure 7.3.2.2). 300 ml of mud from Plucks Gutter was added to each of beakers one, two, seven, eight, thirteen and fourteen. 500ml of water from Plucks Gutter, collected at low water was added to each of beakers two, eight and fourteen. 500ml of water from Plucks Gutter, collected at high water was added to each of beakers one, seven, and thirteen. These beakers will be referred to as the Plucks Gutter beakers.

300 ml of mud from Richborough Castle was added to each of beakers three, four, nine, ten, fifteen and sixteen. 500ml of water from Richborough Castle, collected at low water was added to each of beakers four, ten and sixteen. 500ml of water from Richborough Castle, collected at high water was added to each of beakers three, nine and fifteen. These beakers will be referred to as the Richborough Castle beakers.

300 ml of mud from Richborough Port was added to each of beakers, five, six, eleven, twelve, seventeen and eighteen. 500ml of water from Richborough Port, collected at low water was added to each of beakers six, twelve, and eighteen. 500ml of water from Richborough Port, collected at high water was added to each of beakers five, eleven and seventeen. These beakers will be referred to as the Richborough Port beakers.

The beakers were left overnight to allow the mud to settle. The next morning aeration was set up for each of the beakers, using Whisper 300 aquarium pumps, equipped with air stones. The air lines were fixed to the beakers using gaffer tape, so that the air stones were just below the surface of the water, and did not disturb the mud. The water was aerated for an hour, and then animals were introduced to the beakers.

The three species of corophiid were introduced to the following beakers. Ten *C. curvispinum* were added to each of beakers one, two, three, four, five and six. Ten *Corophium multisetosum* were added to each of beakers seven, eight, nine, ten, eleven and twelve. Seven *C. volutator* were added to each of beakers thirteen, fourteen, fifteen, sixteen, seventeen and eighteen. The complete experimental design is illustrated in Figure 7.3.2.2.

The beakers were checked every two days and any deaths of corophiids were recorded. After ten days the beakers were emptied and the survivors of each beaker were recorded.



Figure 7.3.2.2. Experimental design for the ecological requirements experiment. PGS= Plucks Gutter Sediment, RCS = Richborough Castle Sediment, RPS = Richborough Port Sediment, CC = *C. curvispinum,* CM = *C. multisetosum,* CV = *C. volutator,* PGHW = Plucks Gutter high water, PGLW = Plucks Gutter low water, RCHW = Richborough Castle high water, RCLW = Richborough Castle low water, RPHW = Richborough Port high water, RPLW = Richborough Port low water.

7.3.3 Results

The results were analysed in two ways. Firstly, a correlation was carried out between each of the three species of cumulative mortality, at each site, over the time of the experiment (see Figures 7.3.3.1-7.3.3.3). Secondly a regression graph was plotted to compare the mortality of each species at each site with the mean mortality for all species at each site.

The data for high and low water at each site were pooled and then expressed as a percentage of the population for each species at each site. The results are summarised in Figures 7.3.3.1, 7.3.3.2 and 7.3.3.3.

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In the Plucks Gutter beakers, *C. curvispinum* appeared to respond in a similar way to *C. multisetosum* to conditions over time. 85% of the *C. volutator* in the Plucks Gutter beakers died in the first two days and the remainder did not survive past day six. The remaining two species, *C. curvispinum* and *C. multisetosum* showed a similar response to exposure to the Plucks Gutter environment. At the end of the experiment, however, slightly more *C. multisetosum* then *C. curvispinum* had survived (Figure 7.3.3.1). *C. curvispinum* also experienced the greatest range of mortality over the course of the experiment (55% between day 2 and day 10). *C. multisetosum* lost 50% of the experimental population between day 2 and day 10, and *C. volutator* lost 15% of the experimental population over the course of the study, although with *C. volutator* this result is an artefact of the experiment as the 15% represented all of the animals that survived for the first two days.



Figure 7.3.3.1. Cumulative percentage mortality of three corophiid species exposed to sediment and water from Plucks Gutter

The three species showed a different relationship in the Richborough Castle beakers (Figure 7.3.3.2). Throughout the experiment C. multisetosum suffered the lowest percentage mortality, apart from day 2, when both C. curvispinum and C. multisetosum had yet to suffer any mortality. C. curvispinum showed a similar response curve to C. multisetosum, although apart from day 2, C. curvispinum consistently suffered the higher mortality of the two species. C. volutator demonstrated the greatest stability of the three species at this site over the period of the experiment. After an initial loss of 35% of the experimental C. volutator population in the beakers, there was only a loss of 30% of the population over the remainder of the experiment. In comparison, C. curvispinum showed the greatest range of mortality over the length of the experiment (60%), even though it initially demonstrated the joint lowest mortality of the three species. The middle species in the Richborough Castle beakers was C. multisetosum, with 50% mortality over the study period (between day 2 and day 10). At the end of the experiment, C. volutator had sustained the greatest overall mortality of the three species. None of the species suffered 100% mortality at this site.



Figure 7.3.3.2. Cumulative percentage mortality of three corophiid species exposed to sediment and water from Richborough Castle

Figure 7.3.3.3 shows the results of exposing the three species of corophiids to beakers that imitated conditions at Richborough Port. The species that suffered both the greatest range and the greatest overall mortality in the Richborough Port beakers was *C. multisetosum*, which went from a 0% initial mortality to 95% at day 10. *C. curvispinum* suffered less mortality then *C. volutator*, 30% and 40% respectively, but because of a higher initial mortality in *C. curvispinum*, the species with the highest percentage of survivors was *C. volutator*.



Figure 7.3.3.3. Cumulative percentage mortality of three corophiid species exposed to water and sediment from Richborough Port

As Figure 7.3.3.4 shows, The regression line of mean mortality that *C. curvispinum* showed was the closest, in terms of slope, to the regression line for population mean of all three species. This indicated that *C. curvispinum* was a generalist which did not fare particularly well or particularly badly in any situation. However a particularly eclectic species would have been expected to show the same slope of regression line as the population mean, but with a lower intercept value (Figure 7.3.2.1. line b). *C. multisetosum* produced a regression line similar to that shown by line d in Figure

7.3.3.1. *C. volutator* displayed a regression line that showed it to be a specialist in its environmental requirements (Figure 7.3.3.4).



Figure 7.3.3.4. Regression of mean site mortality for all species with mean site mortality for individual species on the Stour estuary.

7.3.4 Discussion

The results seem to provide some evidence that *C. curvispinum* is eclectic in its environmental requirements. In the Richborough Port beakers, *C. curvispinum* suffered a similar mortality to the native species for that site. Also it consistently showed a closer correlation with the theoretically more eclectic *C. multisetosum*, than with the more conservative *C. volutator*. The results from the Richborough Castle beakers differ from those seen in the rest of the experiment. In these beakers, *C. curvispinum* suffered greater mortality then either of the other species throughout the experiment until day 8. The mean regression (Figure 7.3.3.4) indicated that *C. curvispinum* was the most generalist of the three species, in terms of mortality at the three sites.

Another point to consider is the relevance of the parameters that were covered by this experiment. Each species could have responded to a single limiting factor. For example *C. volutator* could have found the substratum at Plucks Gutter unsuitable, but it might have been able to survive in Plucks Gutter water. Further experimentation is required to answer questions of this nature.

The experiment examined the response of corophilds to a relatively stable system, in terms of fixed values for physico-chemical parameters. Obviously this does not compare with the reality of a tidally fluctuating estuary. According to Attrill (2002), animals exposed to water of a stable salinity will be able to tolerate a greater overall range of salinity than animals exposed to fluctuating stability. This means that the experiment could have suggested a greater tolerance for parameters, like salinity, than would actually be the case in a natural, fluctuating estuary. Conversely, it might be possible for an animal to survive for a short period in a salinity that slightly exceeds its normal range for a short while, but die if it is kept in that salinity for a longer period of time.

Figure 7.3.2.1 was based on the experiment carried out by Finlay and Wilkinson (1963). However, there were, in my experiment, two possibly significant departures from their methodology. Firstly, Finlay and Wilkinson looked at varieties of barley, not different species. It could be possible that the three species were just too different in their environmental requirements to make such comparisons a valid exercise. One way to address this issue would be to use species that are more alike in habitat preference. *C. multisetosum* and *C. curvispinum* are similar in that they are both described as inhabitants of slightly brackish/freshwater. *C. volutator*, on the other hand, is an intertidal species that can survive in saline water. Therefore it might make the experiment more valid if another freshwater/brackish corophiid was used instead of *C. volutator*. *Corophium lacustre* has recently been reported from

the river Medway (Humpheryes 2006 personal communication), and this could be a good species to use. Alternatively the experiment could be run with three or more populations of *C. curvispinum* from different locations, in order to see if there was a particularly eclectic 'variety' of *C. curvispinum*.

Secondly, although Finlay and Wilkinson also used three sites, they used 227 varieties of barley, and repeated the experiment during different seasons. In my experiment there wer only three 'varieties', and it was carried out only once. It might be a good idea to pick three disparate habitats, and then to test mortality of more than three 'varieties' in order to make the analysis more powerful.

Apart from examining whether *C. curvispinum* was eclectic in its environmental requirements, an additional reason for undertaking this experiment was to see if there was any evidence that *C. curvispinum* could survive further down the river than where it is currently found, given the same conditions but with any possibly competing species removed. Some *C. curvispinum* survived in every habitat for the entire ten day study period, which provides evidence that *C. curvispinum* could theoretically survive. However, it is not possible to attribute this success to the absence of other corophilds species without further experimentation.

Mortalities of species in their 'native' habitat could indicate a poor match between the estuarine environment and the laboratory, or insensitive collection of animals in the field. *C. curvispinum* suffered 70% mortality in the Plucks Gutter beakers, *C. multisetosum* suffered 50% mortality in the Richborough Castle beakers, and *C. volutator* suffered 78% mortality in the Richborough Port beakers. This could be a major issue in this experiment. It is possible that given the large percentage mortality of *C. multisetosum* in the Richborough Castle beakers, the conditions of the experiment did not accurately represent the natural conditions in the river next to

Chapter 7 Experimental work to investigate *C. curvispinum* in the Stour Estuary

Richborough Castle. There are certainly areas where the experimental procedures could be improved. An example is the collection of *C. multisetosum* from the Richborough Castle site. Collection took several hours due to the low population density of this species. Effort was made to shade the collection vessel, but it was a hot day. This confounding factor could be avoided in future by carrying a coolbox down to the river. Acclimatising the animals to the water in which they would eventually be put was considered, but discounted, as the time taken could have introduced further stress. There were not enough animals collected to sustain any large losses from the acclimatisation process. In future, it might be a good idea to use more personnel and collect more animals. Full acclimatisation could then be carried out, and survivors of this process could be used. Using larger beakers might also minimise the extent that the environment in the lab microcosm diverged from the environment in the corresponding stretch of river.

The independence of data from the experiment may also have been compromised because each beaker contained a number of corophiids. If any corophiid in one of the beakers died, then the ensuing bacterial build up could impact on the other corophiids in the same beaker. To avoid this complication, it would be better to put each individual corophiid into an individual beaker, and have ten beakers for each of the eighteen sediment/water/corophiid species conditions.

C. curvispinum always built its tubes attached to the glass of the beaker. This was noted as a useful feature of the animal for future experimentation. In the natural environment of Richborough Castle and Richborough Port, hard substratum that remains covered throughout the tidal cycle is rare. The provision of hard substratum, in the form of the beakers themselves may have been a confounding factor, although it is unclear what could be done to ameliorate it.

7.4 Experiment 3: The method by which C. curvispinum may have been introduced into the Stour

7.4.1 Overview

The river Stour is not directly connected to other rivers, nor is it connected by canal to other water bodies. Therefore, *C. curvispinum* is unlikely to have dispersed passively to the Stour estuary. This leaves two obvious avenues of entry into the Stour:

- C. curvispinum could have been carried overland attached to either a boat, or fishing equipment. Plucks Gutter, where C. curvispinum was first found in the estuary, features a slipway for small craft. Boats are also moored at Plucks Gutter, over what is possibly the greatest area of hard substratum in the entire estuary. There are also other slipways which could have been used to launch a boat that was already colonised by C. curvispinum.
- 2. C. curvispinum could have been vectored by sea. A recognised route for a freshwater invader is to be carried through marine conditions via ballast water (Ricciardi and MacIsaac 2000). The Stour is a small estuary and not navigable very far by the sort of boat that would carry ballast water. When the Richborough Power Station was operating, tankers would moor between site 2 and site 3. However, even during this period there would be little reason for a craft of this kind to venture as far up estuary as even the lowest site where *C. curvispinum* is found. Therefore, if a boat did vector *C. curvispinum* through the marine environment, then the most likely occurrence is that *C. curvispinum* would be attached to the boat's hull.

These two possibilities were investigated by simulating them in the laboratory to see which would be the most likely entry route into the Stour.

7.4.2 Method

Approximately 300 *C. curvispinum* were collected from Plucks Gutter using a dredge. A small amount of detritus from above the intertidal zone was also collected. It was reasoned that this detritus would be similar to detritus that was permanently submerged, and as *C. curvispinum* had only ever been found in permanently submerged sites, the experiment was unlikely to be confounded by 'unaccounted for' *C. curvispinum*. 20 litres of water was also collected from Plucks Gutter at low water, and sieved through a 0.3 mm mesh sieve to remove macrofauna.

20 litres of seawater were also collected from a large concrete open air swimming pool at Walpole rocks (TR 37018 71463), near Margate, at low tide.



Figure 7.4.2.1. Outdoor swimming pool at Walpole Rocks, near Margate

Back in the lab, 500ml of water from Plucks Gutter was placed in each of 24 x 2 litre beakers. Approximately 2 grams of detritus was also put into each beaker and the mixture was stirred to break up the detritus. Ten adult *C. curvispinum* were placed in each beaker. The beakers were aerated and the animals were then left overnight to construct tubes. For the duration of the experiment, the beakers were kept in a temperature controlled room at 15°C.

The following day the beakers were split into three groups, each containing eight beakers. Group one was designated the control group, and was left with water and aeration. Group two was designated the 'sea' group. Half the river water was removed and replaced with sea water. An hour later this was repeated, and finally all the water was removed and replaced with sea water. This was to simulate *C. curvispinum* being attached to a boat hull travelling from one estuary to another by sea. The third group was designated the 'land' group. All the water was poured from these beakers. This was to simulate *C. curvispinum* being attached to a solution the sea water. This was poured from these beakers. This was to simulate *C. curvispinum* being attached to a boat hull travelling from one estuary to another by a boat transported overland by trailer.

As soon as the experiment was running, two beakers were taken from each group and live *C. curvispinum* were counted. This was repeated at intervals of 6, 12 and 24 hours.

7.4.3 Results

Exposure to sea water was almost invariably fatal to the *C. curvispinum* involved in the experiment. Exposure to air affected *C. curvispinum* less than both the sea and the control conditions. The results are summarised in Table 7.4.3.1.
<u>A)</u>						
	Mortality of (C. curvispinu	<i>m</i> in eac	h beake	r	
Time (hrs)	Control 1	Control 2	Sea 1	Sea 2	Air 1	Air 2
0	0	3	10	9	1	0
6	4	0	10	10	1	1
12	0	1	10	10	0	1
24	0	2	10	10	0	1

Table 7.4.3.1. The effect of exposure to sea-water and air on mortality of *C. curvispinum* A) mortality (number of individuals), B) percentage mortality from each treatment.

B)			
A	/erage Mo	rtality (%)	
Time (hrs)	Control	Sea	Air
0	15	95	5
6	20	100	10
12	5	100	5
24	10	100	5

A two way ANOVA found a significant difference in Mortality between locations (control, sea and air) (F=150.912, P<0.05). There was no significant difference in Mortality between different times (0, 6, 12, 24 hours) (F=0.353, P=0.7879). There was no significant interaction effect between location and time (F=0.206, P=.09682).

The two way ANOVA was followed by three Fisher's protected least significant difference (PLSD) tests on the control, sea and air groups, which found a significant difference between the control group and the air group (P<0.0001), and between the air and sea groups (P<0.0001), but no significant difference was found between the Control group and the air group (P>0.05) (Table 7.4.3.2).

 Table 7.4.3.2. Summary of PLSD tests for significant differences between Control, Sea and Air groups.

Groups tested	Mean Diff.	Critical Diff.	P-Value
Control/Sea	-8.625	1.297	<0.0001
Control/Air	0.625	1.297	0.3143
Sea/Air	9.250	1.297	<0.0001

7.4.4 Discussion

It would seem that the most likely invasion for *C. curvispinum* into the Stour was overland. Throughout the experiment, there were only five deaths in the 'air' group. There was a possible problem with the experimental design in that even after water has been poured out of a beaker, a small amount always remains in the bottom. This was a possible confounding factor and could be remediated in future by mopping up this water with a tissue. A useful future experiment would be to see how long it took all the *C. curvispinum* left exposed to air to die. This would allow a recommended out of the water 'quarantine' period to be established for boats being transferred from areas with high *C. curvispinum* populations.

The fact that more *C. curvispinum* died in the control group than in the group that was exposed to air suggests that the control conditions may not have adequately reflected natural conditions. Alternatively, this result could simply be an artefact of the small number of individuals in each treatment, and using larger numbers in future experiments might ameliorate this.

Chapter 8

General discussion

8.1 Overview

The aims of the thesis were to investigate how *C. curvispinum* invaded the river Stour, and to investigate the distribution of corophiids in the Stour estuary between 1996 and 2003. These aims were investigated using different datasets to focus on different aspects that could have affected the invasion. Chapters Three to Seven investigated the following aspects in the context of the invasion of the Stour:

- Chapter Three chronicled the distribution of corophilds in the Stour between 1996 and 2003
- Chapter Four investigated the physico-chemical milieu of the Stour over the study period
- Chapter Five examined the native fauna in the sites where C. curvispinum invaded
- Chapter Six considered the Stour invasion in the context of changes in the national distribution of *C. curvispinum*
- Chapter Seven investigated several properties displayed by the *C. curvispinum* population in the Kentish Stour estuary.

Several constraints with each dataset, described within each Chapter, meant that it was difficult to provide definitive answers to most of the questions posed in these chapters. The following points have been noted from each Chapter

8.2 A review of the main conclusions from each chapter

8.2.1 Chapter 1- Literature review

According to the literature available, *C. curvispinum* seemed to preferentially colonise hard substratum, was a recent addition to freshwater ecosystems, and often occurred concurrently with the Ponto-Caspian bivalve *D. polymorpha*. There was also evidence presented in the literature that *C. curvispinum* was a euryhaline organism that could tolerate slightly brackish water.

8.2.2 Chapter 2 – Methodology

There were many problems with the data-sets available for analyzing the invasion of the Stour estuary. Overall, it was decided that it would be dangerous to lend too much weight to any conclusions reached from analysis of the Stour estuary datasets. Any conclusions reached through analysis of this data would need to be confirmed by other sources, either in the form of data from other sources, or in the relevant literature.

8.2.3 Chapter 3 – Invasion

Identification of corophilds to the level of species revealed that the maximum range of *C. curvispinum* encompassed sites 10 to 6. Below this point any corophilds were either *C. multisetosum* or *C. volutator*. A separate one-off survey between sites six and five found only *C. multisetosum*.

There was evidence of a 'boom and bust' pattern to the invasion – the abundance of *C. curvispinum* in samples, and its appearance in samples, were both highest in the two years following the invasion. Abundance then seemed to become relatively steady. This pattern seemed to suggest that there were three time periods with respect to the invasion. A period before the invasion, a period during the invasion, with a high abundance of *C. curvispinum*, and a period after the invasion when numbers of *C. curvispinum* in samples maintained a low steady state. The fact that *C. curvispinum* was only found above the limit of saline penetration suggested that it could only establish a viable population in at most slightly brackish water.

8.2.4 Chapter 4 - Physico-chemistry

Weather data showed that 1996 was an unusual year compared 1995 or 1997-2000.

There were several indicators of a rise in water quality over the entire study period. However, none of the physico-chemical parameters displayed a statistically significant relationship with *C. curvispinum* abundance in the estuary.

No physico-chemical reason was found either for the initial invasion, the population spike, or the crash in population.

8.2.5 Chapter 5 – Native fauna

There was no conclusive evidence of a weakening of the native fauna before the invasion. Any evidence that there was, for example, the reduction in the abundance of filter feeders in net samples, was flimsy, and was not corroborated by similar evidence, for example, abundance of filter feeders in grab samples.

There was also no evidence of a steady increase in native diversity over the period of the invasion. There was no evidence of an impact in native fauna during the invasion either.

Finally, there did seem to be a local correlation between *C. curvispinum* and diversity of native fauna. The reason for this correlation is not known, although it could indicate that either *C. curvispinum* can modify its local habitat to the benefit of its environment, or that *C. curvispinum* colonises micro-habitats that are incidentally more diverse.

8.2.6 Chapter 6 – Geography

Most of the waterbodies in which *C. curvispinum* was found were canals. In the years preceding the invasion of the Stour estuary, the national distribution of *C. curvispinum* appeared to expand southwards, bringing it closer to the Stour estuary. Both the peak abundance of the *C. curvispinum* nationwide and its greatest distribution were found two years before the invasion of the Stour. The cause of this peak was not known.

In 1997, a year after this peak and a year before the invasion of the Stour estuary, the national population of *C. curvispinum* began to decline. The reasons for this decline are also unknown.

At several locations, *C. curvispinum* appeared, explosively increased its number, and then disappeared from a site, all within a few years. At other sites the species persisted for a number of years. This suggests a certain amount of plasticity in the animals life history.

8.2.7 Chapter 7- Experimental work

C. curvispinum did not seem to colonise available substratum particularly quickly, even when the substratum in question was placed in direct contact with a substantial population of *C. curvispinum*. This could explain why it took over seventy years to expand its range from its first report near Tewksbury, to its introduction into the Thames, and subsequently the south-east of Great Britain. Also, the results of this experiment could indicate that the maps in Chapter 6 (Figures 6.2.1-6.2.18) are an not a reflection of true changes in the national distribution of *C. curvispinum*. Instead they could be an artifact of insufficient replicates being taken at each EA sample site, at least for a species that follows a negative binomial distribution, such as *C. curvispinum*. On balance, it is probable that Figures 6.2.1-6.2.18 are a combination of changes in this thesis, the maps should be viewed with caution.

In order to get a complete understanding of the nature of *C. curvispinum* as an invader, it is important to have an idea of how many *C. curvispinum* would be needed in an ecosystem to form a viable population. This could be investigated experimentally or by examining the genome of the *C. curvispinum* population in the Stour estuary.

The experimental evidence highlights the possibility that *C. curvispinum* was present in the river for some time before it was discovered by the monitoring programme.

C. curvispinum from the Stour estuary were shown to be relatively eclectic in its environmental requirements. Further experimentation is needed to determine whether this applies to *C. curvispinum* as a species, or just the single population that was tested.

The most likely invasion route for *C. curvispinum* was overland attached to the hull of a boat.

8.3 Preparing a Habitat suitability/ likely impact index

In the early 1980s, the United States Fish and Wildlife Service produced a number of environmental models that could be used to assess whether a habitat was suitable for a particular suite of target species (Hays *et al.* 1981). The reason for these indices seems to have been the preservation of habitat occupied by rare or endangered species. However, a habitat suitability index could also be used to identify habitats that are potentially vulnerable to invasion by a particular species. An example of a habitat suitability model is one written for the Red Spotted Newt, *Notophthalmus viridescens viridescens* (Sousa 1985). This document lists the habitat requirements of the Red Spotted Newt, then identifies the most crucial elements that influence whether or not a habitat will prove suitable. The following passage is an attempt to do the same thing for *C. curvispinum*:

General: *C. curvispinum* inhabits tubes attached primarily to hard substratum. In all studies where high densities of *C. curvispinum* has been reported, hard substratum was a feature of the habitat (e.g. Van den Brink *et al.* 2002, Musko *et al.* 1998, Musko 1992, Pygott and Douglas 1989, Sebestyen1937).

Food: *C. curvispinum* is a filter feeder, as such it is a generalist feeder. Although one study (Van den Brink *et al.* 1993), investigated the trophic status of *C. curvispinum*, overall not enough is known about the nutritional needs of *C. curvispinum* to include information about food requirements in an index.

Reproduction: Female *C. curvispinum* protect eggs in a brood pouch. Apart from material to construct a tube, which is made out of an excretion combined with a variety of building materials, such as silt and detritus, there are no special habitat requirements for breeding.

Water: *C. curvispinum* is described as a relatively new addition to the freshwater fauna (Harris and Bayliss 1990). In the Stour estuary it was only found above the limit of saline penetration. Furthermore, reports of *C. curvispinum* from national records suggest that it occurs almost exclusively in inland waters. This would suggest that *C. curvispinum* was best adapted to fresh or at most slightly brackish water and it would not survive in seawater or extremely brackish water for any length of time. The only reference that stated that *C. curvispinum* could be found in marine conditions was an un-referenced sentence in a key to Amphipoda by Lincoln (1979). Experiments showed that *C. curvispinum* could survive for a period of time in brackish water, but it is not known at what exact salinity *C. curvispinum* would be prevented from forming viable populations.

Although a positive correlation has been found between *C. curvispinum* abundance and flow speed, healthy populations of *C. curvispinum* have been reported from lakes. At high flow speeds transfer of *C. curvispinum* from a vector to a new habitat could be affected, but there was no data to support this hypothesis. Therefore, flow speed was discounted as a helpful factor in determining the likelihood of habitat suitability.

Movements: *C. curvispinum* is transported into new water bodies either by passive dispersal from connected water-bodies that already contain populations of *C. curvispinum*, or with the aid of a vector. According to the literature (Pygott and Douglas

1989, Bij de Vaate *et al.* 2002), and experimental evidence, that vector is most likely to be a small boat, transported overland.

From these findings it is difficult to produce a comprehensive set of factors that could provide favorable conditions for *C. curvispinum*. However, the following important factors can be identified as making a waterbody particularly vulnerable to invasion by *C. curvispinum*:

- either a direct connection to a water-body colonized by *C. curvispinum*, or a mooring area for boats that could transport *C. curvispinum* from other waterbodies,
- a proportion of the benthos made up of hard substratum,
- a level of salinity slightly higher than that found in freshwater.

Figure 8.3.1 pictures three metrics that might be used to assess the vulnerability of a particular site to invasion. These are not comprehensive, for example there is no metric for whether or not the site is near a connection to a water body containing *C. curvispinum*. Distance to a water body containing *C. curvispinum* would be pointless in a case like the Stour estuary, which has no direct connection to another inland water body



Figure 8.3.1. Three suggested metrics for assessing the vulnerability of a site to invasion by *C. curvispinum*. HSM= Habitat Suitability Metric

Once each value had been interpolated from each of the three graphs the final value for the vulnerability of the site would be given by the equation:

$HSI = HSI1 \times HSI2 \times HSI3$

Where HSI = The Habitat Suitability Index score for the site

HSM1 = Interpolation from the graph of number of boats arriving overland from

other water-bodies every month (Figure 8.3.1a)

HSM2 = Interpolation from the graph of percentage of the benthos composed of

hard substratum (Figure 8.3.1b)

HSM3 = Interpolation from the graph of water salinity (Figure 8.3.1c)

A final point is that a habitat suitability index has to be field tested and calibrated before it is used. At present the metrics suggested above are merely informed conjecture. An examination of the features of several water-bodies successfully invaded by *C*. *curvispinum* could provide other factors that could be included, such as the flow of the river. Other factors could be removed, for example if all records are confirmed as coming from freshwater then the salinity metric could be removed.

8.4 Controlling C. curvispinum invasions

Evidence from the literature and from this thesis shows that an unmodified river, with little hard substratum present will be relatively un-impacted by an invasion of *C. curvispinum*. In this case it would be unnecessary to attempt any management procedures, apart from minimising the chance of escape into other, more vulnerable water courses. One way to minimise the spread of *C. curvispinum* from one river to another would be to encourage boat owners to travel from one catchment to another by sea, instead of overland via a boat trailer. However, this might not be practical in all cases.

In order to prevent spread of aliens in general it would be wise to encourage boat owners transporting boats overland to wash the hull of their boats in Milton, or some other disinfectant, prior to launching them into a new catchment. There is evidence that slightly elevated salinity is conducive to successful establishment of *C. curvispinum*. Controlling salt pollution into river catchments, such as winter run-off from gritted roads, might help to manage or prevent establishment of *C. curvispinum* populations.

8.5 Overall lessons from the thesis

It would seem that *C. curvispinum* is not an aggressive invader on the lines of other Ponto-Caspian species such as *D. villosus*, or *D. polymorpha*. There is no evidence of it having an impact on the native fauna, either directly, or through modification of habitat in the Stour Estuary. In water bodies where it did apparently modify the habitat significantly, such as the Lower Rhine, the water body was already heavily modified (Den Hartog *et al.* 1992).

Examination of datasets *a priori* cannot answer many questions definitively. At best, they can provide evidence that can then be further tested experimentally. A dataset gathered for general monitoring purposes can give general clues about the state of the habitat sampled, for instance the Stour Monitoring Dataset detected the invasion of the Stour by the Corophiidae. The simple question of 'what species were present in the Stour Estuary' could be answered. However, in order to answer more fundamental questions about the habitat, such as the question of why a particular species invaded the habitat, more specialized directed examination involving both intensive surveying of the habitat (exemplified by the survey between sites 5 and 6 in Chapter Three) and complementary experimental work.

The successful range expansion of *C. curvispinum* seems to be largely dependent on human agency. Di castri (1989) suggested parallels between natural and man-made phenomena. In an estuary unmodified by man, hard substratum would probably be a rare habitat, and *C. curvispinum* would be a relatively rare specialist. Once the lower courses of rivers were modified with hard substratum and flow regulation by man, for example during the process of canalization, they would become ideal habitat for *C. curvispinum*.

Bij de Vaate *et al.* (2002) and Critescu *et al.* (2003), described specific invasion corridors channeling invasions by Ponto-Caspian amphipods through Europe. No such invasion corridors were found in connection with the Stour invasion. The majority of the expansion of the species occurred in Great Britain via a series of interconnected canals. In Europe, the spread of *C. curvispinum* was also associated with canals (Jazdzewski 1980).

C. curvispinum seems to exhibit a number of the traits suggested by Ehrlich (1984), Bij de Vaate *et al.* (2002), and Elton (1958). As a filter feeder, *C. curvispinum* will consume any nutrition it filters from the water column, and so can be regarded as having a wide feeding niche. As shown experimentally, *C. curvispinum* was relatively eclectic in its environmental requirements. Finally, the generation time for *C. curvispinum* is a single year and there can be as many as three cohorts reproducing each year (Rajagopal *et al.* 1999), leading to a rapid turnover of generations.

No evidence was found of the phenomenon of invasion resistance suggested by Elton (1958). Also no impact on the native fauna was found after the invasion had taken place. In this respect the Stour invasion was different to the invasion of the Great Lakes in North American by *D. polymorpha* (Rayl 1999), and more like the low impact invasion of woodland by *A. Dorrieni* (Cowling *et al.* 2003).

This thesis is not exhaustive. Many other factors could affect, or be affected by a *C*. *curvispinum* invasion. The effect of physical factors, such as current speed at the micro-habitat level, availability of suitable food, action of predators, and parasite transmission have not been considered, due to a lack of sufficient data to make a detailed analysis worthwhile.

8.6 Final conclusions

The main indicator of a habitat where *C. curvispinum* will thrive seems to be the presence of hard substratum as a dominant feature of the benthos. The literature, data on *C. curvispinum* in the Stour and experimental evidence all corroborate this. In the lowland, slower moving reaches of rivers, where *C. curvispinum* is found, the only substantial source of hard substratum would come from man-made structures. No evidence was found in the Stour estuary data that could link changes in the physico-chemistry of the estuary to the fortunes of the invading *C. curvispinum* population.

Evidence from Chapter six showed that the suspended solid loads in the estuary after the invasion changed to a concentration that matched suspended solid loads in waterbodies nationwide where *C. curvispinum* was present. This drop could have been caused by an increase of filter feeders in the estuary. The drop in suspended solids could equally have been caused by the *C. curvispinum* population, or a change in suspended solids could have precipitated the crash in the *C. curvispinum* population of the Stour. Whether there was a causal link between *C. curvispinum* and suspended solid concentration was not specifically investigated.

No evidence was found to indicate that there was a weakening of the fauna before the invasion. There was also no evidence that the invasion impacted the fauna of the upper three sites in any way. *C. curvispinum* seems to have occupied a vacant niche in the estuary, in a similar way to the land amphipod *A. dorrieni* in broadleaf woodland.

The invasion of the Stour estuary can probably be attributed to a number of factors, the most likely, based on the evidence examined, being the proximity of the estuary to the next nearest source of *C. curvispinum* and the presence of hard substratum at Plucks

Gutter. It is also a possibility that the Corophiidae in the estuary were responsible for the drop in suspended solids in the Stour estuary.

The slow colonization rate in Britain could be due to the specific set of circumstances required for a successful *C. curvispinum* invasion. From the evidence gathered in this thesis, it would seem that for a successful invasion to take place there must be available hard substratum in the river. Also there must either be a direct connection to a waterbody already colonized by *C. curvispinum*, or a vector. If the invasion was precipitated by a vector such as a boat, it would have to travel overland, rather then by sea, and would have to be left in the waterbody for a period of time, possibly weeks, in order to effect the transfer of *C. curvispinum* from the vector to the invaded habitat.

Therefore in final conclusion, *C. curvispinum* requires a specific set of man-made conditions in order to thrive in the lower estuary. It is not a particularly pernicious invader into natural, physically unmodified habitats. There is no evidence that it has any negative impacts on the native fauna. There is evidence that it can modify the suspended solid load of a waterbody, but this evidence is weak and needs further work.

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Appendix 1: BMWP score sheet

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Appendix 2: authority list for species featured in the thesis

Arcitalitrus dorrieni (Hunt, 1925) Anoplolepis custodiens Smith, 1858 Aphanomyces astacii Schikora Austropotamobius pallipes Lereboullet, 1858 Chelicorophium curvispinum (Sars, 1895) Chenopodium album Linnaeus Corophium lacustre Vanhöffen, 1911 Corophium multisetosum Stock, 1952 Corophium volutator (Pallas, 1766) Dikerogammarus villosus (Sowinsky, 1894) Dreissena polymorpha Pallas, 1771 Dreissena bugensis Andrusov, 1897 Echinogammarus ishnus (Stebbing, 1898) Gammarus fasciatus Say, 1818 Linepithema humile Mayr, 1868 Metrioptera roeseli (Hagenbach, 1822) Mimetes cucullatus (Cuvier, 1817) Musculista senhousia (Benson, 1842) Oryctolagus cuniculus (Linnaeus, 1758) Pacifastacus leniusculus (Dana, 1852) Pheidole capensis (Mayr, 1862) Pinus halepensis (Miller, 1768) Potamopyrgus antipodarum Smith, 1889 Senecio squalidus Linnaeus