Distribution and dispersal of the non-native caprellid amphipod, Caprella mutica Schurin 1935

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Distribution and dispersal of the non-native caprellid amphipod, *Caprella mutica* Schurin 1935

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- The work has been done by the candidate.
- All quotations have been distinguished by quotation marks and the sources of information specifically acknowledged.

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"That's a caprellid," said Dr. Jon Moore. To demonstrate its behavior, he and Mercer Brugler invented the "Caprellid dance," holding up both hands and waving them, while shifting hips from side to side. This made clear to everyone what kind of organism they were looking at.

"Oh yes, the caprellid!"

Log from the NOAA Mountains of the Sea exploration:

May 15 2004

I'm glad I'm not the only one that has resorted to 'dance' to describe the renowned caprellid!

Abstract

The introduction of non-native species is the second most important anthropogenic threat to global biodiversity, with the first being habitat destruction. In the UK, we have been fortunate to date, that the majority of marine non-native species have not caused significant adverse ecological impacts. Consequently, the processes of species introduction to the region have been little studied.

Caprella mutica is a native of the Sea of Japan area, but has subsequently been identified as introduced in several globally distributed locations. This thesis first establishes the current global distribution of *C. mutica* and uses molecular evidence to suggest global introduction pathways. A study of post-establishment (secondary) vectors and the environmental tolerance of *C. mutica* provide information regarding the modes of dispersal and potential future range of this and other non-native species. Information regarding the biology and ecology of *C. mutica* was collected through a study of its seasonal population dynamics.

Globally, *C. mutica* has established populations on all oceanic coastlines in the northern hemisphere and has been found at two sites in New Zealand in the southern hemisphere. A phylogenetic analysis, using the cytochrome c oxidase subunit I gene, indicated that the global distribution of *C. mutica* is the result of multiple introduction pathways with stepping-stone events. A high genetic diversity was found in the native region but the source for the non-native populations could not be identified. Individuals from the east Pacific grouped together and were genetically most divergent, indicating a single introduction of a few individuals to this region. Several haplotypes were shared between populations from east and west Atlantic locations, indicating similar source populations, introduction mechanisms and stepping-stone pathways. Human vectors, primarily shipping and aquaculture activities, are likely to be responsible for the species' global distribution.

The distribution of *C. mutica* on the west coast of Scotland indicates effective secondary dispersal of *C. mutica* from sources of introduction. Phylogenetic analysis indicated that the populations may be the result of multiple introductions to the region, and several vectors may be responsible for the distribution. The most likely vectors are aquaculture and shipping activities and recreational boating. Field experiments confirmed the ability of *C. mutica* to disperse by rafting on drifting algae, with localised dispersal (< 5 km) by free-swimming.

Physiological tolerance determines the fundamental niches of marine species. In laboratory experiments, *C. mutica* were tolerant of a broad range of temperature and salinity conditions,

with 100 % mortality at 30 °C (48 h LT_{50}, 28.3 ± 0.41 °C), and salinities lower than 15 (48 h LC_{50}, 18.7 ± 0.24). Although lethargic at low temperatures (2 °C), no mortality was observed and it is known to survive at temperatures as low as -1.8 °C. It is unlikely that salinity will limit the distribution of *C. mutica* in open coastal waters, however, the species will be excluded from brackish-water environments such as the heads of sea lochs or estuaries. The physiological limits of *C. mutica* are beyond the physical conditions experienced in its native or introduced range, thus are unlikely to be the primary factors limiting its present distribution and future spread.

C. mutica has several traits attributed as being characteristic of successful non-native species, such as an abundant and widespread distribution in the native range, broad physiological tolerances, broad food preferences, short lifespan and generation times and high fecundity and growth rate. The seasonal population dynamics of *C. mutica* were studied at four human-impacted sites on the west coast of Scotland. Populations at two fish farm sites were more successful than those at a recreational marina and disused pontoon. At all sites, *C. mutica* was most abundant during late summer (August-September) with reduced abundance or absence during winter (January-April). The maximum recorded abundance was 319,000 individuals m^{-2}, in August 2004. Females were dominant for most of the year and the year-round presence of juveniles at the fish farms indicates either continuous reproduction or delayed growth of over-wintering juveniles. Fecundity was positively correlated with female size; the maximum number of eggs per female was 363. The relative anthropogenic influences at the four sites played an important role in the population dynamics and characteristics of *C. mutica*. Enhanced food supply, availability of space, and intensity, timing and sequence of disturbance events contributed to the relative success of *C. mutica* at each site.

Species native to cold temperate regions, with similar life-history characteristics to *C. mutica* and exposed to human dispersal mechanisms have the potential to be introduced to the UK. Once established, there are several effective dispersal mechanisms that can rapidly spread species along the coastlines. Given the widespread distribution of *C. mutica* in the UK, eradication is not an option.

Acknowledgements

Although this is destined to be the most widely read part of my thesis, there is no method I can follow and there are no statistics I can use to quantify the significance of the contributions. An unrestricted discussion based on my personal opinion, potentially a scientist's dream, but more analogous to a tricky obstacle at the end of a challenging endurance event. An expression of thanks, often considered as a measure of how much I value your contribution to this work and my life over the past 3 years. But if you are not mentioned personally, it does not mean that your contribution meant any less to me, it is probably a reflection of my level of sanity, the decline of which several of you have commented upon. I am guaranteed to forget someone of utmost importance, so thank you to all those who are not mentioned below, your presence has been much appreciated.

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I liken my fellow students to climbing protection: always to hand, dependable, and give you confidence to carry on when you think you're destined for a fall. Clare, Gareth, Lindsay, Lyndsey, Mark, Romain, Sam and Suzie, cheers guys! Adam, Craig, Des, Jenny and Susan- you'd been there, done that, and sticking with the climbing theme, provided the route description. Karin and Richard who pick up the caprellid baton in the continuation of their own PhDs- cheers for the discussions, the field assistance, the laughs and the opportunity to get into

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Contents

Abstract i

Acknowledgements iii

Contents v

Chapter 1

Introduction

1.1 Introduction 1

Distribution 4

Dispersal 6

Characteristics of non-native species 8

1.2 *Caprella mutica* Schurin 1935 10

1.3 West coast of Scotland study sites 13

1.4 Thesis outline 15

Appendix 1.1 16

Chapter 2

Distribution of *Caprella mutica* on the west coast of Scotland and a review of its global distribution

2.1 Introduction 17

2.2 Methods 19

Statistical analysis 20

2.3 Results 20

West coast of Scotland distribution 20

Global distribution 23

2.4 Discussion 27

Scottish distribution 27

Global distribution 28

Vectors 30

Summary 31

Chapter 3

Introduction pathways of *Caprella mutica* populations throughout the northern hemisphere: a phylogenetic analysis

3.1 Introduction 33

Molecular techniques 36

Phylogenetic analysis 40

3.2 Materials and methods 42

Sample collection and preparation 42

Mitochondrial DNA analysis 42

DNA sequence analysis 44
3.3 Results 45
DNA sequence characteristics 45
Native region (Japan) 46
Phylogenetic comparisons 49
3.4 Discussion 56
Japan as an introduction source 56
Global introduction pathway(s) 57
Introduction(s) to the Atlantic 58
East and west coasts of the Atlantic 60
Populations on the west coast of Scotland 63
Phylogenetic analysis as a tool for invasion biology 64
Summary 65
Appendices 3.1 – 3.5 67

Chapter 4

Secondary dispersal of *Caprella mutica* on the west coast of Scotland

4.1 Introduction 75
4.2 Methods 78
Free-swimming dispersal 78
Driftweed-associated dispersal 79
Recreational boating - associated dispersal 80
Statistical analysis 81
4.3 Results 82
Free-swimming dispersal 82
Driftweed-associated dispersal 84
Recreational boating - associated dispersal 87
4.4 Discussion 88

Chapter 5

Environmental tolerance of *Caprella mutica*: implications for its distribution as an invasive species

5.1 Introduction 93
Statistical analysis 95
5.2 Materials and methods 96
Temperature tolerance 96
Salinity tolerance 97
5.3 Results 97
Temperature tolerance 97
Salinity tolerance 98
5.4 Discussion 100

Chapter 6

Seasonal population dynamics of *Caprella mutica*

6.1 Introduction 103
6.2 Methods 106
Study area 106
Experimental protocol 108
Laboratory procedures 109
Statistical Analysis 109
6.3 Results 113
Environmental data 113
Population structure and dynamics 115
Sex Ratio 119
Fecundity 120
Size frequency distribution 122
6.4 Discussion 126
Population structure and dynamics 126
Sex ratio and reproductive activity 129
Fecundity 130
Size frequency distribution 131
Anthropogenic influence 133
Summary 135
6.5 Appendix 137

Chapter 7

Final overview and discussion

7.1 Overview 139
Distribution 139
Dispersal 142
Caprella mutica as a non-native species 145
7.2 Discussion 148
7.3 Note 149

References 151

Chapter 1

Distribution and dispersal of the non-native caprellid amphipod, *Caprella mutica* Schurin 1935

1.1 Introduction

Common names frequently identify the geographical source of a non-native species: for example, the Japanese seastar, *Asterias amurensis*; European green crab, *Carcinus maenas*; Chinese mitten crab, *Eriocheir sinensis*; American comb jelly, *Mnemiopsis leidyi*; Mediterranean mussel, *Mytilus galloprovincialis*; Asian bivalve, *Corbula amurensis*. A look at publications over the last century will reveal that these geographically restrictive names are no longer justified; these species are all marine members of the World Conservation Union's (IUCN) 100 worst invaders (Lowe et al. 2000) that are no longer limited to the native range that their common names imply.

Invasion biology was founded on three basic concepts identified by Simberloff in his foreword to Charles Elton's reprinted seminal work:

> *"First, over hundreds of millions of years, the plant and animal communities of different continents have come to be very distinct from one another. Second, human trade and travel are rapidly obliterating these distinctions. Third, this process has grave implications for the conservation of diversity."*
>
> *Simberloff (2000) foreword to Elton (1958)*

These 'grave implications' make 'alien invasions' the second most important human threat to biodiversity (the first being habitat destruction, SSC 2000). Effects can range from the displacement of native plants and/ or animals, as a result of competition, to a more direct effect where introduced species prey on wildlife (Barton 2003). While most non-native species cause relatively few effects, some species have caused catastrophic ecological impacts (Nichols et al. 1990, Parker et al. 1999, Simberloff & Schmitz 1999, Mack et al. 2000). Introductions receive the most attention when these impacts cause serious economic or social damage to humans. For example, the zebra mussel, *Dreissena polymorpha*, introduced to the Great Lakes of North America was estimated to have incurred £14.4 million of related costs in 2004 (Connelly et al. 2006); the comb jelly, *Mnemiopsis leidyi*, caused the collapse of anchovy fisheries in the Black Sea, representing a dry weight loss valued at £9 million per

year, this does not include the loss of several thousand jobs and millions in annual incomes of the coastal community (Knowler 2005).

Often, it is only once the non-native species has become established and widespread that the effects are recognised, and huge efforts must be mobilised to have any hope of controlling the invasion. While the introduction of non-native species is generally considered to be negatively correlated with biodiversity (Mann & Harding 2003), evidence at landscape to regional scales suggests the contrary (Bruno et al. 2005). One of the most extreme examples comes from New Zealand plant species, three of which have become extinct while 2069 non-native species have been added to the flora (Sax et al. 2002). The addition of non-native fish has increased the total number of fish species in southwestern U. S. streams (Gido et al. 2004). This trend is also seen in the UK, where there is little evidence that the 539 introduced species recorded by Brown (1986) or the 51 non-native marine species (Eno et al. 1997) have caused any species extinctions (Manchester & Bullock 2000). However, on a global scale, biodiversity can be considered to be decreasing as a result of species introductions, with the associated homogenization of previously distinct biotas. For the species that are being spread through introductions, the chances of extinction of all its populations are reduced and establishment in new habitats could be considered as a form of 'bet-hedging' (McPeek & Holt 1992, Holt 1997).

Several authors have tried to describe the successive phases of an invasion, and factors which determine the success of an organism at each stage. Hastings (1996) described invasions as having two distinct phases: initial establishment of a species at a single spatial location, and the spread of a species in space. Wonham et al. (2000) described three phases that occur before establishment: transport, introduction and dispersal. This latter description allows investigation of why some introductions fail before becoming established. Some species are unable to survive, being unsuited to their new environment while others can be out-competed by native populations (Barton 2003). Eno et al. (1997) gave several examples of non-established introductions recorded within the UK, including: *Callinectes sapidus* (Blue crab), *Homarus americanus* (American lobster) and *Brachynotus sexdentatus* (Mediterranean crab). Many examples of non-established introductions are species introduced for commercial cultivation which rely on populations being maintained through the importation of hatchery seed (Eno et al. 1997).

The most common reasons for the success of non-natives in British waters are favourable physical factors; including a suitable temperature range, with the main recipient areas often experiencing elevated sea-water temperatures in relation to regional or local conditions (Eno et al. 1997). Although, with adaptation to their new environment and climate change bringing

the environment within tolerance limits, species that have failed to establish to date may be able to establish in the future, such as the New Zealand mussel, *Tiostrea lutaria*, first introduced to the UK in 1970 (Richardson et al. 1993). Other factors influencing the success of a species include the availability of vacant niches, the presence of suitable food, the general fitness of the species concerned and a lack of predators, parasites and diseases (Eno et al. 1997, Colautti et al. 2004, Bruno et al. 2005). Establishment does not always result in invasion as a great majority of introduced species do not go on to harm species, habitats or ecosystems, and many may even deliver significant benefits (EC_Report_2_to_CBD 2002).

There are several obstacles to assessing the impacts of successful invaders. Firstly, there are often no pre-impact data on native assemblages (Ross et al. 2003), which means that there are no baseline or control data for comparison. Secondly, introduced species are often well established before they are discovered, and so significant impacts may have already taken place at the time of detection (Ross et al. 2003). Thirdly, introduced species often become established in areas that are subject to a broad spectrum of other anthropogenic stressors (Ruiz et al. 1999), such as in the vicinity of aquaculture facilities, ports and harbours. In these locations it is difficult to isolate the effects of the introduced species from other anthropogenic stressors. In addition, concern is usually with impacts over large spatial and temporal scales, at which manipulative experiments are difficult and normally not practical (Ruiz et al. 1999).

In the UK, we have been fortunate to date that the majority of introduced marine non-native species have not caused significant adverse ecological impacts (Manchester & Bullock 2000). Species which have had negative impacts include the American slipper limpet, *Crepidula fornicata*, and the American oyster drill, *Urosalpinx cinerea*, both considered pests among commercial oyster beds in southern Britain (Eno et al. 1997). The lack of widespread non-native species with severe impacts means, however, that the processes of species introduction to the area have been little studied here. Likely source locations of introduced species, how a species might spread once introduced, and potential limits to this distribution are also unknown. The difficulty of controlling an introduced species after it has become widely established far exceeds the difficulty of eradication soon after introduction (Simberloff 2003, Tsutsui & Suarez 2003). Thus, a primary goal of invasion biology is to construct a predictive framework for the prevention of invasions (Lodge 1993, Rejmànek & Richardson 1996, Vermeij 1996).

Distribution

Returning to Elton's first concept, the distributions of species are not continuous, and understanding the processes involved in this concept has long confounded scientists, including Aristotle, Paley, Agassiz, and de Candolle (Cadotte 2006). A recent review of ecological oceanography set out by suggesting:

> *"…the results of 150 years of study of the distribution of the marine flora and fauna are so meager that they permit us to predict comprehensively the characteristic assemblage of species likely to occur in no region of the ocean."*
>
> *(Longhurst 1998)*

Historically, natural physical, climatic and ecological factors were used to explain distributions (e.g. Wallace 1876). Climatically, the seas can be divided into at least 6 latitudinal regions: the Arctic, cold temperate, warm temperate, tropical, sub-Antarctic and Antarctic (Vermeij 1978). Areas within these regions share a similar climate but the boundaries do not necessarily indicate limits to the distributions of marine organisms. In addition to the environmental conditions, physical barriers can also limit the distributions of organisms. Land masses divide the climatic regions longitudinally, with, for example, the cold temperate North Atlantic and North Pacific separated by North America and Eurasia. Ocean currents, either through differences in physical properties (Gaylord & Gaines 2000) or limitations to spread (Gaston 2003) can also act as physical barriers to marine species.

The niche of a species is governed by the abiotic and biotic limitations to its distribution (Grinnell 1917, Hutchinson 1957). It was suggested over 100 years ago that a new location must be climatically similar to that of a species' native range (Drude 1896). Simply considering the environmental attributes of a region fails to encompass the different biotic restrictions on a species (Mack 1996). With modifications to the original niche definition, for example, consideration of dispersal, competition and niche size (Pulliam 2000), the concept is still used to investigate the potential distribution of a species (e.g., Petersen & Vieglas 2001, e.g., Petersen 2003, Thuiller et al. 2005). Darwin (1872) recognised that widely distributed species had already triumphed over many competitors and, therefore, were more likely to succeed when introduced to a new location. A wide distribution in the native range has been suggested as a correlative of success of a non-native species (Crawley 1987, Daehler & Strong 1993), yet wide-ranging species are not necessarily generalists, or tolerant of a wide range of environmental conditions (Hanski et al. 1993).

While climatic and ecological factors may exclude a species from an area (Ekman 1957), these factors alone cannot explain the similarity or dissimilarity of the inhabitants of various regions (Darwin 1872). Whether through rapid climate change (Fields et al. 1993, Oreskes 2004, Mieszkowska et al. 2005), habitat destruction (Tilman et al. 1997), or transportation of species (Vitousek et al. 1997a), humans are increasingly changing the distributions of species (Weaver & Clements 1938, Vitousek et al. 1997b, Wilcove et al. 1998, Baillie et al. 2004). The understanding of these processes is an essential factor in the study of biogeography while the last process, human transportation of species, is central to the study of non-native species.

Maximum species diversity is found in the tropics (Gaston & Spicer 1998, Sax 2001; although amphipod diversity does not necessarily follow this trend, Barnard 1991). It might be expected, therefore, that this would be the largest source and sink of non-native species. There is some evidence of a correlation between native and non-native species richness (Stohlgren et al. 1999, Espinosa-García et al. 2004), although relatively few non-native species have been successfully introduced to mainland tropical areas (Rejmanek 1996, Sax 2001, Fine 2002). It has been suggested that the geographic range of species generally increases with latitude (Rapoport 1982, Stevens 1989), although the generality of this rule is also debated (e.g. Gaston et al. 1998, Gaston & Chown 1999). There are more species invading low temperate latitudes than higher latitudes and the species invading the lower latitudes acquire smaller geographic ranges (Sax & Gaines 2006). When considering human transportation, developments in colonization and trade are also likely to be important (Gaston 2003, Sax & Gaines 2006). There may be several reasons for a correlation between the human population and number of non-native species in an area, such as, introduction effort, disturbance, or climate matching (Sax & Gaines 2006).

Previous introductions can be used to match recipient and donor regions and to predict future introductions of marine species (Gollasch 2002a). In their synthesis, Cadotte et al. (2006) took a conceptual approach, and understood invasions at different scales using simply the population rate of intrinsic increase (*r*). Why then, are invasions continuing to baffle scientists? “Of course other ecological and evolutionary factors will be important for individual invasions” (Cadotte et al. 2006). Further investigation of the importance of both *r* and other ecological and evolutionary factors is necessary to establish scientifically sound methods and approaches in order to construct a predictive framework for the prevention of invasions (Andersen et al. 2004).

Dispersal

When questioning why no mammals were common to Europe and Australia or South America, Darwin (1872) concluded that the mammals were unable to migrate between the two locations. In contrast, some plants, with their varied means of dispersal, were able to migrate across the wide and broken interspaces. Through the maintenance or expansion of a population, dispersal essentially limits the distribution of an organism. It is a crucial element of population dynamics and, consequently, a central issue in conservation biology (Macdonald & Johnson 2001). While historically, long-distance dispersal by wind, ocean currents, or migrating animals were considered anomalies (Darwin 1872), human transportation is increasingly being recognised as the greatest anomaly to date. This is Elton's second concept, "human travel and trade are rapidly obliterating these distinctions" (Elton 1958). Movements of species in association with human transport may now be far more important than any natural means of dispersal (Hodkinson & Thompson 1997) and the rate at which species are being transported is unprecedented (Mack et al. 2000). The relationship between globalisation and invasion pathways is perhaps the most important human dimension of invasive non-native species (McNeely 2001).

Dispersal of non-native species is often considered on two scales: primary spread, originating from outside a region and secondary spread, in other words subsequent dispersal to other areas within the region (e.g. Geller 1994, Leppäkoski & Olenin 2000). Depending on the area under question, a marine region is typically either the marine territories of a country, a coastline (e.g. the east coast of North America) or a defined water system (e.g. the Baltic Sea). The vectors acting at the two scales are generally different. In marine systems, international shipping (including ballast water and hull fouling) and aquaculture practices are the most important primary vectors (Gollasch 2002b). Other mechanisms of introduction include: biocontrol, ornamental escape, research escape and man-made canals as corridors for dispersal (Ruiz et al. 2000).

Assuming that shipping and aquaculture practices are the most important primary vectors (Gollasch 2002b), it might be expected that the frequency of biological introductions could be predicted using measures of these practices. However, the wide application of these prediction methods has been questioned (Drake & Lodge 2004, Verling et al. 2005). With shipping, for example, using the frequency of marine shipping routes and labelling major shipping terminals as hotspots for global invasions could give an indication of introduction pressure (Figure 1-1, Endresen et al. 2003; Figure 1-2, Drake & Lodge 2004). However, estimates based on shipping intensity underestimated the importance of several areas known to have a high number of marine invaders; for example, San Francisco Bay and the North American

Great Lakes (Cohen & Carlton 1998, Ricciardi 2001). The frequency of dispersal vectors between two regions could also be a means of linking recipient and donor regions. From the monthly shipping density plot (Figure 1-1), there appears to be a global shipping conveyor belt for non-native species, with areas in the southern hemisphere and polar regions less likely to be visited than the northern hemisphere and equatorial regions.

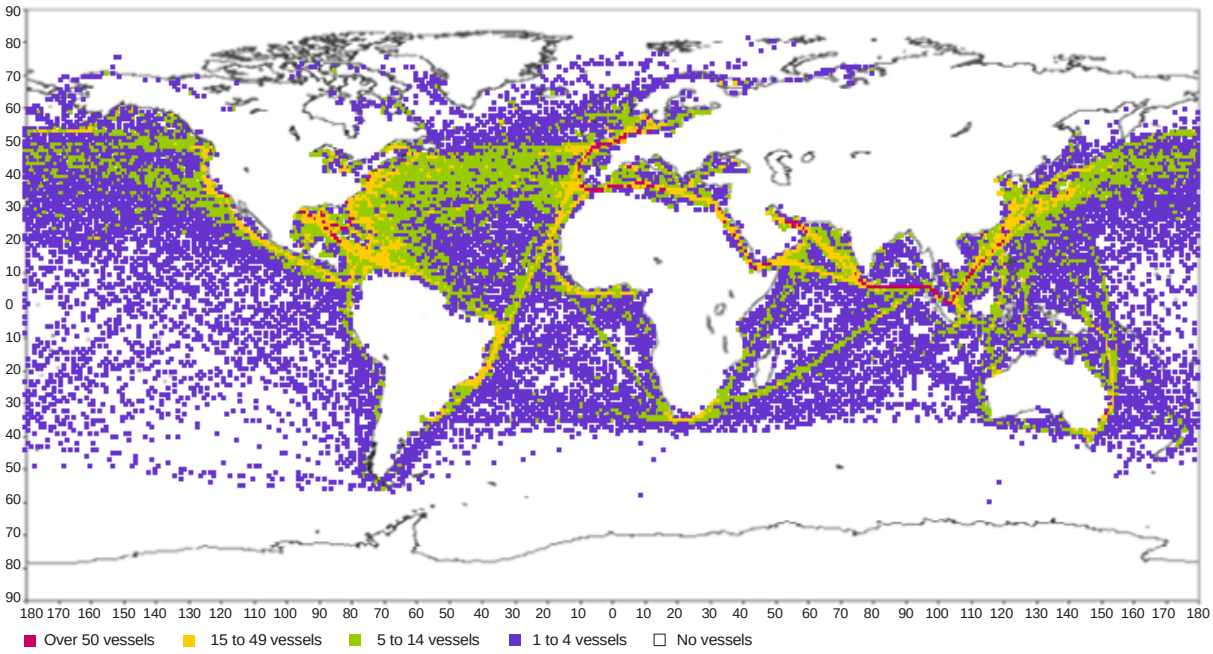


Figure 1-1. Monthly shipping density plot based on traffic density as indicated by AMVER (June 2006) (Automated Mutual-Assistance Vessel Rescue System www.amver.com). Each coloured dot approximates a one-degree cell (60 minutes of latitude by 60 minutes of longitude). Cell fill indicates shipping density.

Figure 1-2. Estimated per-ship probability of causing an invasion (approx. 1/2275) x average number of ship visits per port (taken from Drake & Lodge 2004). Estimated invasion rates range from 0 (blue) to 2.94 $\times 10^{-4}$ (red) species km^{-2} yr^{-1}.

Global aquaculture production is dominated by China, followed in quantity by India, the Philippines, Indonesia, Japan, Vietnam, Thailand and Korea (FAO 2006). These countries should, therefore, have a large number of non-native species. Aquaculture practices contribute to the introduction of both target (e.g. the Portuguese oyster, *Crassostrea gigas* introduced to the UK; Eno et al. 1997) and non-target species (e.g. *Sargassum muticum* introduced to northern France with oyster spat; Eno et al. 1997). In 2004, 65% of the aquaculture production of the Caribbean and Latin America was based on non-native species (FAO 2006). The use of aquaculture production as a predictor of species introductions is hard to assess, as there are relatively few records of non-native species from these predominantly developing countries (*sensu* Yan et al. 2001). The global invasive species database (www.issg.org/database/ welcome) lists 137 invasive species in China, 123 in India, 71 in the Philippines, 87 in Indonesia and 115 in Japan; compared with 331 in the USA and 95 in the UK (these numbers are much less than total numbers of established non-native species).

Secondary spread will ultimately determine the extent of the economic and environmental impact of a non-native species (Lodge et al. 1998). Anthropogenic dispersal mechanisms responsible for primary introduction are also responsible for secondary spread, although over shorter distances natural dispersal mechanisms and recreational boating activity may be more important (Johnson & Carlton 1996, Johnson et al. 2001). Secondary spread can be tracked through the range expansion of an organism or by using genetic or morphological markers (Geller 1994). If a specific vector can be identified for the introduction of a species, likely source regions and future spread can be easier to predict (e.g. Stevens et al. 2002). Frequency of recreational boat transfer from Lake Sinclair, Michigan, USA has been used to predict the spread of the zebra mussel, *Dreissena polymorpha* and other non-native species (Johnson et al. 2001). In many cases, it can be difficult to distinguish between the relative significance of multiple introduction vectors and all plausible mechanisms must be considered.

Characteristics of non-native species

Whether the characteristics of a species makes it more susceptible to human introduction is debatable (Manchester & Bullock 2000). Are there attributes of the distribution or biology of a species which can allow us to predict its dispersal, success in the new habitat and therefore the likely future distribution? The final determinant of non-native success is that the species can successfully “struggle against indigenous plants perfectly adapted to the climate” (Drude 1896 discussing the success of plant introductions, translated in Cadotte 2006). Abiotic and

biotic factors are only limiting because the species has not evolved the morphological, physiological or ecological capacities to overcome them (Gaston 2003).

The Subphylum Crustacea is the most successful for introductions in marine systems, accounting for 28% of reports in marine coastal communities in North America (Ruiz et al. 2000) and 56% of faunal species found in ballast tanks in Europe (Gollasch et al. 2002). Several characteristics of crustaceans may enhance this invasive ability. The small size of many crustaceans, in particular the juvenile stages, and their morphology enhances their ability to survive introduction to new areas. Thus, their protective exoskeleton increases the chance of surviving the stresses of transportation, such as mechanical pressure (Ruiz et al. 2000), and many species tolerate wide ranges in salinity and temperature (MacIsaac et al. 2001). They are easily taken onboard vessels, transported and released (Fofonoff et al. 2003). However, the numerous reports of introduced crustacean species may also be a result of sampling bias as their exoskeletons are preserved, while other organisms may be crushed or distorted beyond identification (Ruiz et al. 2000, Gollasch et al. 2002). Furthermore, the systematics and biogeography of crustaceans are rather better known (Ruiz et al. 2000), thereby enhancing the likelihood of detection of new non-native species. These characteristics also make the Crustacea suitable model species for studying invasion processes.

Several authors have described the characteristics of successful invasive species (Newsome & Noble 1986, Ehrlich 1989, Lodge 1993, Ricciardi & Rasmussen 1998). The following list is based on characteristics listed in the aforementioned papers, and qualities listed for crustacean invaders (Van der Velde et al. 1998):

- Abundant and widely distributed in original range
- Wide environmental tolerance
- High genetic variability
- Short generation time
- Rapid growth
- Early sexual maturity
- High reproductive capacity
- Broad diet (opportunistic feeding)
- Gregariousness
- Possessing natural mechanisms of rapid dispersal
- Commensal with human activity (e.g. ship ballast-water transport).

Human introductions of crustaceans include those made intentionally for aquaculture purposes. These species include, for example, the North American freshwater crayfish, *Pacifasticus leniusculus* (Dana) and *Astacus leptodactylus* (Nordmann) introduced to Britain (Gherardi & Holdich, 1999); and the Pacific red king crab *Paralithodes camschaticus* (Tilesius) introduced to Norway (Jørgensen et al., 2004). Examples of accidental

introductions include the Australasian barnacle *Elminius modestus* Darwin (Crisp 1958), the Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards (Clark et al. 1998) and the Asian shore crab *Hemigrapsus sanguineus* (de Haan) (Galil et al. 2002).

1.2 *Caprella mutica* Schurin 1935

Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Superorder: Peracarida
Order: Amphipoda
Suborder: Caprellidae

Caprellid amphipods are marine crustaceans which inhabit the littoral zone to depths of over 1500m (McCain & Steinberg 1970) and are found on all coastlines globally. They are an important trophic link between unicellular algae and predatory fish in the coastal water ecosystem (Caine 1989, Holbrook & Schmitt 1992). Predators of the Caprellidae include fish (Kvenseth et al. 2003) and invertebrates (e.g. actinarians, *Epiactis* sp. and nudibranchs, *Melibe* sp.) found in the local environment of caprellid populations (Caine 1980). In Britain there are 22 native caprellid species recorded in three families (Hayward & Ryland 2000). This number has increased through the introduction of at least one species, *Caprella mutica* (Figure 1-3).

Caprellids spend their whole life attached to the substratum surface, lacking any planktonic life stage (like all amphipods). Habitat segregation may be due to the degree of wave exposure, or other environmental factors (Hirayama & Kikuchi 1980). Caprellid species appear to be opportunist feeders, consuming organic material which is most readily available at the time (Keith 1969). They implement a number of feeding strategies (browsing, filter-feeding, predation, scavenging, and scraping) and in their natural habitat, food is not considered to be a limiting factor (e.g *C. california* and *C. equilibria*, Caine 1977). Caprellid prey items include nematodes, harpacticoid copepods, amphipods and ostracods (Caine 1980). *C. mutica* has been observed in the laboratory to be omnivorous, grazing on diatoms, scraping the surface of *Ulva lactuca* for epibionts, and scavenging from bodies of dead or dying individuals.

Figure 1-3. *Caprella mutica* male (top, 22.43mm), and female (bottom, 9.30mm) T. Nickell

The common pattern of caprellid reproduction is continuous throughout the year, with peaks in spring and summer (Lewbel 1978, Caine 1979, Takeuchi & Hirano 1992). The duration of the caprellid life-cycle has been estimated at less than 8 months (*C. laeviuscula*, Caine 1979) to around 18 months (Jessen 1969). The caprellid life-cycle appears to be species-specific, *C. penantis* breed all year, whilst *C. septentrionalis* brood annually, with young hatching in June (Geptner 1963). *Pseudoprotella phasma* produce two or more broods in the life-cycle, only reproducing during the spring or summer (Harrison 1940). Breeding activity of *C. equilibria* was found to show peaks in April and September, and a decrease in summer due to both high temperatures (over 20 ºC) and oxygen deficiency; breeding ceased in winter (Sconfietti & Luparia 1995). Female *C. equilibria* produced more than one brood per year. Extended breeding activity in *C. penantis* is noted by Bynum (1978), with a decline in breeding activity in January and February followed by a peak in early spring, and a lesser peak in late summer and early autumn. Fish have been found to selectively predate on females of increasing size and evidence of inter-specific discrimination has also been found (Caine 1979).

C. mutica is a native of the Sea of Japan area, first described in Peter the Great Bay, Vladivostok Russia (Schurin 1935), and subsequently identified in Possjet Bay (Vassilenko 1967) and Akkeshi Bay (Akkeshi High School of Fish. 1968), Japan (Figure 1-4). *C. mutica* has a long history of introductions, having been introduced to the Northeast Pacific before 1979 (Carlton 1979) and discovered in Europe in the early 1990s where Platvoet et al. (1995) recognized it as introduced (although mistakenly identified it as *Caprella macho*). *C. mutica* has also been identified on the western north Atlantic (Cohen et al. 1998), the west coast of

Ireland (Tierney et al. 2004), in Belgium and Norway (ICES 2003). *Caprella mutica* was identified on the west coast of Scotland in 2000 (Willis et al. 2004). Since the publication of Willis et al. (2004), caprellid specimens held in collections of the Scottish Environment Protection Agency have been re-examined, and individuals collected from Roseneath Patch (Clyde Sea, 55º 58.50' N, 4º 47.50' W) in 1999, initially identified as *C. tuberculata*, have been re-determined as *C. mutica* (O'Reilly submitted 2006). For a key to the British Caprellidae see Appendix 1.1 (pg 16).

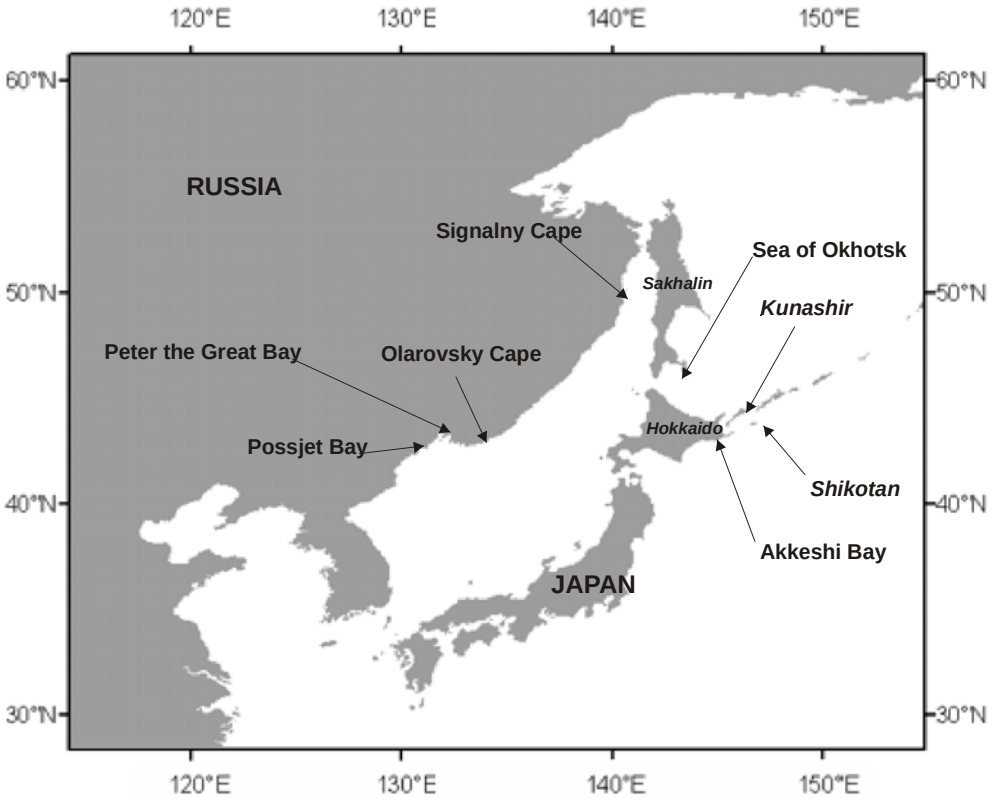


Figure 1-4. Native range of *Caprella mutica*. Labels in capitals refer to countries, those in italics refer to islands, arrows indicate locations where *C. mutica* has been found. See text for references.

In its native habitat, *C. mutica* is found associated with attached macroalgae and drifting seaweeds including *Sargassum* spp. and on aquaculture structures, such as ropes for *Undaria* culture in Otsuchi Bay (Kawashima et al. 1999). In the non-native habitats, *C. mutica* have been identified clinging to a wide range of artificial substrata (aquaculture nets, ropes, pontoons, submerged lines along the shoreline and boat hulls) and on fouling organisms associated with these substrata (including seaweed, hydroids, ascidians, mussels and barnacles). They are often observed in very high densities (> 3,000 m^{-2}, Buschbaum & Gutow 2005), especially during summer months. Most caprellids are not substratum specific, occurring on most suitable substrata (e.g. algae, artificial rope, surface detritus and floating debris; McCain 1968, Laubitz 1970, Laubitz 1972).

The association of *C. mutica* with aquaculture and artificial structures may be responsible for its widespread introduction success. Vectors suggested for the transport of *C. mutica* include association with shipments of Japanese oysters, in ballast water (Cohen & Carlton 1995), hull fouling and rafting on drifting algae (Buschbaum & Gutow 2005). Caprellids have been collected from ballast tanks and plankton tows in marginal seas; shipping is probably an important vector for introductions of *C. mutica* (Takeuchi & Sawamoto 1998, Gollasch et al. 2002).

C. mutica has an exoskeleton armoured with many tubercles and is one of the larger caprellid amphipods, with mature males attaining body lengths of up to 50 mm (Nishimura 1995). The species shows several attributes which may increase its success during transport and introduction: including its abundance (> 1,500 m^{-2} recorded in native habitats; Fedotov 1991), apparent wide environmental tolerance (living within latitudes experiencing a temperate climate), high reproductive effort (long reproductive periods and a maximum of 316 eggs in a single brood pouch; Fedotov 1991) and broad diet preference (Cook et al. 2006).

1.3 West coast of Scotland study sites

Caprella mutica was first described on the west coast of Scotland from fish farm structures in the Lynne of Lorne, near Oban (Figure 1-5; Willis et al. 2004). This area is environmentally similar to the native habitat of *C. mutica*, the Japanese and Russian coastlines of the Sea of Japan. In the Sea of Japan, monthly sea surface temperatures range from below 0 °C to 25 °C (Schevchenko et al. 2004). The west coast of Scotland experiences a reduced, but similar, range of temperatures: in 1959, sea surface temperatures ranged from approximately 3 °C (February) to 15 °C (July); bottom temperatures remained fairly constant around 11 °C (Craig

1959). Between 1995 and 2001, sea water temperatures at a depth of 10 to 14 m at Saulmore Point (between Loch Etive and the Lynne of Lorne, Figure 1-5) ranged from 5.5 to 15.5 °C (February and August respectively; Magill & Sayer 2004).

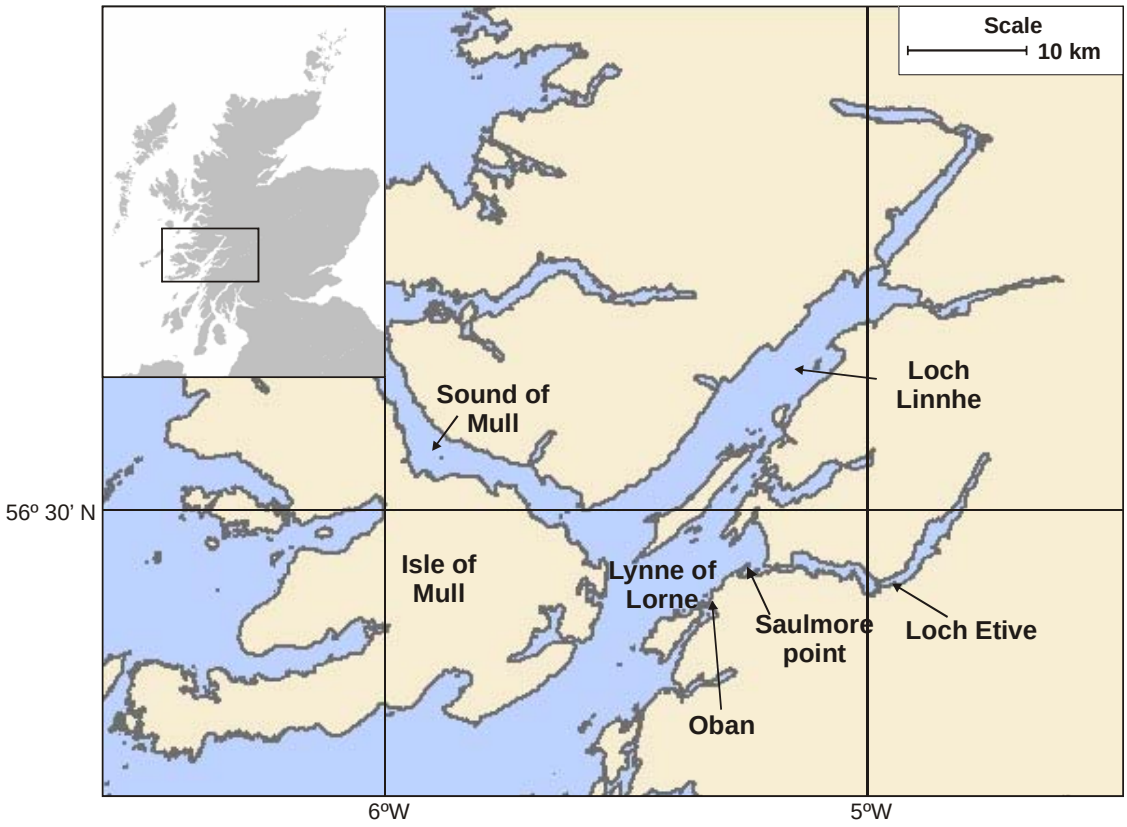


Figure 1-5. Location of the Lynne of Lorne on the west coast of Scotland. Labels refer to features described in the text. Inset map: Scotland.

Both the native habitat and coast of Scotland are in temperate latitudes with a seasonal weather pattern (Wood et al. 1971, Sorokin 1977). Nutrients are mixed into the surface waters during the winter; these nutrients, combined with increasing surface water temperatures and available sunlight in spring initiate a diatom bloom, and productivity is high (Levinton 2001). In late spring, a thermocline establishes and nutrient levels at the surface decline. Heterotrophic microplankton and zooplankton dominate the surface layer, a period of decomposition ensues. The waste products from decomposition fuel a second phytoplankton bloom in late August, productivity levels rise once more and are maintained at a high level until water temperatures and available sunlight decrease, weather systems drive water mixing and the thermocline breaks down. The seasonal dynamics of *C. mutica* on the west coast of Scotland are likely to follow the annual cycle of productivity in the local area and that observed of populations in the native habitat (Fedotov 1991). Local currents, freshwater run-off, anthropogenic inputs etc. influence the cycle in any area.

1.4 Thesis outline

The Japanese skeleton shrimp, *Caprella mutica*, was first recorded on the west coast of Scotland in 2004 (Willis et al. 2004). This thesis first establishes the current global distribution of *C. mutica* and uses molecular evidence to analyse global introduction pathways. A study of post-establishment (secondary) vectors and the environmental tolerance of *C. mutica* provide information regarding the modes of dispersal and potential future range of this and other non-native species. Information regarding the biology and ecology of *C. mutica* was collected through a study of the seasonal population dynamics. Analysis of quantitative data is necessary to understand the integral and fundamental importance of invasions in coastal ecology and evolution (Carlton 1999).

The aims of this study can be represented by four questions:

- The *status quo*, what is the current distribution of *C. mutica*?
- How did it get there? Using a combination of molecular techniques and field studies to establish introduction pathways and potential vectors.
- Where can we expect to see it? What do the current distribution and environmental tolerance limits tell us about the potential distribution of *C. mutica*?
- Does the population biology of *C. mutica* predispose it to be a successful non-native species?

Appendix 1.1

Key to Caprellidae of Britain (Hayward & Ryland, 2000) with the addition of *Caprella mutica*.

1. Gills on segments 2, 3, and 4. Pereopods 3 and 4 six-segmented, pereopod 5 five-segmented ***Phtisica marina***

   Gills on segments 3 and 4 only. Pereopods 3 and 4 greatly reduced or absent **2**

2. Pereopods 3 and 4 minute, reduced to two segments. Mandibular palp of three segments. Head and pereon segments 1 and 2 with strong, forwardly directed spines ***Pseudoprotella phasma***

   Pereopods 3 and 4 absent, or represented only by a single minute seta. Mandibular palp absent **3**

3. Pereopod 5 with six segments, about equal in size to 6 and 7. Pereopods 3 and 4 absent **4**

   Pereopod 5 reduced, minute, with only two segments. Pereopods 3 and 4 represented only by a single seta. Often in association with starfish ***Pariambus typicus***

4. Antenna 2 with short setae ventrally, not arranged in parallel rows. Head bulbous and skull-shaped ***Caprella acanthifera***

   Antenna 2 with long setae ventrally, arranged in parallel rows **5**

5. Prominent ventral spine arising between insertions of gnathopod 2 on each side ***Caprella equilibra***

   No ventral spine between insertions of gnathopod 2. Head may bear one small tubercle, or pair of tubercles ***Caprella linearis***

   No ventral spine between insertions of gnathopod 2. Segments 3-7 with tubercles, males with setation in segments 1 and 2. ***Caprella mutica***

Chapter 2

Distribution of *Caprella mutica* on the west coast of Scotland and a review of its global distribution.

2.1 Introduction

The distributions of taxa are limited by natural barriers to their dispersal, including geographical distance, temperature gradients and current regimes. Since at least the thirteenth century (Petersen et al. 1992) and increasingly since the nineteenth century (Vermeij 1978), humans have been altering and bridging these barriers leading to the increased and unnatural distribution of many species. Knowing the current distribution of an introduced species is critical for determining the range expansion and impact of the species (Parker et al. 1999, Ruiz et al. 2000, Herborg et al. 2003), as well as informing predictions about the potential future range (Arbaciauskas 2002, Telesh & Ojaveer 2002). A species distribution provides the ability to test hypotheses about factors limiting the establishment of sustainable populations in apparently suitable ecosystems (Arbaciauskas 2002).

In 1997, a report by the Joint Nature Conservation Committee recorded 51 non-native marine species in Britain (Eno et al. 1997), including 7 Crustacea (14%). With the addition of *Perophora japonica* (Nishikawa et al. 2000), *Caprella mutica* (Willis et al. 2004), *Ensis directus* (Palmer 2004) and *Tapes phillipinarum* (Jensen et al. 2005), for example, and most likely several other unrecognised non-native species (Ruiz et al. 2000), this number is expected to have increased since the report. Of recorded introductions to Britain, only a few have been described from Scotland (approximately 20% of those recorded in Eno et al. (1997)). These include *Codium fragile* subsp. *tomentosoides* (Oakley 2005), *Sargassum muticum* (Pizzolla 2005), *C. mutica* (Willis et al. 2004), and most recently *Heterosiphonia japonica* (Ware 2004). Most common reasons for the success of non-natives in British waters are favourable physical factors, often being in areas of elevated seawater temperatures in relation to regional or local conditions (Eno et al. 1997). Limits to the distributions of Crustacea include salinity (e.g. *Acartia tonsa*), temperature (e.g. *Elminius modestus*; Barnes & Barnes 1960) and factors influencing dispersal (e.g. *Eusarsiella zostericolor* and *Rhithropanopeus harrisii*; Eno et al. 1997).

Globally, introductions tend to occur at similar latitudes to the source region (Cassey et al. 2004), in areas of similar climatological and physical conditions (Lohrer et al. 2000, Gollasch 2002b). Littoral caprellid species are most abundant and diverse at low latitudes but occur in

low abundances and low diversity at high latitudes (Thiel et al. 2003). In waters adjacent to Japan, *Caprella* spp. are dominant around 30 – 45 °N (Takeuchi & Sawamoto 1998); *C. mutica* has been recorded from 43 – 47 °N. In Peter the Great Bay, Sea of Japan (where *C. mutica* was first described, Schurin 1935), the 1996-1998 sea surface temperature range was -1.8 to 25 °C and salinities from 11 to 35 (Schevchenko et al. 2004). At Akkeshi Bay, where *C. mutica* was described in 1968 (Arimoto 1976), 2003-2004 sea surface temperatures ranged from -1.4 to 21.1 °C and salinities from 21.8 to 32.9 (Nakamura et al. 2005).

Caprellid amphipods inhabit the littoral zone to depths of over 1500 m (McCain & Steinberg 1970) and several species, for example, *C. andreae* and *C. equilibria*, have a worldwide distribution (McCain & Steinberg 1970). Summer surface temperature and the effect of salinity fluctuations have been found to influence the geographical distribution of caprellid species of Atlantic and Arctic Canada (Laubitz 1970). Caprellid species were largely limited to the 10 – 15 °C summer temperature range, and were more frequent along the open coast where salinity fluctuations are less. However, factors influencing the microdistribution of caprellids are poorly known (Caine 1980). Several studies describe characteristic habitat types, for example, *Caprella laeviuscula* is most dense on eelgrass, *Zostera marina* (Caine 1980); and Takeuchi et al. (1987) found caprellid species to be morphologically adapted to hold the algae which it inhabits. *Caprella unica* was found to be an obligate commensal with *Asterias* spp. (Patton 1968) and its distribution follows that of these species. Habitat segregation may also be due to the degree of wave exposure (Takeuchi et al. 1987) and physico-chemical properties of the area (Guerra-García & García-Gómez 2001). In its native habitat, *C. mutica* are found on a wide variety of substrata, and has been described from several species of algae as well as amongst foulings of coasters and sea-going ships (Vassilenko 2006).

To date, the introduction history of *C. mutica* has been sparsely recorded. The first reports of *C. mutica* outside its native habitat in north-east Asia were from the Pacific and Atlantic coasts of North America in the 1970s (Carlton 1979a) and 1980s (Marelli 1981, Cohen & Carlton 1995). The first report of *C. mutica* in the UK is from the west coast of Scotland in 2000 in association with aquaculture activity (Willis et al. 2004). Subsequently, a re-examination of material collected in the Clyde basin in 1999 have also been found to include specimens of *C. mutica* (O'Reilly submitted 2006). *C. mutica* was absent from amphipod-specific listings compiled in 1984 and 2000 (Moore 1984, Smaldon 2000). The distribution of *C. mutica* on the west coast of Scotland has yet to be documented, and many records of its global distribution have not been published.

Invasion patterns can be investigated using two types of methods: records synthesis and field surveys (Ruiz & Hewitt 2002). The data that result from the two methods differ, affecting the inferences that can be drawn and the rigour with which hypotheses can be tested (Ruiz et al. 2000, Ruiz & Hewitt 2002). Surveys are favourable, providing standardised, unbiased data that can be used to test hypotheses about spatial or temporal patterns of invasion (Ruiz & Hewitt 2002). However, they are impractical on large geographic scales. A combination of field surveys on the local scale and records synthesis on the global scale has been used in the current study.

The aim of this study was to determine the distribution of *C. mutica* on the west coast of Scotland and to assemble existing records (both published and unpublished) to determine the current global distribution of this species. The species' current global distribution is then interpreted in relation to potential long-range transport, dispersal mechanisms and temperature gradients for *C. mutica*.

2.2 Methods

In May 2004, aquaculture sites on the west coast of Scotland were surveyed. *Caprella mutica* was previously observed to be abundant on aquaculture structures from May to November (Willis et al. 2004). The survey area ranged from Loch Fyne in the South (56.2 °N, 5.2 °W) to Loch Eriboll in the North (58.5 °N, 4.6 °W), and encompassed sites on the mainland, the Isle of Skye and the Outer Hebrides. Thirty sites were selected from approximately 150 salmon farms on the west coast of Scotland. Where possible at least one site within every 0.5 ° latitude on the west coast was visited.

At each site fouling macroalgae and artificial structures (including cage nets, floating pontoons, mooring ropes and buoys) were inspected to a water depth of 50 cm for the presence of *C. mutica*. At sites where caprellids were present, a sample of 10 females and 10 males was collected and preserved in ethanol (70% absolute). The identity of the specimens as *C. mutica* was later confirmed using diagnostic taxonomic techniques (Arimoto 1976). Temperature and salinity profiles were taken to a maximum of 40 m water depth at each site using a Sea-Bird SBE 19 SeaCat Profiler (Sea-Bird Electronics, Inc, Washington, USA). The results were added to a database of sightings from the west coast of Scotland established in 2003.

To describe potential features limiting the distribution of *C. mutica*, data describing the physical characteristics of the west coast sea lochs visited during the survey were investigated (Edwards & Sharples 1986). Characteristics used were annual rainfall, flushing time and ratio

of runoff to tidal flow (indication of salinity reduction). These factors affect the extent of changes in physical properties in the sea lochs.

An extensive search of published material in peer-reviewed papers, government reports and the internet was conducted for globally reported sightings. Researchers with sightings of *C. mutica* were contacted for further information and if possible, specimens were obtained for confirmation of identification. Global sea surface temperature data were taken from the Global Ocean Surface Temperature Atlas CD-ROM (GOSTA, Atlas8).

Statistical analysis

Kendall's rank correlation (Kendall 1962) was used to provide a distribution-free test of independence between environmental factors (annual rainfall, flushing time and ratio of runoff to tidal flow) and the presence of *C. mutica* at aquaculture sites in sea lochs on the west coast of Scotland. Temperature and salinity data collected during the 2004 survey (for depth intervals: 0 - 5 m; 0-seabed (max 40 m); maximum, minimum and standard deviation of total water column) were also tested for correlation with the presence of *C. mutica.*

2.3 Results

West coast of Scotland distribution

Caprella mutica was found to be widely distributed on the west coast of Scotland, from Ardrossan in the south, to Loch Laxford in the far north, and on the Islands of Mull, Skye and the Outer Hebrides (Figure 2-1). *C. mutica* was present at 17 of the 30 aquaculture sites visited in the survey (59%). To date, *C. mutica* has been confirmed as being present at 38 aquaculture sites on the west coast of Scotland.

The salinity and temperature measurements recorded in May 2004 do not explain presence of *C. mutica* at the survey sites on the west coast of Scotland (Kendall's rank τ; $P > 0.05$; Table 2-1). *C. mutica* were found in sea lochs with salinity and temperature ranges of 3 to 35, and 9.4 to 13.4 °C, respectively. *C. mutica* were found at Loch Carnan (Outer Hebrides), which had the greatest water column salinity range, from 3 to 35, and Loch Erisort and West Loch Roag, which had the highest average salinities (both 35).

In May 2004, *C. mutica* were not found at Loch Fyne where the greatest temperature range (11.2 to 16.5 °C) and highest temperature (16.5 °C) were recorded. However, *C. mutica* has been found in Loch Fyne since the survey. *C. mutica* was also found at Loch Kishorn where

the lowest temperature value (9.4 °C) was recorded. No significant correlation was found between annual rainfall, flushing time or ratio of runoff to tidal flow and the presence of *C. mutica* (Kendall's rank τ; $P > 0.05$; Table 2-1).

In addition to the survey, records of *C. mutica* on the west coast of Scotland include the Clyde and the Isles of Arran and Mull (Figure 2-1). Beyond the west coast, *C. mutica* has been recorded from the Shetland Islands and Scapa Flow, Orkney (Table 2-2).

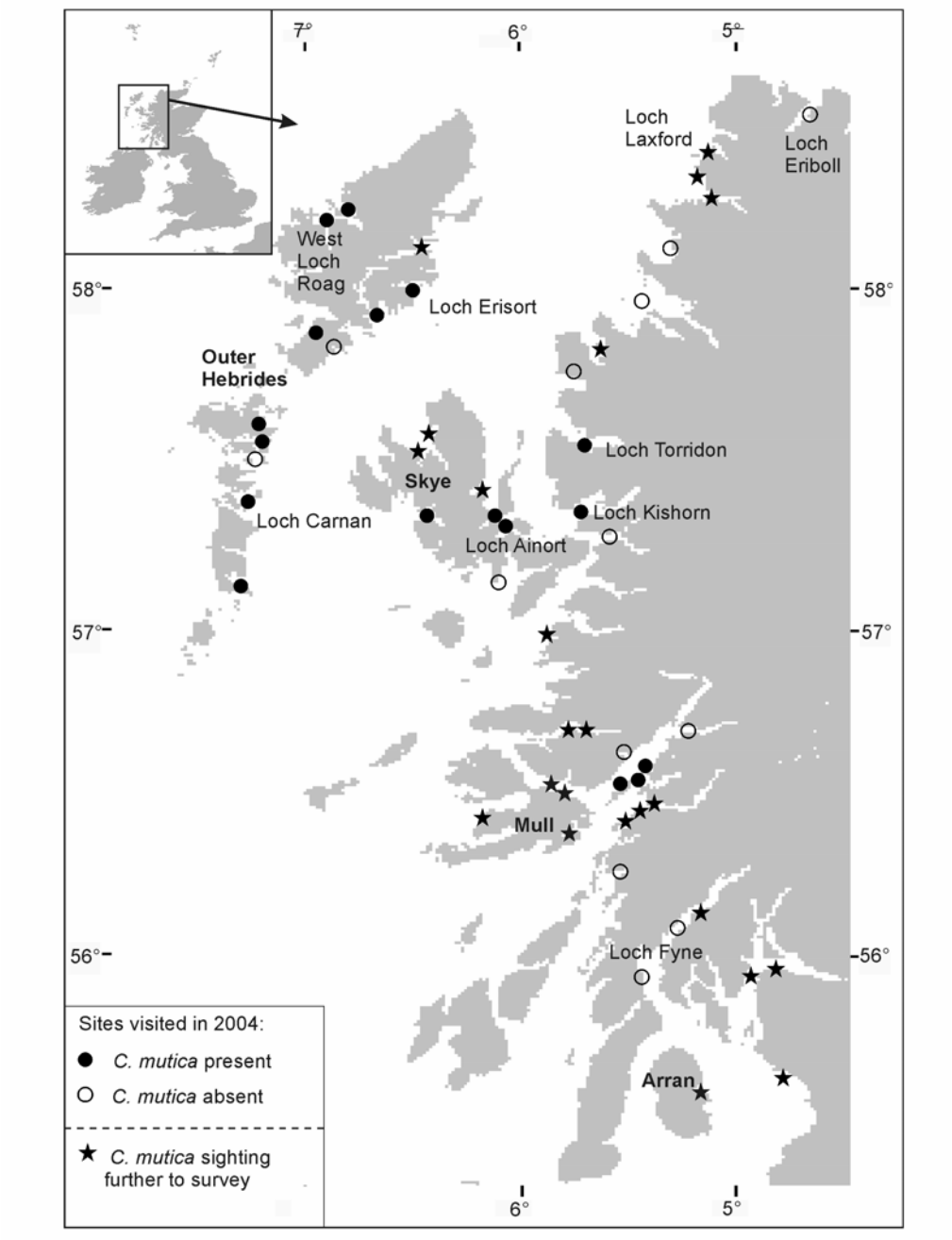


Figure 2-1. Distribution of *Caprella mutica* on the west coast of Scotland. Font in bold are names of islands, normal font are locations referred to in the text. Inset map: The British Isles.

Table 2-1. Distribution records of *Caprella mutica* on the west coast of Scotland; including latitude of sighting, physical data recorded during the survey and taken from Edwards & Sharples (1986). Rows in bold text indicate presence of *C. mutica*. * indicates *C. mutica* was absent during the survey, but has been recorded present subsequently. Kendall's Rank τ indicates correlation between environmental characters and presence of *C. mutica*, all values were not significant (P>0.05).

				Temp (°C)					Sal					Edwards & Sharples (1986)		
Location	Latitude (°N)	*C. mutica* present?	Source (S- survey, C- confirmed sighting)	Average (0-5m)	Average (0-seabed/ 40m)	Minimum	Maximum	Standard Deviation	Average (0-5m)	Average (0-seabed/ 40m)	Minimum	Maximum	Standard Deviation	Annual Rainfall (mm)	Flushing Time (days)	Runoff/Width (m^2/day)
Lamlash	**55.538**	**y**	**C**													
Clyde Basin	**55.968**	**y**	**C**													
Loch Fyne 2	56.247	n	S	16.3	13.4	11.2	16.5	2.0	30.3	32.1	30.1	33.2	1.2	1750	13	1237
Loch Fyne	**56.248**	**y***	**C**	**16.1**	**13.5**	**11.1**	**16.5**	**1.8**	**29.5**	**31.9**	**28.5**	**33.1**	**1.4**	**1750**	**13**	**1237**
Loch Melfort	56.259	n	S	14.4	14.0	13.7	14.9	0.3	33.9	34.0	33.5	34.1	0.1	1750	9	188
Loch Etive	56.451	n	C											2500	14	8584
Fishnish	**56.513**	**y**	**C**													
Lismore W	**56.518**	**y**	**S**	**13.8**	**13.6**	**13.5**	**13.9**	**0.1**	**33.4**	**33.5**	**33.3**	**33.6**	**0.1**	**2200**	**8**	**8314**
Loch Creran	56.524	n	C											2000	3	707
Lismore A	**56.559**	**y**	**S**	**13.5**	**13.4**	**13.4**	**13.6**	**0.0**	**33.9**	**33.7**	**33.6**	**34.0**	**0.1**	**2200**	**8**	**8314**
Shuna	**56.590**	**y**	**S**	**14.0**	**13.5**	**13.0**	**14.1**	**0.4**	**33.1**	**33.4**	**33.0**	**33.6**	**0.2**	**2200**	**8**	**8314**
Kingairloch	56.616	n	S	13.2	12.8	12.3	13.3	0.3	32.5	32.8	32.5	33.4	0.3	2500		
Kinlochleven	**56.699**	**y**	**S**	**12.9**	**12.9**	**12.8**	**13.0**	**0.1**	**31.4**	**31.9**	**30.8**	**32.3**	**0.4**	**2000**	**3**	**2698**
Loch Sunart	**56.704**	**y**	**C**											**2000**	**7**	**868**
Lochboisdale	**57.153**	**y**	**S**	**10.7**	**10.6**	**10.4**	**10.8**	**0.1**	**35.0**	**34.9**	**34.8**	**35.0**	**0.1**	**1200**	**1**	**68**
Loch Slappin	57.211	n	S	13.2	13.2	13.1	13.4	0.1	32.9	33.5	31.2	33.9	0.7	2600	1	486
Loch Duich	57.248	n	S	10.8	10.6	10.4	11.5	0.2	33.2	33.4	32.5	33.6	0.2	2250	7	713
Loch Ainort	57.281	n	S	9.9	9.8	9.6	10.1	0.1	34.6	34.6	34.5	34.8	0.1	2500	2	167
Loch Bracadale	57.338	n	S	13.0	12.7	12.2	13.0	0.4	34.0	34.3	33.8	34.6	0.3			
Loch Carnan	**57.376**	**y**	**S**	**11.4**	**10.8**	**10.3**	**11.9**	**0.5**	**10.5**	**26.8**	**3.3**	**36.1**	**12.9**	**1200**	**4**	**64**
Loch Kishorn	**57.378**	**y**	**S**	**11.2**	**10.8**	**9.4**	**11.2**	**0.5**	**33.8**	**34.0**	**33.6**	**34.6**	**0.3**	**2000**	**3**	**207**
Greshornish	57.500	n	S	10.8	10.6	10.4	11.0	0.1	34.5	34.7	34.0	34.8	0.2	1900	3	434
Loch Torridon	**57.540**	**y**	**S**	**11.4**	**11.0**	**10.6**	**11.5**	**0.3**	**32.4**	**33.5**	**32.1**	**34.2**	**0.7**	**2000**	**9**	**358**
Sidnish	57.556	n	S	11.6	11.4	11.2	11.7	0.2	35.0	34.9	34.8	35.1	0.1	1200	0	273
Loch Maddy	**57.616**	**y**	**S**	**11.4**	**11.4**	**11.3**	**11.5**	**0.1**	**34.6**	**34.7**	**34.0**	**34.8**	**0.2**	**1200**	**1**	**273**
Cheese Bay	**57.660**	**y**	**S**	**10.5**	**10.4**	**10.3**	**10.6**	**0.1**	**34.8**	**34.8**	**34.4**	**34.9**	**0.1**	**1200**		**273**
Loch Ewe	**57.787**	**y***	**S**	**11.2**	**10.7**	**10.3**	**11.4**	**0.4**	**34.4**	**34.5**	**34.3**	**34.6**	**0.1**	**1750**	**4**	**614**
E Loch Tarbert	**57.870**	**y**	**S**	**10.7**	**10.5**	**10.2**	**10.7**	**0.1**	**34.8**	**34.8**	**34.8**	**34.9**	**0.0**			
W Loch Tarbert	**57.870**	**y**	**S**	**13.3**	**13.3**	**13.1**	**13.4**	**0.1**	**32.7**	**33.8**	**29.8**	**34.6**	**1.4**	**1900**	**4**	**120**
Loch Seaforth	**57.955**	**y**	**S**	**11.0**	**10.5**	**9.6**	**11.0**	**0.5**	**34.0**	**34.5**	**33.3**	**35.0**	**0.4**	**1800**	**3**	**698**
Loch Shell	**58.006**	**y**	**C**											**1600**	**4**	**311**
Enard Bay	58.093	n	S	10.5	10.3	9.9	10.6	0.2	34.7	34.7	34.6	34.9	0.1	1500	3	661
Loch Erisort	**58.111**	**y**	**S**	**10.8**	**10.7**	**10.4**	**10.9**	**0.2**	**34.8**	**35.0**	**34.7**	**35.1**	**0.1**	**1300**	**1**	**527**
W Loch Roag	**58.197**	**y**	**S**	**11.7**	**11.7**	**11.7**	**11.7**	**0.0**	**34.9**	**35.0**	**34.5**	**35.0**	**0.1**			
E Loch Roag2	**58.210**	**y**	**S**	**11.9**	**11.9**	**11.8**	**11.9**	**0.1**	**34.9**	**34.9**	**34.8**	**35.0**	**0.0**			
E Loch Roag	**58.219**	**y**	**S**	**12.0**	**11.7**	**11.5**	**12.1**	**0.2**	**21.8**	**28.2**	**13.6**	**31.9**	**4.6**			
Calbha Bay	**58.290**	**y**	**C**											**1750**	**5**	**664**
Loch Duart/Laxford	**58.402**	**y**	**C**											**2000**	**4**	**717**
Loch Eriboll	58.521	n	S	10.7	10.5	10.4	11.2	0.2	34.9	34.8	34.7	35.0	0.1	1500	4	179
Kendall's Rank τ				-0.03	-0.03	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	-0.02	-0.03	-0.02

Global distribution

Thirty-six records of *C. mutica* outside its native range were collected (Table 2-2) with the majority of sightings from the northern hemisphere (Figure 2-2). On the Pacific coast of North America, the total number of records is nine, including the first record of this species on oil platforms, approximately 4 miles off the Californian coast. These records include sightings between latitudes of 37.5 to 48.5 °N, which experience a temperature range of 8 to 18 °C (given ranges are the winter minimum and summer maximum sea surface temperatures; GOSTA Atlas8). There are a total of five records from the Atlantic coast of North America, ranging from Connecticut to Quebec, including two new records for Canada (Table 2-2). These records include sightings from latitudes of 41.5 to 47.9 °N. A total of 18 records were collated for Europe (49.5 to 69.1 °N). This total includes the west coast of Scotland as a single sighting. To date, the temperature range experienced by *C. mutica* in Europe is 6 to 22 °C. There are two records from the southern hemisphere, both from New Zealand (Table 2-2), which experience an annual temperature range of 8.5 to 17.5 °C (Coakley 1970). The global records encompass sea surface temperatures of 0 to 22 °C. Therefore, if temperature limits the global distribution of *C. mutica*, the species' potential non-native distribution is shown in Figure 2-2.

All of the global records are from areas of human activity (marinas, ports, aquaculture sites). Fifty-nine percent of the introduced locations are within 10 km of an international port, and a further 19% are within 50 km (Table 2-2). Seventeen records (52%) are in areas of shipping activity (16 from harbours and marinas, 1 from an oil rig) and can be attributed to either hull fouling, ballast water, or within sea chests. Six records (19%) can be attributed to aquaculture activities and a single record was from an offshore wind farm.

Recent surveys of caprellid species' distributions in Chile (Thiel et al. 2003), Tasmania (Guerra-Garcia & Takeuchi 2004) and South Africa (C. Griffiths, pers. comm.) have not reported *C. mutica* (Figure 2-2). Thus, for the purposes of this paper, these have been presented as records of absence, but should not be considered as unequivocal confirmation that the species is absent from these locations.

Table 2-2. Global distribution records of *Caprella mutica* including date of reporting, site description (if known) and possible mechanism of introduction. Mechanisms of introduction are: NH - Native Habitat; AQ - aquaculture; JO - Japanese Oysters; SH - Shipping; BW - ballast water; SF - ship hull fouling; RB - recreational boats; UN - Unknown. Distance from port is direct distance in kilometres from the nearest International port as recognised by Drake & Lodge (2004). Where coastlines rather than specific sites have been reported, the distance from the nearest port has not been calculated.

Date	Country	Location	Site Description	Introduction Mechanism	Distance from port (km)	Source
1935	Russia	Peter the Great Bay		NH	<10	(Schurin 1935)
1967	Japan	Possjet Bay, Sea of Japan		NH	<200	(Vassilenko 1967)
1968	Japan	Akkeshi Bay		NH	<20	(Arimoto 1976)
1973	USA	Humboldt Bay		JO	<10	(Marelli 1981)
1976	USA	San Francisco Bay		JO	<10	(USGS 2005)
1977	USA	Oakland Estuary		JO	<10	(USGS 2005)
1978	USA	Elkhorn Slough		JO	<150	(USGS 2005)
Pre-1979	USA	Puget Sound	Harbour	SH, BW	<20	(Carlton 1979a)
1983	USA	Coos Bay		JO	<10	(USGS 2005)
1995	Netherlands	Burghsluis	Pontoon & Scaffolding	SH	<100	(Platvoet et al. 1995)
1995	USA	San Francisco Bay		JO	<10	(Cohen & Carlton 1995)
1998	Belgium	Zeebrugge	Marina	SH	<10	(ICES 2003)
1999	Japan	Otsuchi Bay	Ropes for *Undaria* culture	NH		(Kawashima et al. 1999)
1999	Norway	Hordaland	Harbour	SH		(Heilscher 2000)
1999	Scotland	Clyde basin	Harbour Buoys	SH	<10	(O'Reilly submitted 2006)
2000	USA	Pacific coast	Various marinas	SH		(Cohen et al. 2002)
2000	Scotland	Oban	Fish Farm	AQ, SF	<200	(Willis et al. 2004)
2000	Germany	South-Eastern North Sea	Pontoons	SH		(Buschbaum & Gutow 2005)
2002	Belgium	Oostende	Harbour Buoy	SH	<10	(ICES 2003)
2002	Norway	Alesund	Harbours	UN	<10	(ICES 2003, Botnen et al. 2004)
2002	England	Harwich	Harbour	SH	<10	(Ashelby 2005)
2003-present	Scotland	West coast, various (Fig. 1)	Fish Farms	AQ, SF		Various (fish farm staff)
2003	Canada	Passamaquody Bay	Mussel lines	AQ, SF	<150	S. Robinson (pers. comm.)
2003	England	Southampton	Harbour	SH	<10	L. Baldock & M. Marley (pers. comm.)
2003	Scotland	Shetland Is	Mussel lines	AQ, SF	<30	G. Duncan (pers. comm.)
2003	Wales	Anglesey	Mooring line	RB, SF	<20	T. Stoker (pers. comm.)
2003	USA	South Freeport	Marina	SH	<40	MIT Sea Grant, 2003

	Country	Location	Site Description	Introduction Mechanism	Distance from port (km)	Source
2003	USA	Mystic	Marina	SH	<100	MIT Sea Grant, 2003
2003	USA	Atlantic coast, various	Harbours	SH		MIT Sea Grant, 2003
2003	Norway	Various	Aquaculture	AQ, SF		W. Vader (pers. comm.)
2003	Ireland	Betraghboy Bay	Aquaculture	AQ, SF	<50	(Tierney et al. 2004)
2004	Canada	Chaleur Bay, Quebec	Mussel lines	AQ, SF	<10	B. Sainte-Marie (pers. comm.)
2004	France	Le Havre	Harbour	SH	<10	G. Breton (pers. comm.)
2004	New Zealand	Timaru	Harbour	SH	<10	G. Fenwick (pers. comm.)
2004	USA	California	Oil Rigs	SH	<10	J. Hoesterey (pers. comm.)
2005	Denmark	Horns Rev wind farm	Wind Farm	SF	<100	R. Frederiksen (pers. comm.)
2005	New Zealand	Lyttleton	Harbour	SH	<10	O. Floerl (pers.comm.)
2006	Scotland	Scapa Flow	Mooring line	RB, SF	<10	I. Sibley-Calder (pers.comm.)
2006	Scotland	Ardrossan Port Edgar Peterhead Lossiemouth Largs	Harbours	RB		Ashton et al. (in press)

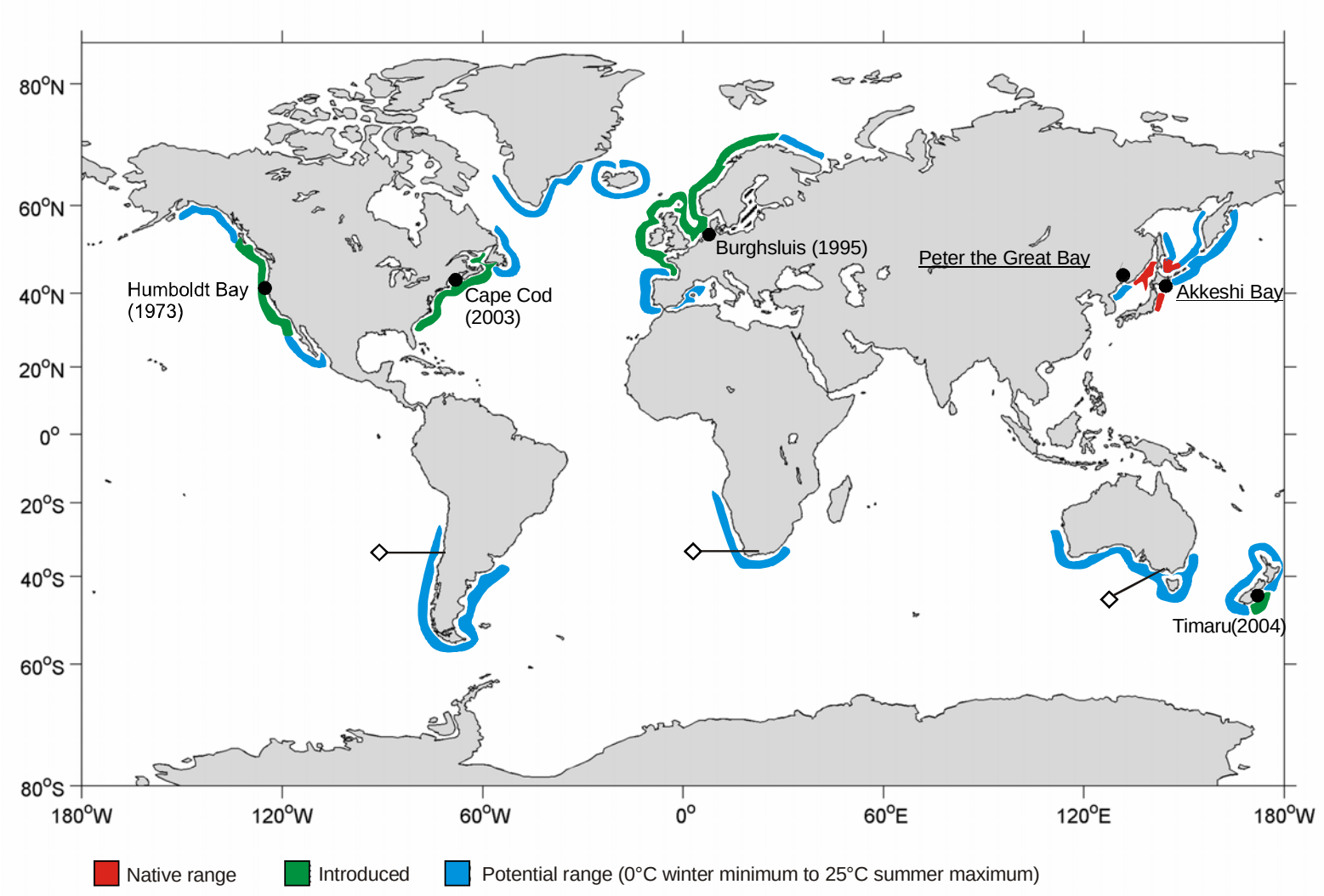


Figure 2-2. Current global distribution of *Caprella mutica*. Labels with underline show the native distribution; normal text labels show dates of first record on each coastline; diamonds indicate locations of surveys which have not found *C. mutica*.

2.4 Discussion

Scottish distribution

Introduced populations of *Caprella mutica* have successfully established and are thriving on artificial structures along the west coast of Scotland. *C. mutica* has been described from Arran in the south (55.5 °N) to Loch Laxford in the north (58.4 °N). It has been found at sites on both the mainland and all islands included in the survey. *C. mutica* was present at 59% of the sites visited in the survey. There is no obvious feature that has limited *C. mutica* from the sites where it was not found.

It has been suggested, that environmental habitat conditions (such as hydrodynamics, sedimentation rate, turbidity and substratum stability) are important in determining the local distribution of littoral caprellids (Guerra-García & García-Gómez 2001, Thiel et al. 2003). *C. mutica* were found in waters with a salinity range of 3 to 35 and surface temperatures ranging from 10 to 16 °C in May 2004. A wide salinity tolerance has been found for many caprellid species and was unable to explain differences in the caprellids' distributions (Takeuchi et al. 2003). In north-east Asia (within the native range), *C. mutica* experiences a salinity range of 11 to 35 and temperatures from -1.8 to 25 °C (Schevchenko et al. 2004). This supports the result that salinity and water temperature do not limit the distribution of *C. mutica* on the west coast of Scotland. However, these results should be treated with caution as they only represent the physical characteristics experienced at the time of the survey, and can not be interpreted as representative of the environment over longer periods of time. The tidal cycle and freshwater input may greatly affect the salinity and temperature of the sea lochs.

The physical variables tested in the present study (annual rainfall, flushing time and ratio of runoff to tidal flow) showed no correlation to the presence of *C. mutica* in the sea lochs. Ninety percent of confirmed marine non-native species in South Africa are restricted to sheltered bays, harbours and estuaries (Robinson et al. 2005); the sheltered nature of sea lochs on the west coast of Scotland may have contributed to the success of *C. mutica* at these sites. Caprellid amphipods have been found to be relatively unselective with respect to their substratum (McCain 1968, Laubitz 1970), however, all sightings of *C. mutica* are from man-made structures, suggesting that this may limit the species to disturbed areas. The availability of dispersal mechanisms and/ or biological parameters may be limiting the distribution of this non-native species on the west coast of Scotland.

Dispersal vectors (e.g. shipping activity) were reported to limit the spread of 33% of the faunal species identified as non-native to Britain by Eno et al. (1997), including 43% of the Crustacea. *C. mutica* have no free-living planktonic larval phase (Buschbaum & Gutow 2005), and therefore, require either natural or artificial vectors for long distance dispersal. Drifting algae and recreational boating have been found to influence the dispersal of *C. mutica* in the local area (Chapter 4). On the Pacific coast of continental Chile, Thiel et al. (2003) suggested hydrodynamic conditions affect dispersal of littoral caprellid species, resulting in a relatively uniform distribution pattern within a bay system. Hydrodynamic constraints to the distribution of *C. mutica* may include the influence of wind driven currents on drifting algae (Thiel & Gutow 2005), and the complex patterns of circulation and surface mixing in Scottish sea lochs (e.g. Watts et al. 1998). Hydrodynamic influences and frequency of vessel activity (including recreational and commercial vessels) may also influence the local distribution described.

The wide distribution of *C. mutica* on human-impacted sites on the west coast of Scotland suggest that this species was introduced before the first record, in 1999 (O'Reilly submitted 2006). This record was published when publicity concerning the introduction of *C. mutica* to Scotland led O'Reilly to re-examine samples taken in 1999. Anecdotal evidence from discussions with staff at the fish farms indicates that *C. mutica* may have been present on the Outer Hebrides for 15 - 20 years before the survey.

Global distribution

On a global scale, *C. mutica* has spread from its native habitat in north-east Asia to 31 sites in the northern hemisphere in less than 40 years. The first records of introduced populations of *C. mutica* are from various locations along the Pacific coast of North America in the 1970s (Carlton 1979a, Marelli 1981, USGS 2005). It is not known whether these records are the result of several independent cross-oceanic introductions with oyster spat (Carlton 1979a), or the result of local small-scale transport following the first introduction (Carlton 1996). The next series of records are from Europe, with the first sighting in the Netherlands (Platvoet et al. 1995). *C. mutica* has also been discovered on the Atlantic coast of North America where it was identified from South Freeport to Mystic in 2003 (MIT. Sea Grant 2003). The establishment of *C. mutica* in all these locations indicates environmental similarity between these and its native region (Gollasch 2002a). They are 'highly probable' matched recipient and donor regions for introductions of other marine species.

As yet, *C. mutica* has not been reported from the Pacific coast of South America, southern Australia, and South Africa. All these areas experience a similar temperature range to regions where *C. mutica* has already become established and are exposed to high levels of shipping activity (Drake & Lodge 2004). South America and Australia, in particular are linked by major shipping routes to north-east Asia, where *C. mutica* is known to originate, and as a consequence may provide a suitable region for colonisation of this species in the future.

Introduced populations of *C. mutica* have been reported from temperate latitudes between 35 and 70 °N, an extension of the native latitudinal range. These areas experience an annual seawater temperature range of 0 to 22 °C, comparable to those experienced in the native range. While temperature did not limit the local distribution of *C. mutica* during the survey in May 2004 on the west coast of Scotland, it may influence the global distribution (Laubitz 1970, Thiel et al. 2003). The observed distribution follows the natural distribution of amphipods and more specifically littoral caprellids, which are generally found in greater abundance and diversity in temperate latitudes (Stevens 1989, France 1992). Laubitz (1970) found temperature to be the main factor limiting the distribution of caprellids in the North Pacific, with most occurring in the 10 - 15 °C temperature range and only a few in the subantarctic 5 – 10 °C range. The fact that *C. mutica* has not yet occupied all regions which experience temperatures within the 0 to 22 °C range, indicates that either the range expansion is still underway and *C. mutica* sightings will continue to increase, or factors aside from temperature are affecting the establishment of *C. mutica* on a global scale. Factors which have been found to limit the distribution of *Caprella* spp. and need further investigation with respect to *C. mutica* include wave exposure (Takeuchi et al. 1987, Guerra-García 2001), substratum features (Caine 1978), inter-species competition (Caine 1980) and predation (Guerra-García 2001).

Disturbance is known to affect the invasibility of communities (Hobbs & Huenneke 1992, Hooper et al. 2005). To date, *C. mutica* has only been found in areas of human activity (e.g. aquaculture sites, marinas/harbours and moorings), indicating that human interference plays an important role in the establishment of *C. mutica*. These areas undergo much disturbance (for example lifting of aquaculture nets and lines, or movement associated with boat traffic). Location of records near to human activity may also be due to the increased intensity of surveys, rather than restriction of the species to these areas. It is important to determine whether *C. mutica* has become established in natural habitats, as this may have implications for the identification of secondary vectors and ecological impacts.

Vectors

Marine introductions are most common in areas of intense shipping activity (Ricciardi, 2001). The most important pathway for region-to-region global exchange of non-indigenous species in ballast water is between Asia and Europe, followed in importance by Asia-Africa, Africa-North America and Africa-Europe (Drake & Lodge 2004). Drake & Lodge (2004) identified global hotspots for invasion to include large regions of Southeast Asia, northern Europe, the Mediterranean Sea, and some coastal areas in North and South America; although some areas known to have a large number of non-indigenous species' e.g. San Francisco Bay (Carlton 1979a), were not recognised by this study. However, using the study of Drake & Lodge as a guideline, the absence of *C. mutica* from some of these hotspots, and its presence outside these hotspots suggests the importance of factors other than shipping intensity to its dispersal. Alternative explanations include either a bias in sampling effort or that the invasion is still undergoing and *C. mutica* could be identified from these areas in the future. It will be particularly interesting to observe whether the introductions continue to spread in the southern hemisphere.

Shipping has been identified as the likely vector for the transfer of other introduced crustacean species (Clark et al. 1998, Galil et al. 2002) and *C. mutica* has been identified from all temperate coastlines in the northern hemisphere which support major shipping routes (Endresen et al. 2003). Ballast water and hull fouling are likely to be important introduction vectors. Macroalgae, including *Ulva* spp. and *Cladophora* spp., which are associated with *C. mutica* in its native habitat, are common hull-fouling species (Mineur et al. 2004) providing suitable substrata for *C. mutica*. *Caprella* spp. have also been found to survive transport in ballast tanks (Carlton 1985) and an unidentified species of caprellid was found during a survey of sea-chests in New Zealand (Coutts et al. 2003). The global spread of *C. mutica* is a relatively recent event and can probably be attributed to the increase in frequency and speed of global marine trade and transport (Carlton 2003b). Assuming that shipping is an important vector, *C. mutica* will continue to establish along coastlines in the northern hemisphere and it is likely to be introduced to further sites in the southern hemisphere.

Aquaculture transfers are the second most important vector for the introduction of non-native species globally (Eno et al. 1997, Ruiz et al. 2000). Transfers of shellfish spat have resulted in the transport of *C. mutica* beyond its native range, and have been responsible for the introductions to Pacific North America (Carlton 1979a). The global distribution of *Sargassum muticum* (Yendo) Fensholt has been attributed to international transportations of the Pacific oyster *Crassostrea gigas* (Thunberg), as the algae was used as packing material for the spat

(Scagel 1956, Druehl 1973, Critchley 1983). *C. mutica* is associated with *S. muticum* in its native habitat (Sano et al. 2003), so may have been inadvertently introduced to new areas along with this macroalga.

Once *C. mutica* has established in a new area, further artificial and natural dispersal mechanisms may extend its distribution locally (secondary dispersal). Of the global sightings reported here, 22% are over 50 km from an international port. It is considered to be unlikely that individuals will travel this distance independently (Chapter 4), indicating that secondary transport is important in the distribution of *C. mutica*. In Norway, the range of *C. mutica* has extended against the direction of the dominant coastal current, suggesting either vector driven transport, or multiple introduction events (ICES 2001). Secondary vectors are often similar to those of the primary introduction (Eno et al. 1997), therefore, shipping and aquaculture are likely to be important secondary vectors (Willis et al. 2004). Floating macroalgae can persist for long durations (Ingolfsson 1995) and may be a significant vector responsible for localised distribution (Sano et al. 2003, Chapter 4). Recreational boating is also possibly an important vector (Chapter 4).

The production of marine debris, which is then colonised by marine species, provides a further mode of human-mediated transfer of non-native species. The potential for non-native species to spread in coastal marine habitats is increasing with the increase in floating anthropogenic debris (Barnes 2002).

Summary

In less than 40 years, introduced populations of *Caprella mutica* have become widely established along temperate oceanic coasts between latitudes of 35 and 70 °N. These waters experience an annual temperature range of 0 to 22 °C. *C. mutica* have only been identified as introduced in areas of human activity including ports, aquaculture facilities and an oil rig and have not yet been found in undisturbed natural habitats outside their native range. Shipping and aquaculture transfers are the most likely long distance vectors. It is clear from the survey in Scottish waters, that once introduced, *C. mutica* has the potential to disperse quickly via natural and/or artificial vectors. The studied physical environmental parameters, including temperature and salinity do not explain the distribution of *C. mutica* on the west coast of Scotland in May 2004. Due to the stochastic nature of the invasive process (Herkül et al. 2006), it is likely that *C. mutica* has not yet colonized all suitable habitats on the west coast of Scotland. Records continue to be added to the database and this work is on-going. Whilst negative environmental impacts have yet to be observed, it is important that the spread of this

species is monitored in the event that adverse effects do occur and to help predict the spread of future non-natives.

Note

Aspects of this Chapter have been published:

Ashton G. V., Willis, K. J., Cook, E. J., Burrows, M. T. (in press) Distribution of the introduced amphipod, *Caprella mutica* Schurin on the west coast of Scotland and a review of its global distribution. *Hydrobiologia*

Chapter 3

Introduction pathways of *Caprella mutica* populations in the northern hemisphere: a phylogenetic analysis

3.1 Introduction

The long-distance transport of organisms by humans is breaking species' boundaries in the sea that were traditionally defined by geographical (physical) barriers to natural dispersal and establishment (Elton 1958, Vermeij 1978, Holland et al. 2004, Zardus & Hadfield 2005). As a result, the divide between native and non-native has become distorted by human-mediated introductions (Carlton 1987, Carlton 1999, Stevens et al. 2002). The rate of this distortion has increased over time with the increasing frequency and speed of shipping (Carlton & Geller 1993, Everett 2000, Carlton 2003b). It is important for policy and management purposes, to identify the pathways along which a species has been introduced and to predict and prevent future introductions (Lodge et al. 2006). This is rarely possible using traditional understanding of the biology and ecology of a species (Holland 2000), and is further confounded by the complicated network of marine shipping and international trade (Endresen et al. 2003).

Through estimation of genetic divergence, molecular techniques can help to reconstruct introduction histories (Patti & Gambi 2001, Wares et al. 2002, Cristescu et al. 2003); for example, to identify the origins of non-native species (e.g. Hebert & Cristescu 2002, Zardus & Hadfield 2005), modes of introduction (e.g. Pollux et al. 2003, Turon et al. 2003), and the extent of linking/movement between non-native populations (e.g. Zardus & Hadfield 2005). Molecular techniques also offer an indirect approach for addressing the mechanism of invasion (Stoner et al. 2002). Several techniques, the statistics used to analyse them, and applications of the analysis will be discussed below (molecular techniques and phylogenetic analysis). The characteristics of invading populations can complicate the process of constructing geographic histories (Hicks & Tunnell 1993, Davies & Roderick 1999). In some instances, use of a single technique has given poor resolution (Duran et al. 2004, Le Goff-Vitry et al. 2004, Petersen 2006) and it is becoming increasingly common to use more than one molecular technique to investigate a hypothesis. In this case, techniques which investigate processes with different evolutionary rates are normally selected.

Population introductions are rapid evolutionary events in which populations are usually subjected to founder effects during the colonisation event, followed by rapid expansion (Sakai

et al. 2001). The genetic characterisation of non-native populations can be informative in several ways. The non-native population is a sample of individuals from the source population, and hence retains a sub-sample of genetic characters (Holland 2001, Kolbe et al. 2004). A low level of genetic divergence may imply that the populations are still linked (e.g. Holland 2001), or allow identification of a single source population, as described for populations of the cladoceran, *Bythotrephes longimanus*, in the Great Lakes (Berg et al. 2002). A high degree of genetic divergence between populations may indicate several attributes: long-term isolation, as suggested for endemic springtails and mites in Antarctica (Stevens & Hogg 2003, Stevens et al. 2006, Stevens & Hogg 2006); different selection pressures in the habitats, as described for populations of the fly *Rhagoletis pomonella* developing on apple or hawthorn bushes (Filchak et al. 2000); multiple origins, or a limited gene-flow between populations, both described for native populations of the Atlantic barnacle, *Chthamalus proteus*, in the Caribbean and west Atlantic (Zardus & Hadfield 2005).

There are several relevant processes that can alter genetic diversity. In a newly established population, a low level of genetic diversity, less than the source population, is expected (Barrett & Kohn 1991, Allendorf & Lundquist 2003). Genetic diversity may be further reduced by inbreeding (Holland 2000) and different selective pressures in the new habitat (Zuoros & Foltz 1984, Koehn 1991). Following introduction, the population sizes will most likely increase, with an associated increase in genetic diversity, described as a bottleneck (Nei et al. 1975). High levels of genetic diversity in a non-native population have also been described to indicate a large propagule size and/or several introduction events from a single or multiple origins (Kolbe et al. 2004, Zardus & Hadfield 2005). For example, high genetic diversity indicated multiple geographic origins for non-native populations of the brown anole, *Anolis sagrei* in Florida (Kolbe et al. 2004). When independent populations are introduced to a new area and interbreed, genetic diversity in the new area will be higher than that in either source location, described as a Wahlund effect (Ayala 1982, Hartl & Clark 1997). An erosion of genetic diversity may be experienced during successive founder or stepping-stone events (Hänfling et al. 2002). When using genetic techniques to evaluate introduction processes, it is important to genetically characterise the diversity and distribution of populations over the global range of the species, including both native and non-native populations (Stoner et al. 2002, Grapputo et al. 2005, Voisin et al. 2005).

Caprella mutica Schurin was first described from Peter the Great Bay (Vladivostok, Russia, 1935) and subsequently identified in the neighbouring Possjet Bay and Akkeshi Bay, Japan (Vassilenko 1967, Arimoto 1976). In 2006, Vassilenko described the native distribution of *C. mutica* to include bays in the latitudinal range of 43 to 47 °N in East Asia, including sites

in the Seas of Japan and Okhotsk. Publications in the last 40 years indicate that the distribution of *C. mutica* has expanded to include all oceanic coasts in the northern hemisphere (Chapter 2). This distribution is similar to that described for the Chinese mitten crab, *Eriocheir sinensis*, which has also been introduced widely in the northern hemisphere via ballast water and/ or intentional introduction (Herborg et al. 2003). Although the Asian shore crab, *Hemigrapsus sanguineus*, and European green crab, *Carcinus maenas*, have only been introduced to one coast of North America (Jensen et al. 2002), surprisingly, neither has been introduced to the American coast closest to their respective native habitats. The brown mussel, *Perna perna* was introduced from the southern Atlantic to the Gulf of Mexico in the early 1990s (Hicks & Tunnell 1993). However, the exact source of the introduction of *P. perna* is unknown; although, evidence indicates that the introduced and native populations are still linked, with high gene flow between populations (Holland 2001).

The first record of a non-native population of *C. mutica* was during the 1970s from Humboldt Bay on the Pacific coast of North-America (Marelli 1981). Since then numerous populations have become established in Europe (Platvoet et al. 1995, Heilscher 2000, ICES 2003, Tierney et al. 2004, Willis et al. 2004, Buschbaum & Gutow 2005) and on the east coast of North-America (MIT. Sea Grant 2003). There are important trade shipping routes between all these locations (Drake & Lodge 2004) and the first known non-native population, on the Pacific coast of America, was introduced either with oyster spat from Japan or in ballast water (Cohen & Carlton 1995). Aquaculture and shipping transport, including ballast water and hull fouling, are the most important modes of introductions of marine species (Eno et al. 1997, Ruiz et al. 2000). These, and rafting on drifting algae, are likely modes of transport for the non-native populations of *C. mutica* (Willis et al. 2004, Buschbaum & Gutow 2005, Chapter 2). However, the most likely pathway and origin of each introduction remains unclear. In particular, it is uncertain whether populations have developed from multiple introductions from the native range or from a succession of 'stepping stone' events. Following the establishment of the first non-native population of *C. mutica*, each new population has had at least two potential donor regions.

In this study, the genetic variability of *C. mutica* throughout its native and non-native range in the northern hemisphere have been investigated. Firstly, the data were used to test the hypothesis that populations of *C. mutica* Japan could be the source for introduced populations. The data were further used to investigate the introduction pathways of *C. mutica* at several geographic spatial scales:

- Global (Pacific and Atlantic populations)
- Oceanic (populations in the Atlantic)
- Coastal (East and West coasts of the Atlantic)
- Country (west coast of Scotland)

It has been suggested that a null-hypothesis approach is ill-suited for addressing many scientific questions, including range expansion, and a more appropriate approach is to integrate the genetic analyses with theoretic information (i.e. incorporating knowledge of the circumstances that might have led to the observed results; Anderson et al. 2000, Petersen 2006). The results are therefore discussed in relation to vectors and introduction pathways of other non-native species within the northern hemisphere, to discern the most likely scenario(s) for the introduction(s) of *C. mutica*.

Molecular techniques

Prior to Smithies (1955), genetic studies involved analysis of morphological and behavioural traits to infer gene variation. Protein electrophoresis was the first technique which allowed scientists to compare the enzymatic products of the genes. Protein electrophoresis uses migration of proteins through an agarose matrix under the influence of an electric field (gel electrophoresis) to separate proteins based on amino acid composition, the first products of gene translation. Differences between the proteins of individuals are used to estimate genetic divergence. There may be multiple biochemical processes that produce a single amino acid or enzyme, hence this technique is relatively conservative and provides limited resolution (Hamrick et al. 1979, Davies & Roderick 1999); although it is cheap and fast (May 1998). Protein electrophoresis has been used to show that founder populations of several non-native bird species generally have fewer alleles and lowered heterozygosity than source populations (Baker & Moeed 1987, Baker 1992), and to indicate multiple population bottlenecks of the invasive Mediterranean fruit fly, *Ceratitis capitata* (Huettel et al. 1980).

The analysis of Deoxyribose Nucleic Acid (DNA) is increasingly used as a tool in studies of population genetics (Avise 1994). The advantages of DNA analysis over proteins include: 1) techniques appropriate to a problem can be selected on the bases of evolutionary rate or mode of inheritance (Dowling et al. 1996); 2) the methods are generally applicable to any type of DNA (Dowling et al. 1996); 3) DNA can be prepared from small amounts of tissue and is relatively stable (Hillis et al. 1996). There are a number of genetic techniques available for DNA analysis, those which have been applied to phylogenetic analysis are described below.

Restricted fragment length polymorphisms (RFLPs) use restriction endonucleases to 'cut' the entire DNA strand into multiple fragments within a specific 4 or 6 base pair (bp) recognition sequence (restriction digestion). Agarose gel electrophoresis is then used to separate fragments of different charge, size and concentration, giving a genetic 'fingerprint' for the study organism. Amplified Fragment Length Polymorphisms (AFLPs) are based on a similar initial process, but use adapters to introduce primer annealing sites onto each DNA fragment generated by restriction digestion. A subset of fragments that have the correct combination of two adapters (a forward and a reverse primer) are amplified using the polymerase chain reaction (PCR) on an automated thermocycler. Gel electrophoresis or an automated reader is used to produce a fingerprint of the markers. In both RFLP and AFLP, changes in the fingerprint indicate insertions, deletions or substitutions in the DNA and are used to infer genetic divergence between individuals. Darling et al. (2004) used AFLP analysis to show that the genetic structure of non-native infaunal anemone, *Nematostella vectensis*, populations was significantly influenced by both anthropogenic dispersal and reproductive plasticity. Fragment analysis (AFLP) was able to distinguish between Australian and Mediterranean strains of the invasive green alga, *Caulerpa taxifolia* (Murphy & Schaffelke 2003), which could not be separated previously (Schaffelke et al. 2002). One drawback to using fragment analysis is that the techniques rely on high quantities and quality of DNA. This often results in problems with repeatability and homology (Dowling et al. 1996).

Microsatellite analysis relies on the presence of short, tandemly repeated, sequences consisting of repeat units of 1-6 bp in length. Genetic inferences can be drawn because the genetic basis of their evolution is apparent (single- or multiple-step changes in repeat number); they have high mutation rates, are abundant within the genome, generally exhibit considerable population variability, and are non-coding and, therefore, selectively neutral (Goldstein & Pollock 1997, Stoner et al. 2002). Analysis of microsatellites showed that the east coast of North America was not the source of non-native populations of *Botryllus schlosseri* to California (Stoner et al. 2002). Microsatellite analysis also revealed a high level of gene flow between, and panmixia within, populations of the brown mussel, *Perna perna*, in native and non-native locations in the south and central Atlantic and south-western Indian Oceans (Holland 2001). This evidence supported the suggestion that high genetic variability is an important characteristic of successful invasive populations (Ehrlich 1986). Several repeat unit combinations have been used, but the technique requires much development for each new species (Dowling et al. 1996, Holland 2001).

However, with the techniques described above, relatively little information is known about the source or function of the DNA analysed. For this reason, nucleic acid sequencing is a preferred technique for inferring phylogenetic history (Hillis et al. 1996). The characters (nucleotides or bases) are the basis of information coded in organisms and the potential size

of the data sets is immense (Hillis et al. 1996). A pair of short (10-20 bp) single strands of DNA (oligonucleotide primers) is used to restrict (cut) and amplify the target section of DNA (using PCR). Thousands of copies of the target DNA can be generated in a short period of time and sequenced on an automated sequence reader. In order to design the two primers to match the start and end of the sequence, and to set the PCR conditions, some information about the target sequence must be known. Providing this is the case, direct sequencing provides the most basic and reliable source of genetic information. There are several global databases that compile sequence data, the most widely used being GenBank (maintained by the U. S. National Center for Biotechnology Information at the National Library of Medicine; www.ncbi.nlm.nih.gov). Combined with the large number of published primer sequences (over 900 Primer Notes in the journal Molecular Ecology since 1996), starting a new sequencing project can be relatively straightforward. DNA sequencing was used in about one-quarter of phylogenetic studies reviewed in 1993 (Sanderson et al. 1993) and GenBank holds nucleotide sequences for over 130,000 organisms.

Having decided to use direct sequencing, an appropriate gene must be chosen, guided by information regarding the volatility and success of sequencing and presence of potential pseudogenes (non-functional, near-exact copies of the target gene, but at a different locus within the genome). Mitochondrial DNA (mtDNA) is considered to be one of the best markers for revealing phylogenetic relationships between eukaryotic groups (Okamoto et al. 1997) and is the most commonly used marker for population studies (Avise 1994). The highest rates of nucleotide substitution generally occur in mtDNA (Dowling et al. 1996). Substitutions generally occur at neutral sites in the genome. This, coupled with the clonal inheritance of mtDNA via the maternal parent in most groups, means that the genetic ‘tag’ is preserved for multiple generations. These features allow discrimination between populations showing high levels of genetic divergence, as for example, among short-lived organisms in long-distance transport phenomena (Bucklin et al. 1998). Because pseudogenes are non-coding, mutations are equally likely to occur at any nucleotide in the gene (there is no codon position bias), thus a higher proportion of replacements result in a change to the translated amino acid code (non-synonymous replacements; Sunnucks & Hales 1996); they can be dismissed by checking for open reading frames where the sequence translates into an amino acid code with no stop codons in the middle of the gene (Snyder & Gerstein 2003). However, the suitability of mtDNA for studying introduction events has been questioned due to the reduction in genetic variation following bottleneck events, amplified for mitochondrial loci due to the reduced effective population size relative to nuclear genes (Hartl & Clark 1997, Holland 2000).

Mitochondrial DNA contains both protein-coding and non-coding regions and it has been used in studies at both population and species levels (e.g. Wilson et al. 1985, Avise 1994,

Bucklin et al. 1998). Its use for inferring phylogenetic relationships has been well established for a number of years (Simon et al. 1994). The coding regions (e.g. cytochrome *c* oxidase subunit I, COI; cytochrome b, Cyt b) are relatively conserved, as a change here may alter the function of the organism and not be preserved in future generations. Substitutions will most likely occur in the third codon position, which is of less consequence for the amino acid code. Non-coding regions (e.g. Internal Transcription Spacer, ITS1; ITS2) can show a high diversity between closely related individuals and often suffer from multiple different copies within individuals. This diversity may be too high to be informative and the reliability of alignment of these sequences has been questioned (Okamoto et al. 1997). Depending on the expected or known level of divergence, different mitochondrial genes have been used for population and species-level questions. For example, 16S ribosomal RNA was used to demonstrate cryptic multiple invasions of *Carcinus maenas* and *C. aestaurii* in Japan and South Africa (Geller et al. 1997); ITS2 sequences showed strong differences between Atlantic (non-native) and Mediterranean (native) populations of the invasive polychaete, *Sabella spallanzanii* (Patti & Gambi 2001), although the source of the introduction from Europe to Australia could not be identified.

The cytochrome *c* oxidase subunit I (COI) gene is present in most eukaryotes and codes for enzymes critical to cellular metabolism (Capaldi 1990). It is among the most conserved protein-coding genes in the animal mitochondrial genome (Brown 1985) and shows a fast rate of nucleotide sequence divergence (Otto & Wilson 2001). This can be explained primarily by changes at the third codon-position, described as silent substitutions (Palumbi 1996), which makes it appropriate for intraspecific phylogenetic reconstructions. The COI gene has been used to help elucidate the invasion histories of species from many taxa (e.g. Mollusca, Renard et al. 2000, Gastropoda, Wares et al. 2002, Insecta, Grapputo et al. 2005, Crustacea, Zardus & Hadfield 2005). Multiple origins and incursions of the Atlantic barnacle, *Chthamalus proteus*, in the Pacific were determined by the sequencing of a 650-bp fragment of the COI gene (Zardus & Hadfield 2005). The COI gene provided evidence of a European source for American populations of the Chinese mitten crab, *Eriocheir sinensis* (Hänfling et al. 2002). It was also used to refute human-mediated introductions of *Littorina littorea* to North America, in favour of a historically established, ecologically constrained, population (Wares et al. 2002). The COI gene has been suggested as the most appropriate gene for barcoding of insects for biosecurity purposes (Armstrong & Ball 2005) and is the basis of the Barcode of Life initiative (http://www.barcodinglife.org/).

Several of the above techniques were considered for the analysis of the introduction pathways of *C. mutica*. The extracted DNA was of inadequate quality and quantity for AFLP analysis (for method see Appendix 3.1, pg 67). Microsatellites have not been developed for caprellid species and the use of this technique was not considered suitable for the aims of the project.

Due to its wide history of application, reliability of results, and relative ease of implementation, sequencing was the preferred technique. Several genes were chosen for present analyses, based on the availability of primers and previous success in studying the phylogeography of Crustacea.

Phylogenetic analysis

With the development of new genetic techniques and the increasing volume of genetic data, analysis of the data has also progressed. Gene sequences can be edited and aligned visually using, for example, ContigExpress (Vector NTI Advance 10, Invitrogen). Programs such as PAUP* (Swofford 2002) and ARLEQUIN (Excoffier et al. 2005) enable phylogeny to be inferred and provide statistical analysis of the genetic data. Several models of the evolutionary process have been proposed (e.g. K2P, Kimura 1980, TrN, Tamura & Nei 1993), and the Akaike information criterion (AIC, Akaike 1974) is a common method of choosing the most appropriate model for the observed data (Swofford et al. 1996). AIC quantifies the relative 'Goodness-of-fit' of a previously defined model, given a sample of data. The preferred model is that with the lowest AIC. This will be the simplest model (with the fewest parameters) that most closely explains the data.

A neighbour-joining phylogram (phylogenetic tree) describes the evolutionary relationships between sequences of the same gene and is constructed by joining the closest related sequences in the population (neighbours). The neighbours are combined at successive levels of divergence until all individuals in the population are represented in the phylogram (Saitou & Nei 1987); neighbours can therefore be considered evolutionarily close or similar. Lengths of the branches in the phylogram indicate genetic divergence and have been used to estimate the rate of molecular evolution (Kimura 1968, Knowlton & Weigt 1998). The bootstrap provides an estimate of confidence in the branches (nodes) of a given phylogram (Felsenstein 1985). Further confidence in the base of the phylogram can be gained by assigning the most distantly related sequence as an outgroup (Watrous & Wheeler 1981). Outgroups are often members of a different species or a geographically isolated population.

Phylograms present the data as terminal nodes on a branching tree; however, with population studies, individuals may be internal nodes or ancestors to other individuals in the population (Clement et al. 2002). Networks allow incorporation of non-bifurcating genetic information and presentation of more complex evolutionary scenarios (e.g. recombination, hybridization; Huson & Bryant 2006). Identical sequences are grouped as a haplotype, the frequency of a given haplotype is taken as an estimate of haplotype age (Castelloe & Templeton 1994).

Pairwise comparisons between all haplotypes are used to generate a network, with nodes representing a mutational step. The number of nodes (mutational steps) between two haplotypes, rather than the length of the branch, is an estimation of genetic divergence.

Sequencing is the most sensitive molecular technique and hence can generate a huge amount of variation, the meaning of which can be difficult to discern. Because substitutions are most likely at the third codon position in protein-coding genes (rather than the first or second in the triplet) and a change here is often of no consequence for the amino acid code, phylogenetic studies have recently started to dilute the importance of the third codon in the analyses (Delsuc et al. 2003, Phillips & Penny 2003, Phillips et al. 2004, Stevens et al. 2006), thus reducing the variation in the data set. There are several levels of dilution (also known as weighting), the most extreme of which is exclusion of third codon positions from the analysis. Excluding the third codon positions has generally been used in investigations of higher levels of phylogenetic classification e.g. among avian species (Paton et al. 1995), xenarthrans (armadillos, anteaters and sloths; Delsuc et al. 2003) and Collembola (Frati et al. 1997). A less severe approach is to code the third codons as purines (R; nucleotides A and G) and pyrimidines (Y; nucleotides C and T) (RY coding), thus weighting transversions (R - Y or Y – R substitutions, which are evolutionarily less frequent) greater than transitions (R – R or Y – Y substitutions) in the analyses. RY coding the third codon positions reduces the amount of 'noise' whilst retaining the phylogenetic information (Harrison et al. 2004, Phillips et al. 2004). This method was used to investigate the root of the mammalian tree (Phillips & Penny 2003), to review hexapod phylogeny and to resolve deep branches (Delsuc et al. 2003, Stevens et al. 2006), and to investigate the divergence of modern avian lineages (Harrison et al. 2004). These methods can be useful when investigating alternative relationships (i.e. phylogeography) which may be confounded by the level of diversity found when including all nucleotide positions.

Pairwise F-statistics (F_{ST}) analyse the observed genetic variation within a sample of individuals that can be partitioned by dividing the individuals into two defined populations. F_{ST} estimate the genetic 'distance' between the populations; a high F_{ST} value indicates a large genetic divergence. Fixation indices (Φ-statistics) incorporate a further level of grouping, and enable analysis of genetic divergence within, versus between populations (Harrison et al. 2004). Φ-statistics generate 3 values: Φ_{ST} estimates the amount of divergence in the whole sample explained by the lowest grouping of individuals (e.g. by sample site; equivalent to F_{ST} analysis of more than two groups); Φ_{CT} is an estimate of the amount of divergence in the individuals explained by the second level grouping of individuals (e.g. by continent; once again similar to F_{ST}); and Φ_{SC} is an estimate of the amount of divergence explained by the

two levels of groupings (e.g. sample site within continent). A high Φ-value indicates that the majority of the variation is explained by the grouping. Assumptions of both methods (e.g. random sampling to create the initial subdivisions, genetic drift and no migration) are rarely met in natural populations; however, the methods remain useful ways of investigating phylogenetic data (Excoffier et al. 1992).

3.2 Materials and methods

Several extraction techniques, sequencing primers, and the use of AFLP analysis were assessed during the study. The techniques used for the final analysis are described below. Supplementary information concerning the other techniques is presented in Appendix 3.1 (pg 67).

Sample collection and preparation

Specimens of *Caprella mutica* were collected from 26 sites in 9 countries in the northern hemisphere (Table 3-1); this included 4 sites in Japan and 22 non-native sites, 11 of which were on the west coast of Scotland. Populations could be grouped at several spatial scales: northern-hemisphere (native vs non-native), oceanic (native, Atlantic and Pacific), coastline (native, east Pacific, east and west Atlantic) and on the west coast of Scotland. *Caprella mutica* were collected from filamentous algae from the surface water and isolated from the algae using forceps. At each site, 10 females and 10 males were collected (dependent on availability) and immediately preserved in 100% ethanol. Individuals were confirmed to be *C. mutica* (Arimoto 1976) under a stereomicroscope. *Caprella acanthogaster* and *C. equilibria* were used as out-groups (Cap1 and Cap2, Table 3-1).

Tissue for DNA extraction was removed from the caprellid appendages (gnathopods, pereopods, antennae and gills) using a stereomicroscope, taking care to avoid contact with the gut. DNA was extracted using a combination of the 'salting-out' procedure (Sunnucks & Hales 1996) and a GenElute Mammalian DNA Miniprep Kit (SIGMA). DNA concentration was estimated using a Nanodrop Spectrophotometer (Nanodrop Technologies).

Mitochondrial DNA analysis

A ~600-bp fragment of the cytochrome *c* oxidase subunit I gene (COI) was amplified using the polymerase chain reaction (PCR; Saiki et al. 1988) on a Biometra gradient thermocycler. The 10 µL reaction volume consisted of 1 µL (~20 – 50 ng) DNA, 10x PCR buffer (Roche), 1.75 mM $MgCl_2$, 0.2 mM dNTP, 0.4 µM of each primer and 0.3 U *Taq* DNA polymerase

(Roche). Thermal cycling conditions were: 94 ˚C for 1 min followed by 35 cycles of denaturation at 94 ˚C for 1 min, annealing at 45 ˚C for 1 min and extension at 72 ˚C for 1 min, followed by a final extension at 72 ˚C for 5 min (modified from Witt & Hebert 2000). Negative controls were included with at least 1 per 10 reactions.

The COI3F (forward; 5' AGG AGA TCC TAT CCT TTA CC 3'; this study, Appendix 3.1, pg 67) and COI2R (reverse; 5' GGR TAR TCW GAR TAW CGN CGW GGT AT 3'; Otto & Wilson 1991) primer pair were used. Agarose gel electrophoresis using SYBR stain (Invitrogen) was used to visualise PCR products, which were then purified using SAP/Exo (USB Corp.; Hanke & Wink 1994). Sequencing reactions were performed using BigDye™ Terminator chemistry (Applied Biosystems Inc.), with the forward primer (COI3F) for all samples, and with the reverse primer (COI2R) for approximately 25 ambiguous/unique samples. Sequencing was performed using an ABI3730 automated sequencer (Applied Biosystems Inc.) at the Allan Wilson Centre Genome Service, Massey University, New Zealand.

Table 3-1. Sample information including oceanic province, source country, site location and description and number of COI sequences obtained per site (n). Samples in italics are non-*Caprella mutica* sequences (outgroups). Each population has a unique location code.

Oceanic Province	Country	Site	Location code	Site description	n
Sea of Japan	Japan	Port of Nagoya, Honshu	Jap1	Aquarium	19
		Mutsu Bay, Honshu	Jap2	Natural habitat	4
		Usujiri, Hokkaido	Jap3	Natural habitat	10
		Saroma Lake, Hokkaido	Jap4	Natural habitat	18
Pacific	USA	San Francisco	SaF	Nearshore Oil Plaform	20
	Australia	*Hobart, Tasmania*	*Cap1*	*Salmon Farm*	*2*
	New Zealand	*Akaroa*	*Cap2*	*Salmon Farm*	*3*
Atlantic	Belgium	Zeebrugge	Bel	Harbour	10
	Canada	Passamaquoddy Bay	Can1	Mussel Farm	18
		Chaleur Bay	Can2	Mussel Farm	10
	England	Poole	Eng	Harbour	2
	France	Le Havre	Fra	Harbour	22
	Germany	Helgoland	Ger	Harbour	20
	Ireland	Betraghboy Bay	Ire1	Aquaculture	17

	Dun Laoghaire Bay	Ire2	Harbour	8
Norway	Raunefjorden	Nor	Harbour	19
Scotland	All sites	Scot	Salmon farms	103
	Lismore	Lis	Salmon farm	17
	Calbha Bay	Cal	Salmon farm	12
	Cheese Bay	Che	Salmon farm	5
	Loch Carnan	Car	Salmon farm	7
	Loch Erisort	Eri	Salmon farm	7
	Loch Kishorn	Kis	Salmon farm	13
	Loch Melfort	Mel	Salmon farm	10
	Loch Roag	Roa	Salmon farm	5
	Loch Seaforth	Sea	Salmon farm	9
	Lochboisdale	Boi	Salmon farm	10
	Lochmaddy	Mad	Salmon farm	8

DNA sequence analysis

Sequences were verified as Caprellidae DNA using the GenBank™ BLASTn search (Altschul et al. 1990). In addition, sequences were checked for open reading frames (using MacClade ver. 4.05; Maddison & Maddison 2000) to confirm the absence of nuclear copies or other unintended sequence types (e.g. pseudogenes). Preliminary analyses using mtDNA (COI) sequence fragments for three species in the genus *Cyamus* (Suborder Caprellidea) obtained from GenBank™ (*Cyamus erraticus,* accession no. DQ095135; *C. gracilis,* DQ095105; *C. ovalis,* DQ09150) identified *Caprella equilibria* as the most divergent outgroup, and this was used as the outgroup for subsequent analyses. Sequences were edited and aligned using ContigExpress (Vector NTI Advance 10, Invitrogen) and trimmed to a common length. Sequences were analysed using PAUP* 4.0b10 (Swofford 2002) and ARLEQUIN 3.0 (Excoffier et al. 2005).

χ^2-tests (implemented in PAUP*) were used to test the hypothesis of homogeneity of base frequencies among sequences. MODELTEST 3.7 (Posada & Crandall 1998) was used to determine the appropriate model parameters for maximum likelihood (ML), the TrN+Γ model (-lnL = 1205.14 (AIC); rate matrix: A-C = 1.00, A-G = 39.93, A-T = 1.00, C-G = 1.00, C-T = 14.77, G-T = 1.0; with base frequencies set to A = 0.2339, C = 0.2940, G = 0.1090, T = 0.3631; Γ = 3.1385) was found to be the best fit to the data; all other options in PAUP* remained as default for the ML heuristic analyses. Sequences with the 3rd codon RY coded (A & G→R; C & T→Y) and the 3rd codon excluded were also analysed in PAUP*. The TrN+Γ model was the best fit to all data sets. ML bootstrap analyses were conducted with 100 replicates (Felsenstein 1985). Relationships among mtDNA sequences were estimated via a

haplotype network using the statistical parsimony method (Templeton et al. 1992) in TCS version 1.13 (Clement et al. 2000).

ARLEQUIN 3.0 was used to calculate pairwise F_{ST} measures between sites, and Φ-statistics to compare genetic differentiation among geographic regions using analysis of molecular variance (AMOVA). To analyse the geographic extent of population differentiation, three groupings were used for the AMOVA tests: (A) native vs non-native, (B) oceanic province (native, east Pacific and Atlantic), and (C) coastline (native, east Pacific, east and west Atlantic). Sites in Scotland were treated as a single sample for comparisons among geographic regions, further pairwise F_{ST} measures were calculated between sites on the west coast of Scotland.

3.3 Results

DNA sequence characteristics

DNA was extracted, amplified and sequenced from a total of 51 *Caprella mutica* individuals from the native area in Japan, and 249 individuals from non-native populations. All unique sequences for each species are published on GenBank™ (accession numbers: DQ466220-466523). Following editing and alignment, sequences were trimmed to a length of 563-bp. No insertions or deletions were detected in the data. Overall base frequencies were biased towards A and T (A = 26%, T = 36%, C = 20%, G = 18%), common for arthropod mtDNA (Simon et al. 1994). We were unable to reject the hypothesis of homogeneity of base frequencies among sequences for all sites ($\chi^2_{153} = 5.05$, $P = 1.00$), for the 170 variable sites ($\chi^2_{153} = 19.23$, $P = 1.00$), or the third codon sites ($\chi^2_{153} = 17.17$, $P = 1.00$).

When the outgroups were included in the analysis, there were 170 polymorphic sites; 60 of these were variable among the 300 *C. mutica* sequences (Table 3-2); 13.33%, 3.33% and 83.33% were at the first, second and third codon positions, respectively. This ratio is very similar to that found in the COI gene of *Chthamalus proteus* barnacles (13.2%, 3.3% and 83.6%; Zardus & Hadfield 2005). Sequence divergence of *C. mutica* haplotypes ranges from 0.2% to 3.4% (Appendix 3.2, pg 71). Thirty-one of the thirty-eight unique haplotypes were observed in the native range, none of these was shared between locations. There were two amino acid changes among sequences in the native range (Table 3-2), one in haplotype 1 (Threonine - Alanine) and one in haplotype 23 (Threonine - Isoleucine). The geographic distribution of the haplotypes is shown in Figure 3-1. Haplotypes F and G (unique to San Francisco) also had an amino acid change from Threonine to Isoleucine at a different position.

In the Atlantic populations there were five unique haplotypes (A - E) with no amino acid changes.

Native region (Japan)

In the native range, high variability but no genetic structure was identified (Figure 3-2 & Figure 3-5). No major clades were resolved and haplotype groups did not represent clear source locations for non-native populations. Haplotype diversity was lower at Port of Nagoya (Jap1, $h \pm SD = 0.621 \pm 0.121$;Appendix 3.3, pg 73) and Usujiri (Jap3, $h \pm SD = 0.867 \pm 0.085$; Appendix 3.3, pg 73) compared with the 100% diversity in samples from the other two sites. Nucleotide diversity was fairly constant at all four sites (Figure 3-2 & Figure 3-5). Pairwise F_{ST} values were small between all native sites (-0.015 to 0.258), and statistically significant between Port of Nagoya, Usujiri and Saroma Lake (Jap1, Jap3 and Jap4; Appendix 3.4, pg 74). The non-significance of pairwise F_{ST} values with Mutsu Bay (Jap2) was most likely due to the small sample size.

Table 3-2. The 60 variable nucleotide sites among *Caprella mutica* haplotypes. *C. mutica* haplotype 1 is used as a reference sequence. Locations where each haplotype was found are indicated using location codes from Table 3-1 and haplotypes are in parentheses. Identical character states are indicated by dots. Node numbers and nucleotides in bold indicate amino acid changes.

	1111111111112222222222223333333333444444**44**45**5**5555555
	1677889001222333558023345677888012456678802344**77**80**0**1344455
Taxon/Node	6832878608703925806645156013602595427098178984725**6**31**3**6406723
Jap1 (1)	ATACCATTTTAGCTACTCCAAGTCATGAATAGTTAAGCCGCCTCCGCC**GC**AT**C**TTAGCGT
Jap1 (2)	.C....C.........T.............................**A**.......A..C
Jap1 (3)	......C.........................C.............**A**....C..A.AC
Jap2 (4)	......C.........................C.............**A**.......A..C
Jap3 (5)	......C.........................C..........A..**A**.......A...
Jap3 (6)	G.....C.........................C...T.....T....**A**.........C
Jap2 (7)	...T..C.........................CG............**A**........TTC
Jap1 (8)	......C...G.....C..............CG............**A**.........C
Jap3 (9)	...T..C..................A......CT............**A**........T.C
Jap3 (10)	...T..C.................G.A......C............**A**........TCC
Jap3 (11)	...T..C....A.............A......C............**A**.........C
Jap4 (12)	......C.........................CG..........**A**..C.......C
Jap4 (13)	......C.........................CG.....T.C.....**A**..C.....T.C
Jap1 (14)	...TT.C..................A......C..........T...**A**........T.C
Jap4 (15)	......C...........T...........G..CG....A........**A**.........C
Jap4 (16)	.C....C...G.......T....T......GA.CG....A.T......**A**..........
Jap4 (17)	...T..C.C.......................C.G...........**A**.........C
Jap4 (18)	...T..C.C.......................C.G...........**A**......G...C
Jap4 (19)	...T..C.C.....................CC.G...........**A**.........C
Jap4 (20)	...T..C.........G.....C...A......C.............**TA**.....C.....
Jap2 (21)	......C.........G..G......A......C.............**TA**.........C
Jap1 (22)	......C.........G.........AG.....C.............**TA**.G..G.....C
Jap4 (23)	......C.....T..TG.........A......CC............**AT**..........
Jap4 (24)	......C.........G...G.....A......CG............**A**..........
Jap3 (25)	......C.........G.....C...A.....CCG............**A**..........
Jap4 (26)	......C...G.....A.....C...A......CG...T.........**A**..........
Jap4 (27)	......C.........G.....C...A.G....CG............**A**..........
Jap4 (28)	......C.........G.....C...A......CG............**A**..........
Jap4 (29)	..G...C......C..G.....C...A......CG...........T.**A**..........
Jap4 (30)	......C.........G....AC...A......CG............**A**..........
Jap4 (31)	......CC........GT........A......CG.A.T.........**A**..........
Bel (A)	......CC.....................C...CG...T.........**A**..C..C....C
Bel (B)	......CC...A...T.............CT..CG.........T...**A**..C..C....C
Can1 (C)	......C.........T.......C.......CG............**A**..C......TC
Can1 (E)	......C...................A......CG...T.........**A**..C..C....C
Can1 (A)	......CC........T............C...CG...T.........**A**..C..C....C
Can1 (B)	......CC...A...T.............CT..CG.........T...**A**..C..C....C
Can2 (A)	......CC........T............C...CG...T.........**A**..C..C....C
Can2 (B)	......CC...A...T.............CT..CG.........T...**A**..C..C....C
Eng (A)	......CC.....................C...CG...T.........**A**..C..C....C
Eng (B)	......CC...A...T.............CT..CG.........T...**A**..C..C....C
Fra (A)	......CC.....................C...CG...T.........**A**..C..C....C
Ger (A)	......CC.....................C...CG...T.........**A**..C..C....C
Ger (B)	......CC...A...T.............CT..CG.........T...**A**..C..C....C
Ire1 (A)	......CC.....................C...CG...T.........**A**..C..C....C
Ire2 (B)	......CC...A...T.............CT..CG.........T...**A**..C..C....C
Nor (A)	......CC.....................C...CG...T.........**A**..C..C....C
Nor (C)	......C.........T.......C.......CG............**A**..C......TC
Scot (D)	......C.........A.........A......CG...T.........**A**..C.......C
Scot (A)	......CC.....................C...CG...T.........**A**..C..C....C
Scot (B)	......CC...A...T.............CT..CG.........T...**A**..C.......C
SaF (F)	.........C....G...T...........GACCG....A........**A**...**T**.T...AC
SaF (G)	.....G...C....G...T...........GACCG....A........**A**...**T**.T...AC

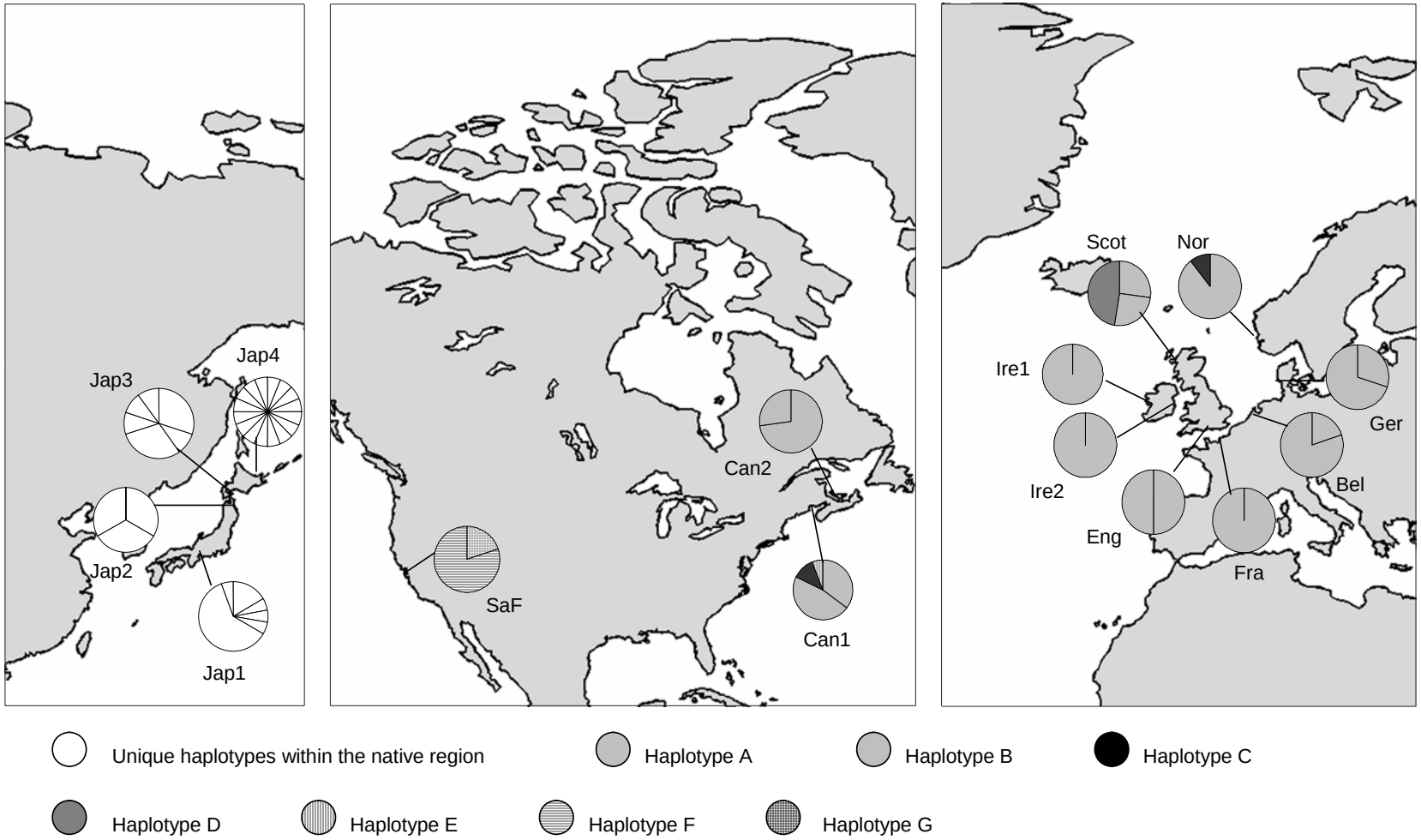


Figure 3-1. Geographic distribution of the 31 COI haplotypes of *Caprella mutica* in the northern hemisphere. Each site is represented by a pie chart showing haplotype frequency. Site codes correspond to Table 3-1. All haplotypes in the native range are unique to a single location, represented by white pie fill. Pie shading in the non-native sites corresponds to the haplotypes in Table 3-2 (see legend).

Phylogenetic comparisons

F_{ST} values were statistically significant and greater between native and non-native sites (Appendix 3.4, pg 74). The exception to this was the non-significance of the F_{ST} between England and Mutsu Bay (Jap2), most likely due to the small sample sizes at both these locations. AMOVA tests resulted in significant genetic differences within regions, among sites within regions, and among regions at all geographic levels (Table 3-3).

Seven haplotypes were identified in the non-native populations (Figures 3-1 & 3-2), and there was considerable genetic differentiation between populations (total non-native $\Phi_{ST} = 0.612$). Figure 3-1 shows the geographic distribution of the native and non-native haplotypes. Haplotypes F and G, found only in San Francisco, were the most divergent with genetic distance (TrN+Γ model) ranging from 1.2% to 3.4% compared to all other haplotypes (Appendix 3.2, pg 71). All three population groupings were significant (native vs non-native, oceanic province, and coastline; Table 3-3). Grouping by oceanic province (i.e. native, east Pacific and Atlantic) explained the most (59%) among-region variance ($\Phi_{CT} = 0.592$, $P < 0.001$; Table 3-3).

The neighbour joining phylogram with all nucleotide positions included indicates that haplotype C was the most basal haplotype sampled (Figure 3-2). Independent of the treatment of the data, the non-native haplotypes consistently clustered into four groups: A & B (1), F & G (2), D & E (3) and C (4; Figures 3-2, 3-3 & 3-4). However, the rooting of each group within the phylogram changed depending on the weighting of the 3rd codon. With no weighting (Figure 3-2) haplotypes A and B, F and G grouped closely with haplotypes from Jap4. Haplotypes D and E did not link closely with any of the haplotypes from Japan. Haplotype C was independent and basal. RY coding differentiated haplotypes A and B from the Japanese haplotypes, with haplotypes F and G then grouping most closely with Jap1; haplotypes D and E group with Jap1 and Jap2; haplotype C remained independent (Figure 3-3). Removing the 3rd codon from the analysis maintained the general groups of the RY coding analysis, although haplotypes D and E regrouped with haplotypes from all locations in Japan (Figure 3-4). The four groupings were also apparent in the haplotype network, constructed without weighting (Figure 3-5). The low bootstrap support most likely reflects the small genetic differences between these intraspecific sequences.

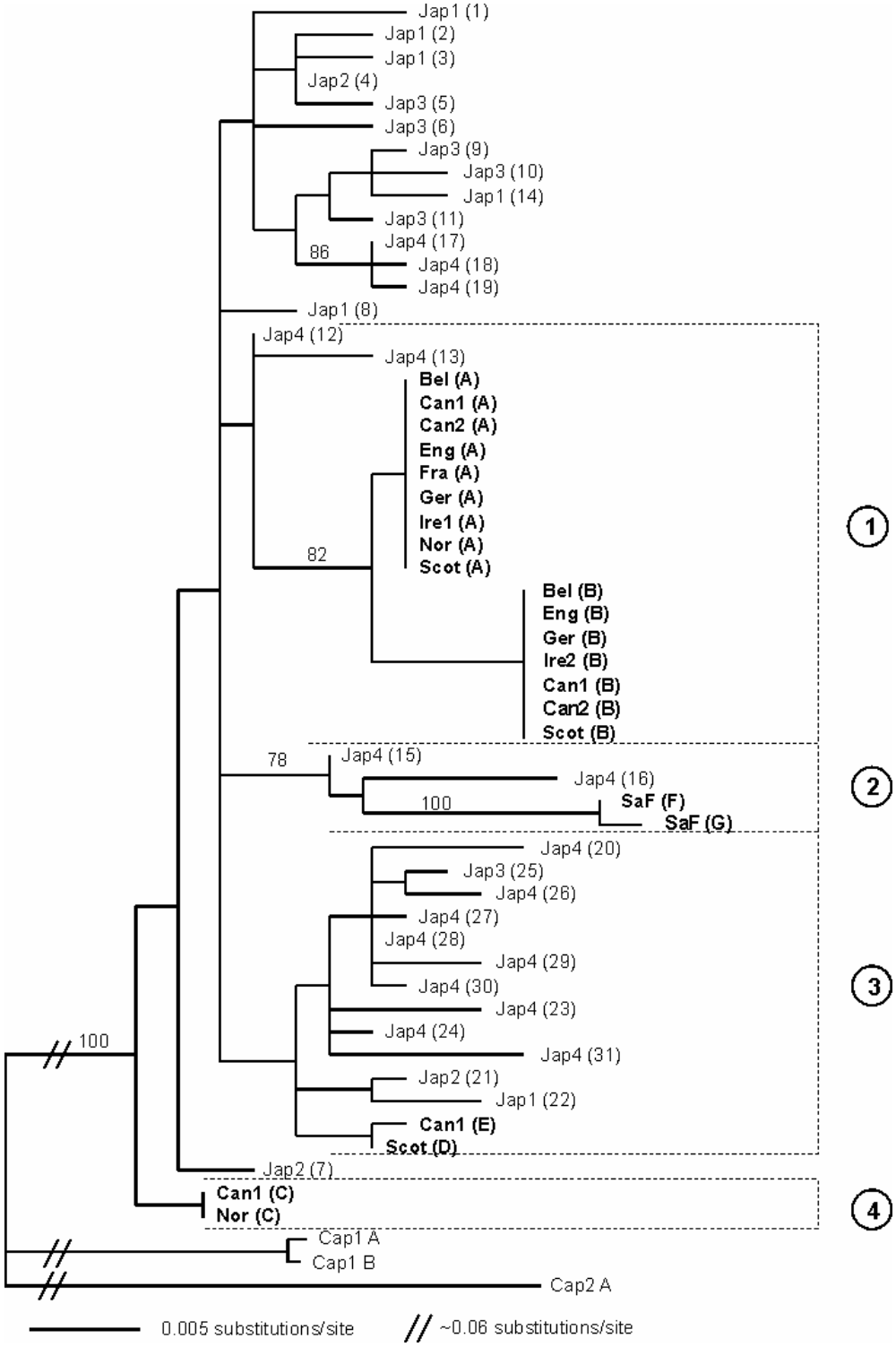


Figure 3-2. Neighbour joining phylogram of 15 *Caprella mutica* populations from its native and non-native range (Scottish data are represented as a single population). *Caprella acanthogaster* and *Caprella equilibria* haplotypes were included as outgroup taxa (Cap1 and Cap2 respectively). Numbers above branches are bootstrap confidence limits (100 replicates) greater than 70%. Population codes correspond to Table 3-1, characters in parentheses correspond to the haplotypes in Table 3-2, bold text indicates non-native locations, dashed lines and numbers indicate groups referred to in the text.

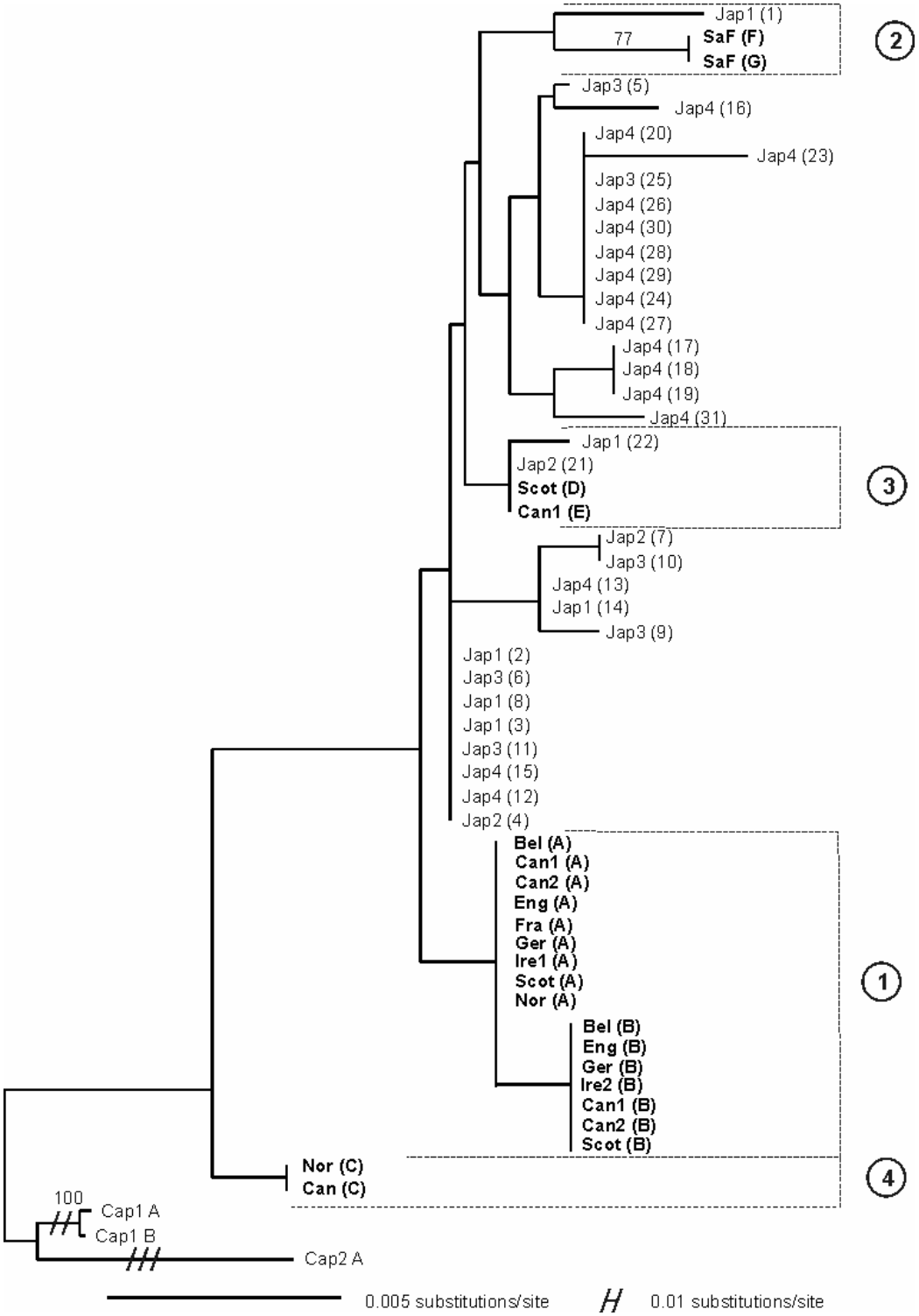


Figure 3-3. Neighbour joining phylogram, 3rd codon position RY coded, of 15 *Caprella mutica* populations from its native and non-native range (Scotland data are represented as a single population). *Caprella acanthogaster* and *Caprella equilibria* haplotypes were included as outgroup taxa (Cap1 and Cap2 respectively). Numbers above branches are bootstrap confidence limits (100 replicates) greater than 70%. Population codes correspond to Table 3-1, characters in parentheses correspond to the haplotypes in Table 3-2, bold text indicates non-native locations, dashed lines and numbers indicate groups referred to in the text.

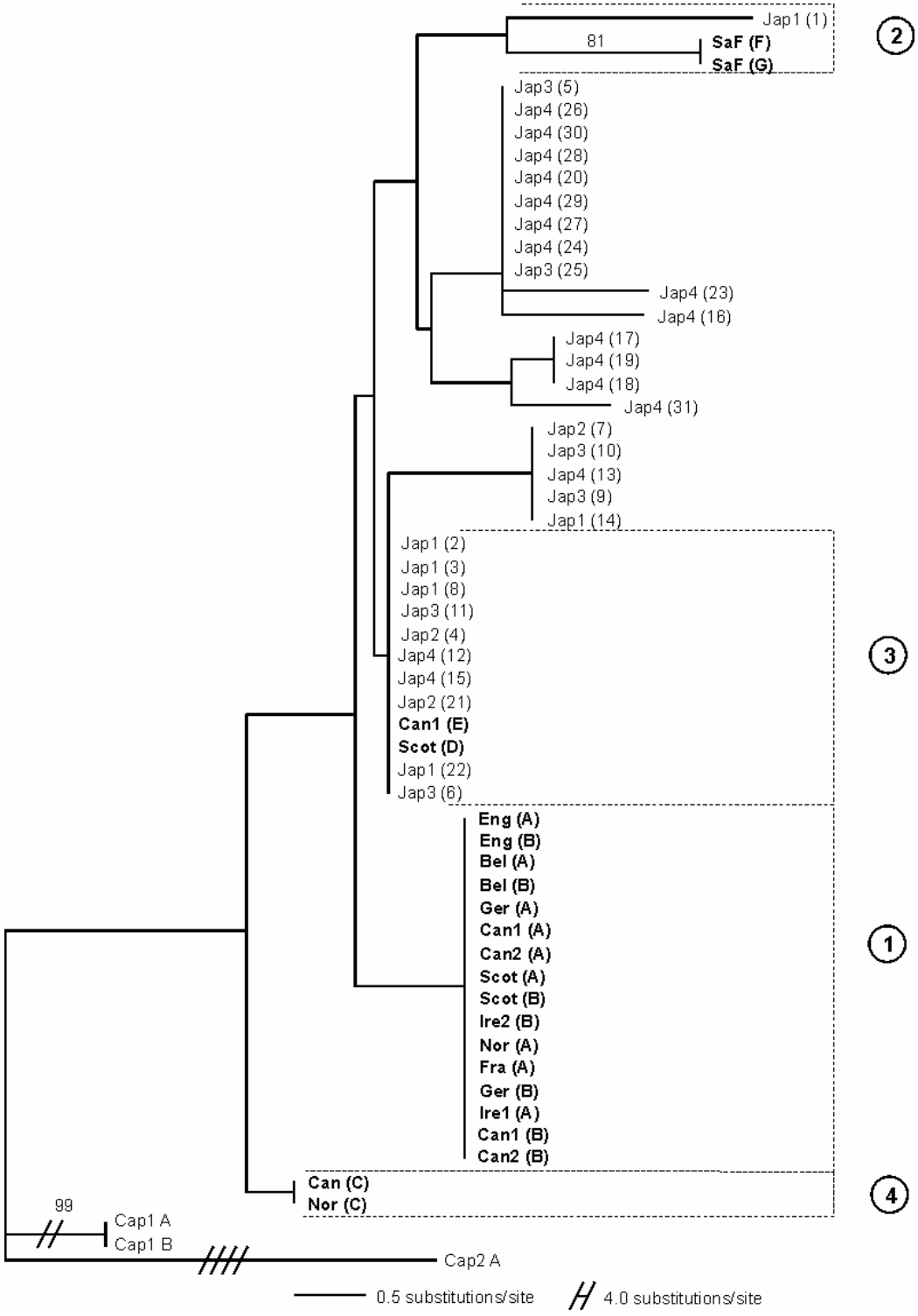


Figure 3-4. Neighbour Joining phylogram of 1st and 2nd codon positions only of 15 *Caprella mutica* populations from its native and non-native range (Scotland data are represented as a single population). *Caprella acanthogaster* and *Caprella equilibria* haplotypes were included as outgroup taxa (Cap1 and Cap2 respectively). Numbers above branches are bootstrap confidence limits (100 replicates) greater than 70%. Population codes correspond to Table 3-1, characters in parentheses correspond to the haplotypes in Table 3-2, bold text indicates non-native locations, dashed lines and numbers indicate groups referred to in the text.

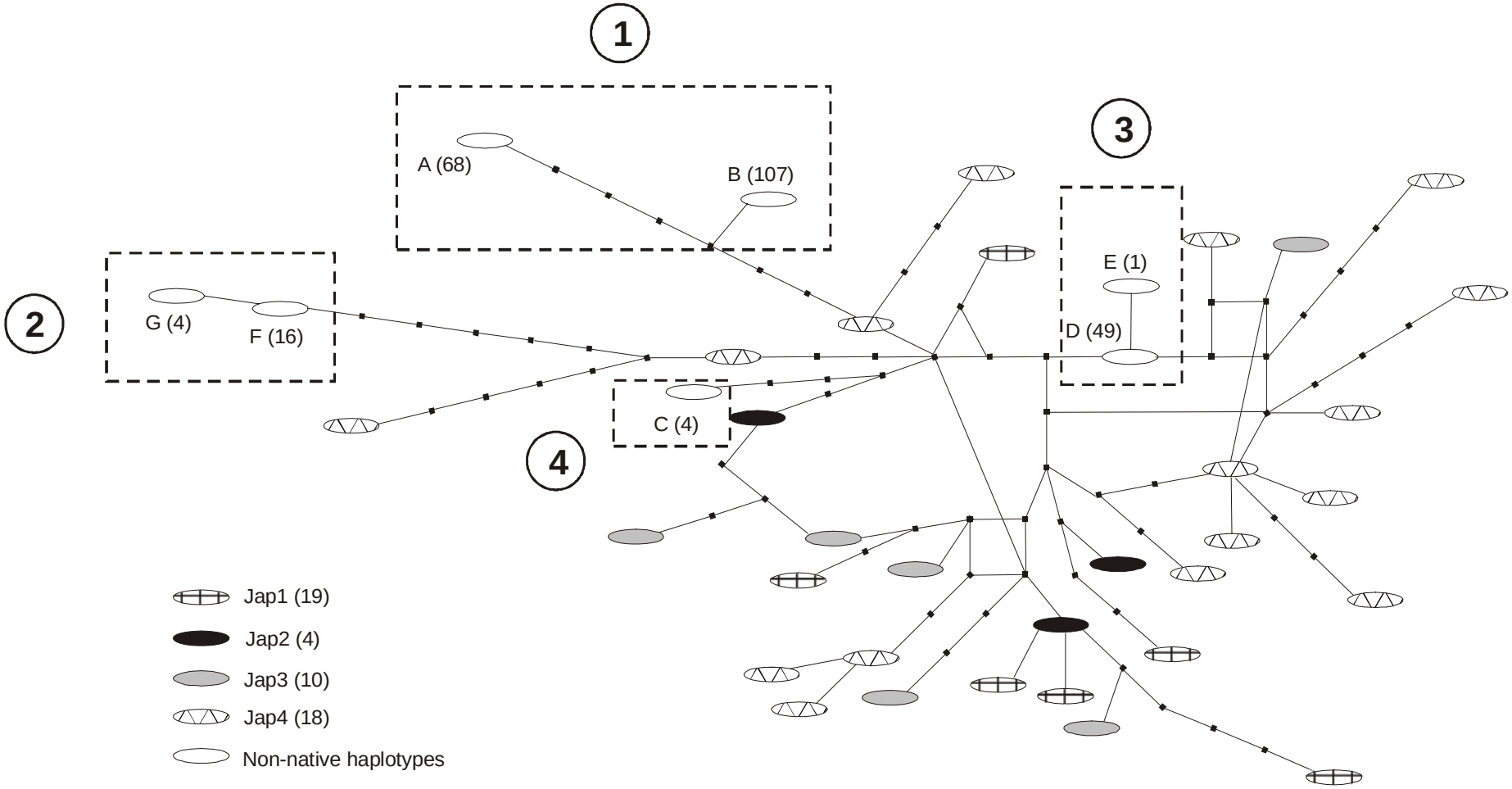


Figure 3-5. Haplotype network for the 38 COI haplotypes of *Caprella mutica* from its native and non-native range, estimated by statistical parsimony (Templeton et al. 1992). Lines show most-parsimonious relationships between individual haplotypes, represented by ovals. Nodes along each branch designate the number of base pair differences between haplotypes. Ovals representing native haplotypes have a fill pattern corresponding to the geographic source (see legend). Non-native haplotypes are labeled corresponding to Table 3-2 (A-G). Numbers of sequences are in parentheses. Dashed lines and numbers indicate groups referred to in the text.

Table 3-3. Results of the AMOVA tests comparing variation in COI sequences of *Caprella mutica* grouped at 3 geographic levels: (A) native vs non-native, (B) oceanic province (native, east Pacific and Atlantic), and (C) coastline (native, east Pacific, east and west Atlantic).

Group	Source of variation	d.f.	SS	Variance components	% of variation	*Φ*ST	*Φ*SC	*Φ*CT
A	Among regions	1	155.610	1.46553	30.01			0.300*
Native vs Non-native	Among sites within regions	13	441.875	1.84307	37.75		0.539*	
	Within sites	281	442.340	1.57416	32.24	0.678*		
	Total	295	1039.824	4.88277				
B	Among regions	2	415.594	3.43525	59.21			0.592*
Oceanic province	Among sites within regions	12	181.890	0.79207	13.65		0.334*	
	Within sites	281	442.340	1.57416	27.13	0.728*		
	Total	195	1039.824	5.80149				
C	Among regions	3	422.480	2.45480	50.31			0.503*
Coastline	Among sites within regions	11	175.004	0.85031	17.43		0.351*	
	Within sites	281	442.340	1.57416	32.26	0.677*		
	Total	295	1039.824	1.87927				

* Significant at $P < 0.0001$; statistical probabilities derived from 1023 permutations

The five haplotypes of *C. mutica* present in the Atlantic (A-E) were genetically distinct from those found in San Francisco (2.4 - 3.2% genetic divergence, Appendix 3.2, pg 71; 0.782 - 0.994 significant pairwise F_{ST} values, Appendix 3.4, pg 74). Passamaquoddy Bay (Can1) had the highest haplotype diversity followed by Scotland ($h \pm SD = 0.677 \pm 0.075$ & 0.642 ± 0.022 respectively, Appendix 3.3, pg 73). Haplotypes A and B were widely distributed on both coasts of the Atlantic (Figure 3-1). Haplotypes D and E were present only in Scotland and Canada, respectively. All individuals from France, and from the two locations in Ireland, only had a single haplotype (A or B, the populations in Ireland were not the same haplotype). Populations from Scotland, Norway, Belgium and Germany had at least 2 haplotypes, shared with individuals from at least 1 other location. Pairwise comparisons of F_{ST} values within the Atlantic returned varied and not always significant results, ranging from 0 to 1 (Appendix 3.4, pg 74). The Φ_{ST} value for all populations in the Atlantic was 0.363, and in Europe was 0.417.

Three haplotypes (A, B, D) were present on the west coast of Scotland (Figure 3-2 & Figure 3-5). Haplotype A was widely distributed on the mainland and at two sites on the Outer Hebrides; Haplotype B occurred at southern mainland sites and two locations on the Outer Hebrides. Haplotype D was only present at northern mainland sites and dominated on the Outer Hebrides (Figure 3-6). The Φ_{ST} value for all populations on the west coast of Scotland was 0.560. Pairwise comparisons of F_{ST} values between sites returned a wide range of values (-0.055 to 0.848) with varying levels of significance (Appendix 3.5, pg 74).

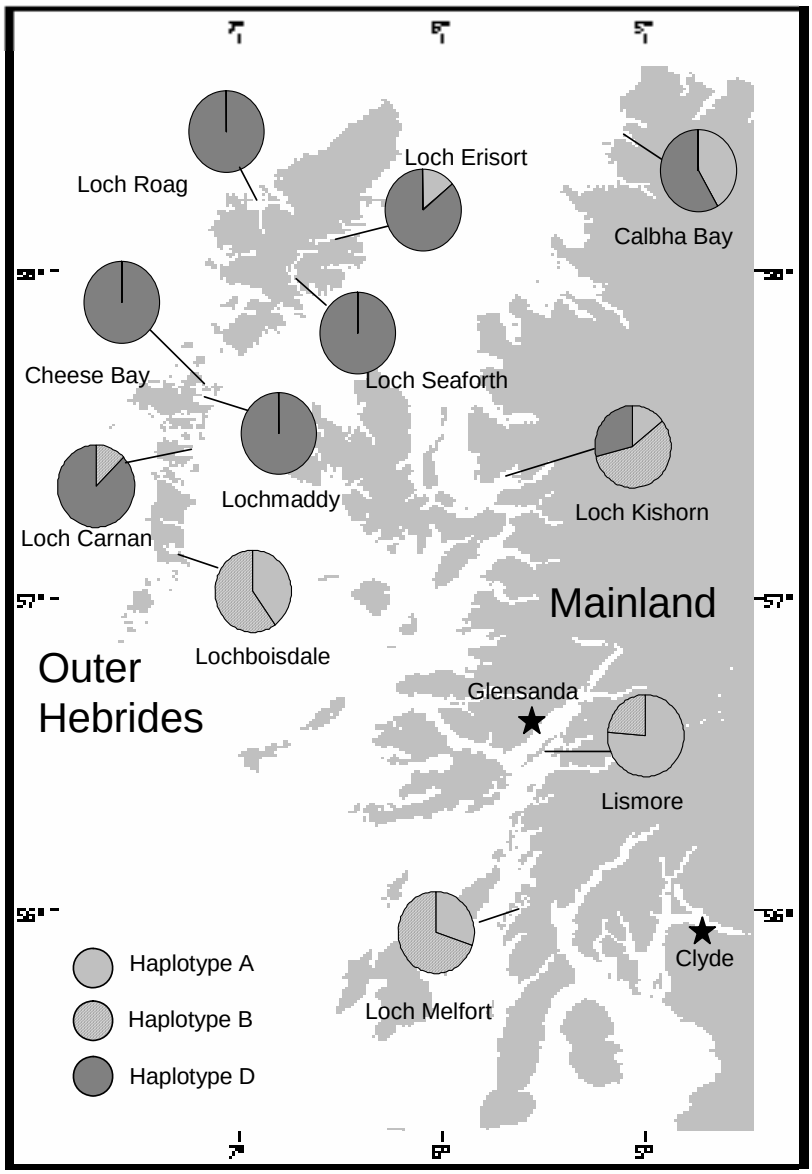


Figure 3-6. Geographic distribution of the 3 COI haplotypes of *Caprella mutica* on the west coast of Scotland. Each site is represented by a pie chart showing haplotype frequency. Pie fills correspond with haplotypes in Figure 3-2 (see legend). Stars indicate major commercial ports (Scottish_Transport_Statistics 2005).

3.4 Discussion

The aims of this study were to test if populations in Japan could be the source for introduced populations of *Caprella mutica*, and to investigate the introduction pathways of *C. mutica* on several geographic scales: Global, Oceanic, Coastal and within a single country (Scotland). In most geographic locations, the data could not identify the entry point of *C. mutica*, nor confirm subsequent pathways; however, several pathways will be discussed and it is likely that multiple introduction pathways and stepping stone events exist. The genetic information will be integrated with that of previous studies to provide the most likely introduction scenario(s).

Japan as an introduction source

The assumption from the beginning of the study was that Japan was in the native range of *C. mutica*. In this respect, native populations of *C. mutica* in Japan had a high level of genetic diversity with negligible geographic subdivision. The low F_{ST} values suggest that gene flow between populations in Japan is high, and although the lack of shared haplotypes contradicts this somewhat, haplotype groups generally contained individuals from more than one populations (e.g. group 3, Figure 3-2). The haplotype network suggests large diverse population sizes that are inter-connected in Japan (Figure 3-5). High levels of genetic diversity in the native range have been described as a characteristic of invasive species (Ehrlich 1986, Ehrlich 1989), which may be a result of the widespread and gregarious nature of many invasive species in their native habitats (Duda 1994). Thus, it is important to include a large number of individuals from sites throughout the native range in order to identify source populations with any confidence.

Several of the introduced haplotypes group closely with those from the populations in Japan (indicated by groupings in Figures 3-2 to 3-5), suggesting that these might be the source populations (e.g. Jap4). This is also supported by the results when the importance of the third codon is diluted (Figures 3-3 & 3-4), when haplotypes D and E show no divergence from populations in Japan. However, several factors suggest that the exact source population(s) have not been sampled, or that the sample size from these potential source populations was too small: i) the groupings are not consistent to all phylograms, ii) there is a high level of divergence between native and non-native populations, and iii) the non-native populations do not share haplotypes with any of the sites in Japan. Furthermore, haplotype C is basal to the other *C. mutica* haplotypes in the phylogram (Figure 3-2), indicating that the populations sampled in

Japan were definitely not the source for this haplotype. The high level of genetic divergence within the native populations sampled in Japan, and between these and the non-native populations, suggests geographic isolation with limited exchange between populations in the native range.

Global introduction pathway(s)

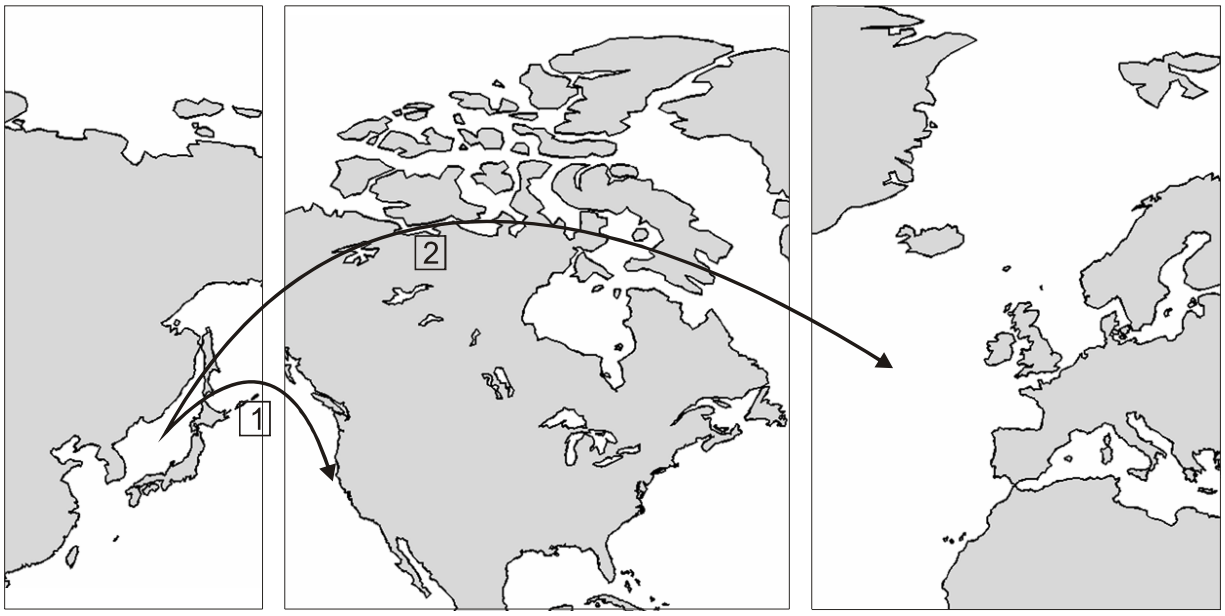


Figure 3-7. Independent introduction pathways of *C. mutica* to the Atlantic and Pacific. Numbers indicate pathways described in the text.

The analyses indicate that the San Francisco population is independent of the other introduced populations and, therefore, more than one introduction pathway is operating globally. Low genetic diversity and high divergence from other populations characterized the COI sequences from San Francisco. The haplotypes here (F & G) represent an amino acid change, and fixation indices for *C. mutica* were highest when the introduced populations were grouped by oceanic province.

An introduced population was described from the west Pacific at least 20 years before introduction to any other geographic location (Carlton 1979a). Most likely vectors for this introduction were either with oyster spat introductions (Cohen & Carlton 1995), in ballast water, or on oil platforms (or similar structures) towed slowly across the ocean (Rodríguez & Suaréz 2001). Japan has been considered the most likely source of other non-native species introduced to

California (Lambert & Lambert 1998, Stoner et al. 2002), including at least 22 invertebrate species introduced to San Francisco Bay (Cohen & Carlton 1995).

The data indicate that there is no clear link between populations of *C. mutica* on the Pacific and Atlantic coasts of North America (group 2, Figures 3-3 to 3-5), and that there have been at least two independent introduction pathways of *C. mutica* (routes 1 & 2, Figure 3-7). Independent pathways to the east and west coasts of the USA were also suggested for non-native populations of *Botryllus schlosseri* (Stoner et al. 2002). The low genetic diversity of the *C. mutica* population in San Francisco indicates that the introduction was a single introduction event, probably of a small propagule size; that ecological selection pressures have reduced the original introduced gene pool; or reflect founder effects, i.e. inbreeding leading to a fixing of low diversity.

Introduction(s) to the Atlantic

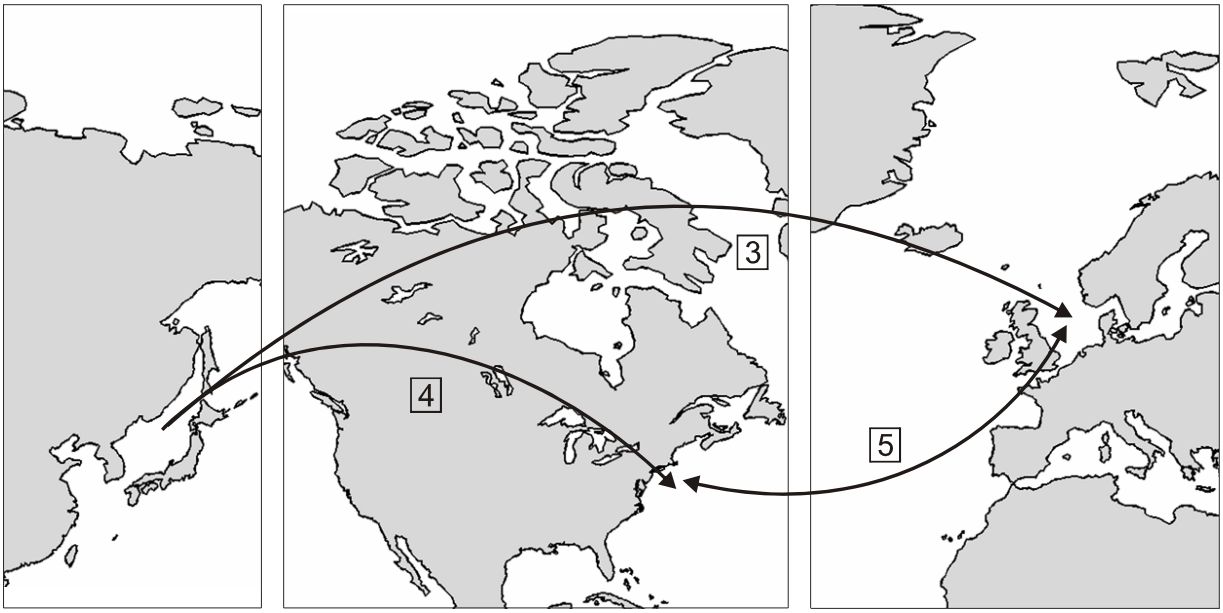


Figure 3-8. Likely introduction pathways of *C. mutica* to the Atlantic. Numbers indicate pathways described in the text.

Populations of *C. mutica* in the Atlantic consistently grouped together in both phylogram and network phylogeny estimates. Collectively, the Atlantic populations have a relatively high genetic diversity, similar to that seen in Japan, and several populations share haplotypes. The geographic distribution of the haplotypes (Figure 3-1) and the statistically significant within-region variation, suggest multiple introduction pathways and stepping-stone events (routes 3, 4 &

5, Figure 3-8). The high genetic diversity could also reflect different selection pressures operating at the non-native sites (Zuoros & Foltz 1984, Koehn 1991). The phylogram and haplotype network (Figure 3-2 & Figure 3-5) indicate at least 3 independent lineages within the Atlantic (A & B; C; D & E). Given the high diversity observed in the native region, the non-native populations could feasibly all originate from a single source population.

Haplotypes A and B are present in populations on both sides of the Atlantic and suggest either multiple introductions from the same source, or that the populations are stepping stones along the same pathways (routes 3 & 5 or 4 & 5, Figure 3-8), or that the populations are linked (route 5, Figure 3-8). The population in Passamaquoddy Bay has haplotypes from each of the four groups of non-native individuals (Figures 2, 3 & 4) and may be the first step in a single introduction pathway to the Atlantic (route 4 and not 3, Figure 8). However, the dates of first record of *C. mutica* do not support this theory, with individuals being identified in Europe in 1995 (Platvoet et al. 1995) and Atlantic North-America in 2003 (MIT. Sea Grant 2003). There are several examples of crustaceans having been introduced in both directions across the Atlantic, for example *Eriocheir sinensis* from Europe to North America (Geller et al. 1997) and *Hemigrapsus sanguineus* in the opposite direction (Breton et al. 2002). The alga *Codium fragile* ssp. *tomentosoides* was also probably introduced across the Atlantic from Western Europe as a fouling organism on ships' hulls (Carlton & Scanlon 1985). Shipping routes have existed across the Atlantic for more than 500 years (Stoner et al. 2002), and shellfish transfers have also historically been made across the Atlantic (Loosanoff 1975). Both these mechanisms are likely to be responsible for introductions of *C. mutica*. The mixing of populations, via human activities such as these, is known to have reduced genetic differentiation in non-native populations of *Crepidula fornicata* (Dupont et al. 2003) and may be responsible for the low divergence within populations of *C. mutica* in the Atlantic.

The haplotype network indicates that haplotypes D and E are closely related (Figure 3-5), suggesting that individuals established in Scotland may have subsequently been introduced to Canada. Canada and Norway share haplotype C and this may be evidence that these populations are from the same introduction pathway. Salmon aquaculture is an important industry in Scotland, Norway and Canada (FAO Fisheries Statistics 1999-2006), and transfers of species and equipment between these countries may unintentionally be responsible for introductions of *C. mutica*. Although, these methods of transport were only inferred for intra-country transfers of Infectious Salmon Anaemia (ISA; Murray et al. 2002).

East and west coasts of the Atlantic

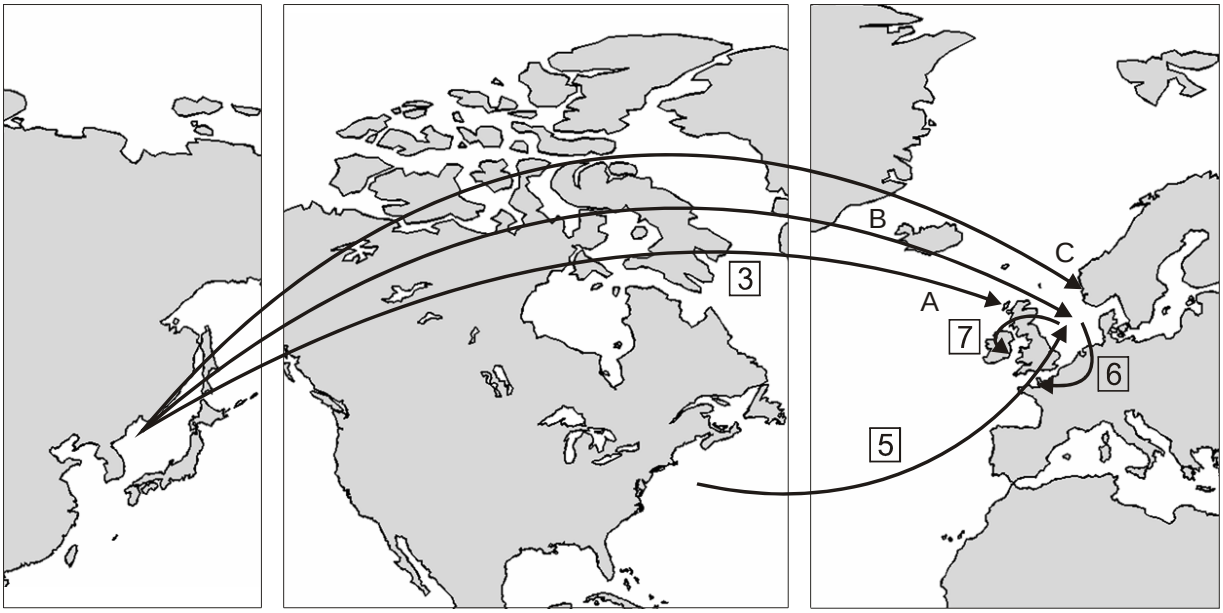


Figure 3-9. Potential introduction pathways of *C. mutica* to the east Atlantic. Numbers indicate pathways described in the text.

On the East coast of the Atlantic (in Europe), haplotypes A and B dominate *C. mutica* populations (Figure 3-1). The highest haplotype diversity in the European populations occurred in Scotland (although this was also the most intensively sampled region). Both Scotland and Norway have unique haplotypes within Europe and may represent first steps in introduction pathways to Europe (routes 3A & C, Figure 3-9). Both countries have major international ports, active aquaculture industries and recreational boating facilities. These features are not unique to Scotland and Norway, however, and there are no obvious reasons as to why these locations might be initial entry points for *C. mutica* to Europe (route 3B, Figure 3-9). At a recent meeting of the ICES Working Group on Introductions and Transfers of Marine Organisms, reports of transfers of live marine organisms came from all member countries (ICES 2006). For example, *Crassostrea gigas*, the Pacific Oyster, has been introduced to the Netherlands, Germany, England and France and from these countries to Denmark; with an associated parasite (*Bonamia ostreae*) only found in populations in France and Scotland to date. Buschbaum & Gutow (2005) suggested shipments of the Pacific Oyster as an introduction mechanism for *C. mutica.* Germany reportedly imports the greatest number of marine species in Europe; second to Canada amongst the ICES members (ICES 2006). Germany also imported species from the highest number of source

regions, although these data should be treated with caution as there was no consistency in the reporting practice. In Europe, the risk of invasion in association with shipping is characterised by large clusters of ports with intermediate levels of trade (Drake & Lodge 2004). However, the transportation hotspots that cover most of north-western Europe do not include Scotland and Norway (Drake & Lodge 2004) and there are still relatively low numbers of introduced species in these countries.

The southern North Sea has several important ports (e.g. Antwerp, Felixstowe, Hamburg, Le Havre, Rotterdam) where various non-native species have become established (Reise et al. 2002) and are potential entry points of *C. mutica* in Europe. England has been identified as the gateway to continental Europe for several marine species, such as *Elminius modestus* (Crisp 1958), *Sargassum muticum* (Critchley 1983) and *Styela clava* (Davis & Davis 2004). In contrast, the Chinese mitten crab, *Eriocheir sinensis*, was first introduced to continental Europe, and only subsequently to England (Herborg et al. 2005). Historically, several species have been introduced to the Netherlands with imports of shellfish (Vaas 1975). The first report of *C. mutica* in Europe was from the Netherlands (Platvoet et al. 1995); also in an area where shellfish are farmed.

The populations of *C. mutica* in France and Ireland had no haplotypic diversity and may represent the most recent steps in this species' introduction pathways (routes 6 & 7, Figure 3-9). The same could be argued for haplotype A in Norway (Figure 3-1). Founding populations were most likely of a small number of individuals derived from a single source location and introduction event (Holland 2000, Zardus & Hadfield 2005). The same pathway may have been responsible for the populations in France and Dun Laoghaire Bay, on the east coast of Ireland (haplotype A); however, an independent pathway was responsible for haplotype B in Betraghboy Bay, on the west coast of Ireland. It is not clear if these introductions were from the same or multiple sources in Europe as several populations have haplotypes A and B. Populations in Belgium, England, Germany and Scotland have both of these haplotypes, so may be intermediate steps on the same introduction pathway, similar to that seen in *Eriocheir sinensis* (Hänfling et al. 2002).

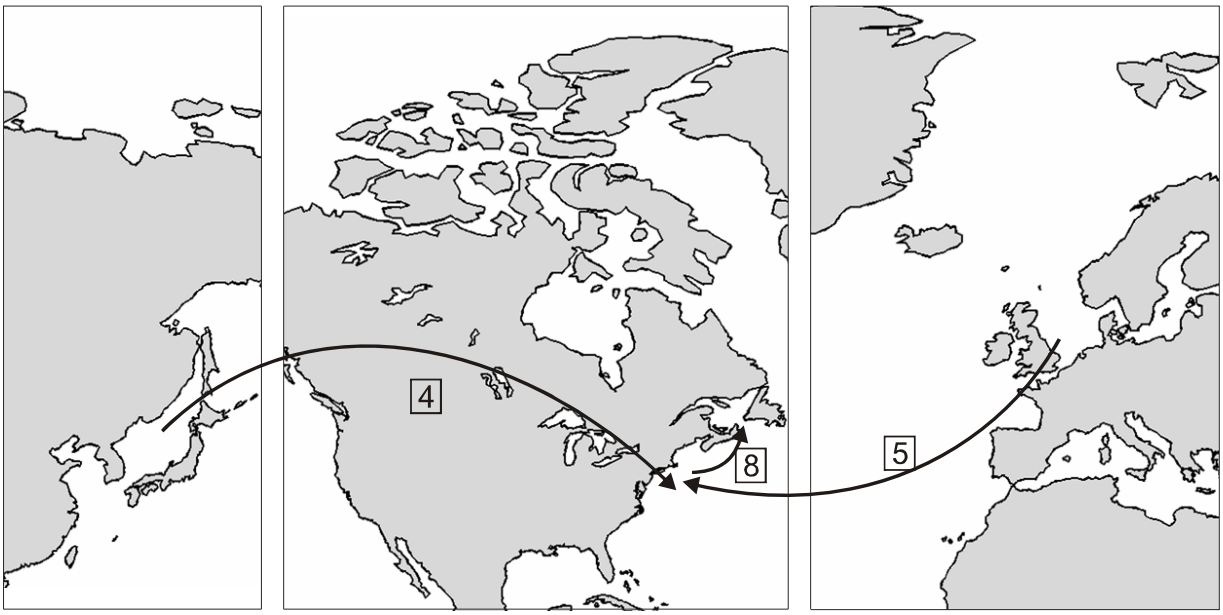


Figure 3-10. Likely introduction pathways of *C. mutica* to the west Atlantic. Numbers indicate pathways described in the text.

On the west coast of the Atlantic, *C. mutica* from Passamaquoddy Bay, Canada, showed high within-site diversity, suggesting either a large propagule size or multiple introductions of *C. mutica* to the area. However, a Wahlund effect (when independent populations are introduced to a new area and interbreed resulting in a higher genetic diversity in the new area compared to either source location) cannot be dismissed. The east coast of North America has been colonized by numerous introduced species (Pederson et al. 2003) with over 150 being listed by Carlton (2003a). Passamaquoddy Bay is within 150km of the international port of Saint John, which has been identified as a global hotspot for species transportation and release by Drake and Lodge (2004). The main industries in Passamaquoddy Bay are salmon farming and a container port at Eastport, Maine. Recreational boating is also a popular activity. International shipping, aquaculture activities and recreational boating are all potential vectors for introductions of *C. mutica* to Passamaquoddy Bay. The green crab (*Carcinus maenas*) spread progressively northwards along the Atlantic coast of America from Massachusetts to Passamaquoddy Bay and Nova Scotia in the late twentieth century (Audet et al. 2003). This was also the direction of spread of *Codium fragile* spp. *tomentosoides*, accelerated by transplantation of oysters, movement of fishing nets and ship fouling (Bird et al. 1993). The data suggest that Canadian populations of *C. mutica* exhibit characteristics of a similar stepping-stone pathway, with the first step being Passamaquoddy Bay, followed by a further step north to Chaleur Bay, where genetic

diversity was reduced (route 8, Figure 3-10). Chaleur Bay is also an important area for the fishing and aquaculture industries, which are potential vectors for *C. mutica* to the area.

Populations on the west coast of Scotland

Eleven populations of *C. mutica* were sampled and three haplotypes were identified on the west coast of Scotland (Figure 3-1). The three haplotypes have different distributions, with Haplotype A mainly in mainland sites (and two sites on the Outer Hebrides), Haplotype B at southern sites and Haplotype D at northern sites. Loch Kishorn was the only site where all 3 haplotypes were present (Figure 3-6). Between 1975 and 1987, an oil-platform fabrication yard was located in Loch Kishorn. Thus, it is possible that Loch Kishorn may be the initial entry point of *C. mutica* to Scotland. Alternatively, the haplotype distribution in Scotland may be the result of multiple introductions (and a Wahlund effect). There are two major commercial ports on the west coast of Scotland, Glensanda and Clyde (Figure 6; Scottish_Transport_Statistics 2005). International shipping to either of these ports may have introduced non-native *C. mutica* to the southern Scottish sites. Haplotype A was found at sites on the Outer Hebrides which are closest to the two main ferry terminals connecting to the mainland (Lochboisdale and Stornoway; Scottish_Transport_Statistics, 2005). Ferry transport may have introduced *C. mutica* to these sites. Haplotype D is unique to Scotland, and found exclusively in the north (including the Outer Hebrides). This suggests a pathway only operating to the North and West of Loch Kishorn, this could be aquaculture or shipping related. The presence of only one of haplotype A or B at several sites (Loch Erisort, Calbha Bay and Loch Carnan) probably indicates either erosion of genetic diversity during successive founder events (Hänfling et al. 2002) or separate introductions from the same source.

All sites sampled on the west coast of Scotland were salmon farms. Boat transport between salmon farms on the west coast of Scotland has been demonstrated to be an important vector for the transmission of ISA (Murray et al. 2002) and may also be responsible for the widespread distribution of *C. mutica* in Scotland. Thus, likely vectors on the west coast of Scotland include ferry transport, aquaculture movements and recreational boating activities (Chapter 4).

Phylogenetic analysis as a tool for invasion biology

Genetic drift and natural selection are major forces that modify genetic architecture (Wright 1948, Lee 2002) and influence adaptation of a species to a new habitat. An initial small population size will reduce genetic variation (Barrett & Kohn 1991, Allendorf & Lundquist 2003), and a population with severely reduced variation faces a greater risk of extinction (Barton & Charlesworth 1984, Allendorf & Lundquist 2003). Various events can alter genetic characters, and the conclusions of phylogenetic analysis can be hampered by the inability to distinguish between these events. Both the effective population size and the genetic diversity of the source populations influences the genetic structure of a species (Holland 2000). Therefore, it is important to characterize genetic variation in both native and non-native populations (Stoner et al. 2002, Grapputo et al. 2005, Voisin et al. 2005).

Unfortunately, the native source population of *C. mutica* was probably not encountered in this study, which has limited the conclusions that can be drawn from the phylogenetic analysis. Recently founded populations may have experienced one or more bottlenecks which will have reduced genetic diversity. Founder effects may explain the reduced genetic variation in non-native populations of *C. mutica*. For example, populations in Canada and Scotland may be the result of more than one introduction and thus demonstrate a Wahlund effect. Ecological selection can also affect genetic divergence (Filchak et al. 2000) and the genetic characters of *C. mutica* may reflect adaptations that are responsible for its success throughout the northern hemisphere, an indication that this species will continue to succeed as a non-native species. Individuals with the dominant haplotypes A and B may be more successful in the northern hemisphere and as a result have out-competed individuals with other haplotypes. To confirm this, it would be necessary to demonstrate that the genetic changes are an evolutionary advantage to the individuals during the dispersal process or in the different habitats.

The groupings of the non-native haplotypes of *C. mutica* were consistent and independent of the weighting of the third codon, however their association with populations from Japan altered with the weighting. The haplotype network suggests that the phylogenetic relationships between populations of *C. mutica* may be more complex than a bifurcating tree allows (Huson & Bryant 2006) and network analysis should continue to be included in future studies. The phylogenetic analysis supported several introduction pathways, although the exact relationships between populations, especially those in the Atlantic, could not be discerned. Increasing the number of individuals sequenced from both native and non-native populations could help establish

relationships and increase confidence in the layout of the phylograms and haplotype network. Future analyses should ideally include individuals from other sites in the native range, including Russia.

The presented phylogenetic analysis, using the mitochondrial COI gene, has contributed to our understanding of the complex introduction pathways of *C. mutica*. Confidence in these phylogenetic relationships might be increased by sequencing another gene with a higher evolutionary rate, such as ITS1. Microsatellite or fragment analyses would give higher resolution to the results and may, for example, divide populations in Europe further (particularly haplotypes A and B). These latter techniques are a powerful complement to sequencing (Duran et al. 2004, Le Goff-Vitry et al. 2004).

Summary

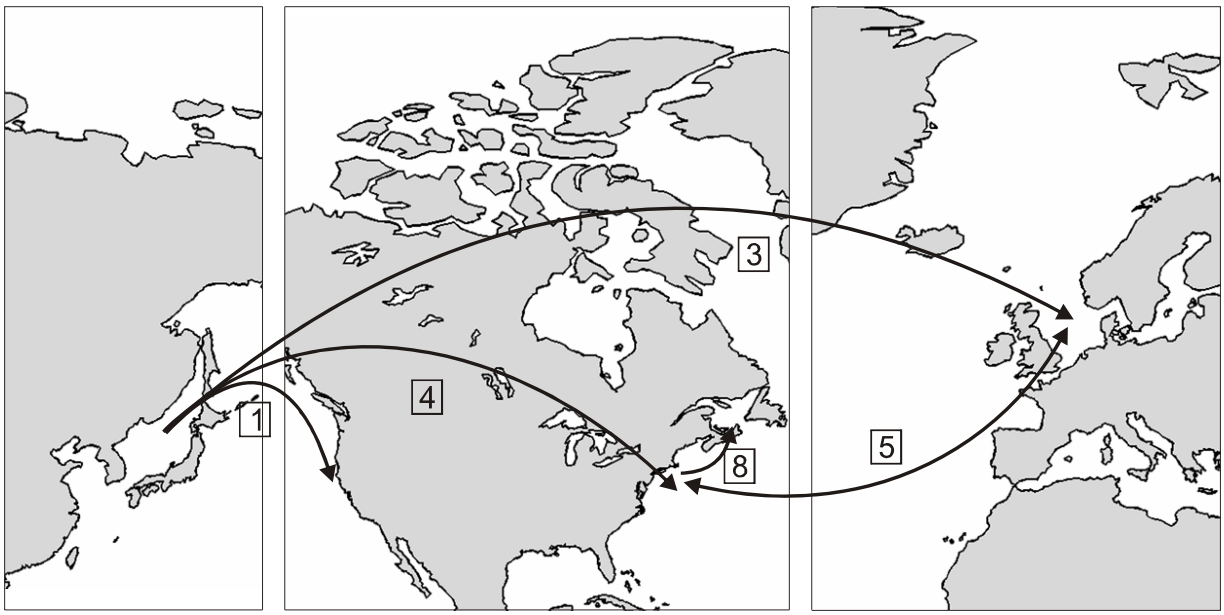


Figure 3-11. Most likely introduction pathways of *C. mutica* in the northern hemisphere. Numbers indicate pathways described in the text.

C. mutica populations in Japan have been described as being within the native range (Vassilenko 1967, Akkeshi High School of Fish. 1968). The presented data indicate that the native populations sampled displayed the most genetic diversity but none of the populations sampled was the source for the non-native populations. Identification of single or multiple source populations in the native region would almost certainly alter the layout of the phylograms and

haplotype network. However, the non-native populations consistently clustered into four groups, one of which contained solely individuals from San Francisco. The most likely introduction pathways of *C. mutica* are shown in Figure 3-11. The San Francisco population of *C. mutica* is independent of the non-native populations in the Atlantic and was probably the result of a single introduction of a few individuals. The remaining groups comprise populations from the Atlantic, which share a number of haplotypes indicating similar source populations, introduction mechanisms, and most likely stepping stone pathways. The pattern of shared haplotypes in the Atlantic may also represent ongoing mixing between populations.

3.5 Appendices

Appendix 3.1.

Method development

To extract the DNA, initially, an animal DNA extraction kit was used (Macherey-Nagel). This method generated a poor return of low quality DNA which did not amplify to produce a clear gel band. Therefore a second protocol, CTAB extraction procedure was tested (Milligan 1998). Once again, extracted DNA did not amplify or sequence. The salting-out technique (Sunnucks & Hales 1996) produced a high quantity of quality DNA, which gave good results from PCR and clear, reliable sequencing results. This method was used for extractions completed in the UK. When the study was continued in New Zealand, a GenElute Mammalian DNA Miniprep Kit (SIGMA) was also found to produce a high quantity of quality DNA, and was used for the remaining extractions.

Seven universal primer combinations (Table 1; described to amplify a wide range of taxa), five aimed at mitochondrial DNA genes (Figure 1), and two aimed at the nuclear DNA, were tested (Table 1). The COI2F and COI2R primer pair was the most reliable at producing an identifiable product during PCR and sufficient quantity of quality material for sequence analysis.

To further increase the quantity and quality of the results, several of the products sequenced from the COI2F and COI2R were used to develop a new forward primer, specific to *Caprella mutica* (Figure 1). This involved stepping the primer in 17 bases, so the first 9 bases were the same as COI2F, with an additional 11 new bases (COI3F: 5' AGG AGA TCC TAT CCT TTA CC 3').

Table 1. Primer sequences

Name	Sequence (5'-3')	Target	Ref
COXB F	GCT TCC ATC CAA CAT CTC AGC ATG ATC A	mtDNA	(Simon et al. 1994)
COXB R	CAG CCC CTC AGA ATG ATA TTT GTC CTC A		
16S F	CGC CTG TTT AWC AAA AAC AT	mtDNA	(Palumbi et al. 1991)
16S R	GGT YTG AAC TCA AGA TCA TGT		
18S F	TAA TGA TCC TTC CGC AGG TTC A	nDNA	(Otto & Wilson 2001)
18S R	TCC CTG GTT GAT CCT GCC AGT A		
COI 2F	TTY GAY CCI DYI GGR GGA GGA GAT CC	mtDNA	(Palumbi et al. 1991)
COI 2R	GGR TAR TCW GAR TAW CGN CGW GGT AT		
CYTB F	ATA TTT GCC GAG ATG TAA ATT AYG GYT GA	mtDNA	(Machida et al. 2004)
CYTB R	GCA AAT AGA AAA TAT CAT TCW GGT TG		
ITS4 F	TCC TCC GCT TAT TGA TAT GC	nDNA	(Folmer et al. 1994)
ITS5 R	TCC TCC GCT TAT TGA TAT GC		
LCO	GGT CAA CAA ATC ATA AAG ATA TTG G	mtDNA	(Folmer et al. 1994)
HCO	CCT CCT CCT GAA GGG TCA AAA AAT GA		

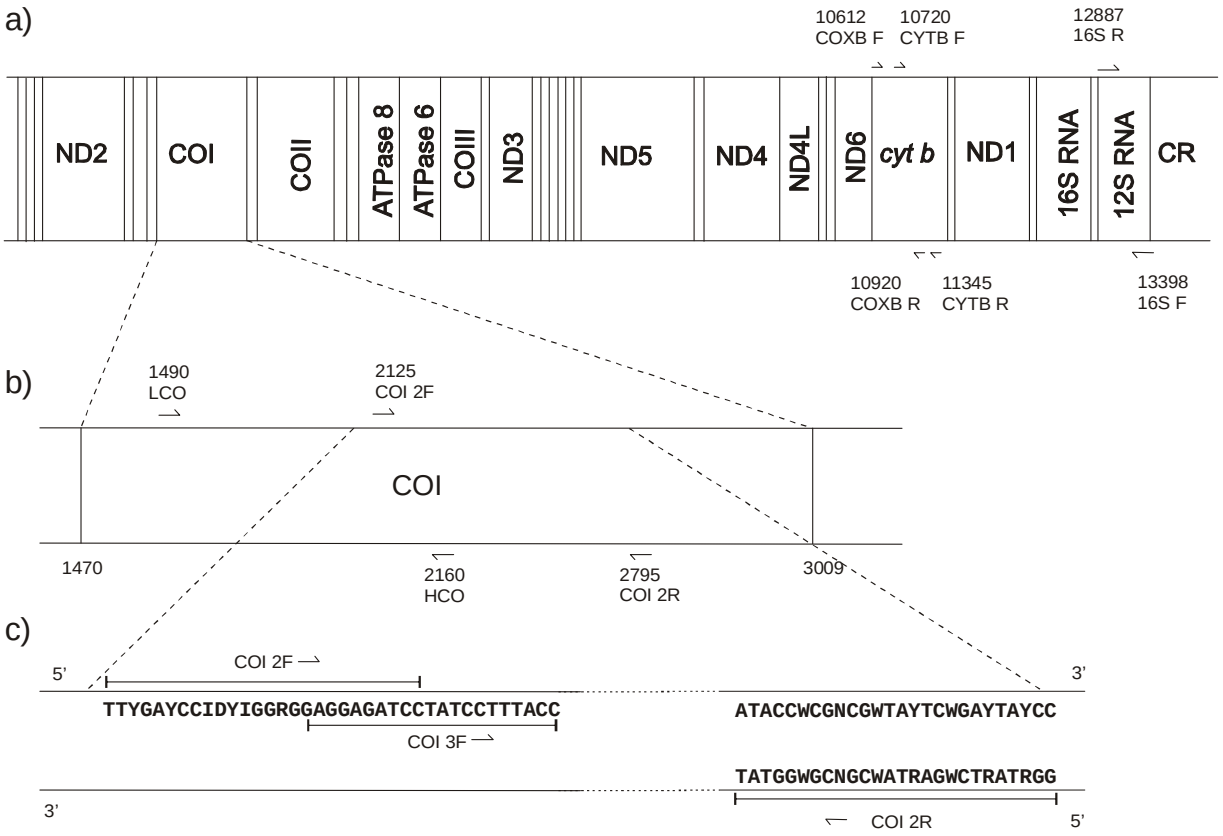


Figure 1. Linear representation of mitochondrial gene arrangement in the putative ancestral arthropod (Cook 2005). a) Protein-coding genes and two ribosomal RNA genes are labelled in bold. Gaps indicate transfer RNA genes. The position of three pairs of PCR primers (COXB F & COXB R; CYTB F & CYTB R; 16S F & 16S R) are shown. Numbers indicate the start position relative to the *Drosophila yakuba* complete mitochondrial sequence (Genbank accession no: X03240). COI–III indicates genes for cytochrome oxidase subunits I–III; ATPase 6 and 8, ATPase subunits 6 and 8; ND 1–6 and 4L, NADH dehydrogenase subunits 1–6 and 4L; cyt b; cytochrome b; srRNA and lrRNA, small and large mitochondrial ribosomal subunit RNAs; CR, putative A-T rich control region. b) Position of two pairs of PCR primers in the COI gene (LCO & HCO; COI 2F and COI 2R). c) Position and sequence of primers COI 2F, COI 2R (Otto & Wilson 2001) and the new primer COI 3F.

AFLP protocol

Reactions were performed following modifications of the Vos et al. protocol. (1995) PE Applied Biosystems restriction site adapters and fluorescently labelled AFLP primers were used. All other enzymes were obtained through Roche. Care was taken to complete digestion, linker ligation, pre-selective PCR amplification within 12 hours. Products were then stored at +4 ºC overnight before performing the selective PCR amplification reaction.

Restriction endonuclease digestion reactions were performed on approximately 250 ng DNA with 0.1 U *Mse*I, 0.1 U *Eco*RI, 1 µL 1x Reaction Buffer with a total reaction volume of 25 µL. Reactions were incubated at 37 ºC for 2 hours, then 70 ºC for 15 min to irreversibly denature the restriction enzymes.

Adapter ligation reactions contained 5 µL Digested DNA, 2 µL 10x Ligation Buffer, 1 U T4 DNA Ligase, 1 nM *Eco*RI adapter and 10 nM *Mse*I adapter (Vos et al. 1995). Total reaction volume was 20 µL. Reactions were incubated at 37 ºC for 3 hours.

Pre-selective amplification was carried out using 1µL ligated DNA in a 20µL reaction volume containing: 1 M Betaine, 0.25 mM dNTPs, 1x PCR Buffer, 0.5 µM *Eco*RI+A primer (5' GAC TGC GTA CCA ATT C**A** 3'), 0.5 µM *Mse*I+C primer (5' GAT GAG TCC TGA GTA A**C** 3') and 1 U *Taq* Polymerase. Reactions were run on a Biometra thermocycler with the following program: 20 cycles of 30 s at 94 ºC, 1 min at 56 ºC, 1 min at 72 ºC.

Selective amplifications used 1 µL of the pre-amplified DNA in a 20 µL reaction volume containing: 0.25 mM dNTPs, 3.125 mM $MgCl_2$, 1x PCR Buffer, 0.5 µM flourescently-labelled *Eco*RI+ANN primer, 0.5 µM *Mse*I+CNN Primer and 1 U *Taq* Polymerase. Reactions were denatured at 94 ºC for 2 min; followed by 10 cycles of 94 ºC for 30 s, 30 s at temperatures decreasing from 66 ºC to 56 ºC by 1 ºC each cycle and 72 ºC for 60 s; finally 30 cycles of 94 ºC for 30 s, 56 ºC for 30 s and 72 ºC for 1 min. The reactions were then held at 72 ºC for 30 min.

32 primer combinations were tested (Table 2). The four producing the clearest gel bands were selected for continuation of the protocol. Products were pooled in the ratio 1:1:1:2 (6-FAM: VIC: NED: PET) to a total volume of 10 µL for capillary electrophoresis. An ABI 3730 automated sequencer (Applied Biosystems Inc.) was used to analyse the fragments.

Table 2. Flourophores and selective primer combinations tested. Bold indicates those chosen for continuation of the protocol

Flourophore	*Eco*RI +	Mse +
6-FAM (blue)	**ACT**	CAA, -CAC, -**CAG**, -CAT, -CCC, -CGG, -CTG, -CTT
VIC (green)	**AGC**	CAA, -CAC, -**CAG**, -CAT, -CCC, -CGG, -CTG, -CTT
NED (yellow)	**ACG**	CAA, -CAC, -**CAG**, -CAT, -CCC, -CGG, -CTG, -CTT
PET (red)	**AAG**	CAA, -CAC, -CAG, -**CAT**, -CCC, -CGG, -CTG, -CTT

Profiles were imported into GeneMapper Software v3.7 (Applied Biosystems). Fragments between 100 and 400 bp were scored. However, at this time it became clear that the fragments could not be reliably scored. Peaks were not shared between sequences, and duplicate runs did not produce the same profile. Therefore, it was decided not to continue with this method.

Appendix 3.2.

Genetic distance based on sequence variation in the mtDNA COI sequences (563 aligned sites) among the 38 identified *Caprella mutica* haplotypes and two outgroup taxa (Cap1- *Caprella acanthogaster*; Cap2- *Caprella equilibria*). Above the diagonal are maximum likelihood distances calculated using the Tamura-Nei plus gamma model; below the diagonal are uncorrected P distances. The haplotype codes refer to those used in Figure 3-2.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1		0.012	0.012	0.009	0.009	0.012	0.014	0.012	0.014	0.016	0.012	0.010	0.016	0.016	0.014	0.021	0.012	0.014	0.014	0.016	0.014	0.017
2	0.012		0.007	0.003	0.007	0.010	0.012	0.010	0.012	0.014	0.010	0.009	0.014	0.014	0.012	0.019	0.010	0.012	0.012	0.017	0.012	0.016
3	0.012	0.007		0.003	0.007	0.010	0.010	0.010	0.012	0.012	0.010	0.009	0.014	0.014	0.012	0.023	0.010	0.012	0.012	0.017	0.012	0.014
4	0.009	0.004	0.004		0.003	0.007	0.009	0.007	0.009	0.010	0.007	0.005	0.010	0.010	0.009	0.019	0.007	0.009	0.009	0.014	0.009	0.012
5	0.009	0.007	0.007	0.004		0.010	0.012	0.010	0.012	0.014	0.010	0.009	0.014	0.014	0.012	0.019	0.010	0.012	0.012	0.014	0.012	0.016
6	0.012	0.011	0.011	0.007	0.011		0.012	0.010	0.012	0.014	0.010	0.009	0.014	0.014	0.012	0.023	0.010	0.012	0.012	0.017	0.012	0.016
7	0.014	0.012	0.011	0.009	0.012	0.012		0.009	0.005	0.007	0.009	0.007	0.009	0.009	0.010	0.021	0.009	0.010	0.010	0.016	0.014	0.017
8	0.012	0.011	0.011	0.007	0.011	0.011	0.009		0.010	0.014	0.010	0.005	0.010	0.014	0.009	0.016	0.010	0.012	0.012	0.016	0.010	0.014
9	0.014	0.012	0.012	0.009	0.012	0.012	0.005	0.011		0.005	0.005	0.009	0.010	0.005	0.012	0.023	0.009	0.010	0.010	0.012	0.010	0.014
10	0.016	0.014	0.012	0.011	0.014	0.014	0.007	0.014	0.005		0.007	0.012	0.014	0.007	0.016	0.026	0.010	0.012	0.012	0.014	0.012	0.016
11	0.012	0.011	0.011	0.007	0.011	0.011	0.009	0.011	0.005	0.007		0.009	0.014	0.007	0.012	0.023	0.007	0.009	0.009	0.010	0.009	0.012
12	0.011	0.009	0.009	0.005	0.009	0.009	0.007	0.005	0.009	0.012	0.009		0.005	0.012	0.007	0.017	0.009	0.010	0.010	0.016	0.010	0.014
13	0.016	0.014	0.014	0.011	0.014	0.014	0.009	0.011	0.011	0.014	0.014	0.005		0.014	0.012	0.023	0.014	0.016	0.016	0.021	0.016	0.019
14	0.016	0.014	0.014	0.011	0.014	0.014	0.009	0.014	0.005	0.007	0.007	0.012	0.014		0.016	0.026	0.010	0.012	0.012	0.014	0.012	0.016
15	0.014	0.012	0.012	0.009	0.012	0.012	0.011	0.009	0.012	0.016	0.012	0.007	0.012	0.016		0.010	0.012	0.014	0.014	0.019	0.014	0.017
16	0.021	0.020	0.023	0.020	0.020	0.023	0.021	0.016	0.023	0.027	0.023	0.018	0.023	0.027	0.011		0.023	0.024	0.024	0.026	0.024	0.028
17	0.012	0.011	0.011	0.007	0.011	0.011	0.009	0.011	0.009	0.011	0.007	0.009	0.014	0.011	0.012	0.023		0.002	0.002	0.014	0.012	0.016
18	0.014	0.012	0.012	0.009	0.012	0.012	0.011	0.012	0.011	0.012	0.009	0.011	0.016	0.012	0.014	0.025	0.002		0.003	0.016	0.014	0.017
19	0.014	0.012	0.012	0.009	0.012	0.012	0.011	0.012	0.011	0.012	0.009	0.011	0.016	0.012	0.014	0.025	0.002	0.004		0.016	0.014	0.017
20	0.016	0.018	0.018	0.014	0.014	0.018	0.016	0.016	0.012	0.014	0.011	0.016	0.021	0.014	0.020	0.027	0.014	0.016	0.016		0.009	0.012
21	0.014	0.012	0.012	0.009	0.012	0.012	0.014	0.011	0.011	0.012	0.009	0.011	0.016	0.012	0.014	0.025	0.012	0.014	0.014	0.009		0.007
22	0.018	0.016	0.014	0.012	0.016	0.016	0.018	0.014	0.014	0.016	0.012	0.014	0.020	0.016	0.018	0.028	0.016	0.018	0.018	0.012	0.007	
23	0.016	0.018	0.018	0.014	0.014	0.018	0.018	0.014	0.014	0.018	0.014	0.014	0.020	0.018	0.018	0.025	0.018	0.020	0.020	0.014	0.012	0.016
24	0.012	0.014	0.014	0.011	0.011	0.014	0.012	0.009	0.011	0.014	0.011	0.009	0.014	0.014	0.012	0.020	0.014	0.016	0.016	0.011	0.009	0.012
25	0.016	0.018	0.018	0.014	0.014	0.018	0.016	0.012	0.014	0.018	0.014	0.012	0.018	0.018	0.016	0.023	0.018	0.020	0.016	0.011	0.012	0.016
26	0.016	0.018	0.018	0.014	0.014	0.018	0.016	0.009	0.014	0.018	0.014	0.012	0.018	0.018	0.016	0.020	0.018	0.020	0.020	0.012	0.014	0.018
27	0.014	0.016	0.016	0.012	0.012	0.016	0.014	0.011	0.012	0.016	0.012	0.011	0.016	0.016	0.014	0.021	0.016	0.018	0.018	0.009	0.011	0.014
28	0.012	0.014	0.014	0.011	0.011	0.014	0.012	0.009	0.011	0.014	0.011	0.009	0.014	0.014	0.012	0.020	0.014	0.016	0.016	0.007	0.009	0.012
29	0.018	0.020	0.020	0.016	0.016	0.020	0.018	0.014	0.016	0.020	0.016	0.014	0.020	0.020	0.018	0.025	0.020	0.021	0.021	0.012	0.014	0.018
30	0.014	0.016	0.016	0.012	0.012	0.016	0.014	0.011	0.012	0.016	0.012	0.011	0.016	0.016	0.014	0.021	0.016	0.018	0.018	0.009	0.011	0.014
31	0.020	0.018	0.021	0.018	0.018	0.021	0.020	0.016	0.018	0.021	0.018	0.016	0.021	0.021	0.020	0.027	0.018	0.020	0.020	0.018	0.016	0.020
A	0.018	0.016	0.016	0.012	0.016	0.016	0.014	0.012	0.016	0.020	0.016	0.007	0.012	0.020	0.014	0.025	0.016	0.018	0.018	0.020	0.018	0.021
B	0.023	0.021	0.021	0.018	0.021	0.021	0.020	0.018	0.021	0.025	0.018	0.012	0.018	0.021	0.018	0.028	0.021	0.023	0.023	0.025	0.023	0.027
C	0.016	0.011	0.012	0.011	0.014	0.014	0.009	0.011	0.014	0.016	0.014	0.009	0.014	0.018	0.012	0.023	0.014	0.016	0.016	0.021	0.016	0.020
D	0.016	0.014	0.014	0.011	0.014	0.014	0.012	0.009	0.011	0.014	0.011	0.009	0.014	0.014	0.012	0.023	0.014	0.016	0.016	0.012	0.011	0.014
E	0.014	0.012	0.012	0.009	0.012	0.012	0.011	0.007	0.009	0.012	0.009	0.007	0.012	0.012	0.011	0.021	0.012	0.014	0.014	0.014	0.009	0.012
F	0.023	0.025	0.021	0.021	0.025	0.025	0.021	0.021	0.025	0.027	0.025	0.020	0.025	0.028	0.012	0.020	0.025	0.027	0.023	0.032	0.027	0.030
G	0.025	0.027	0.023	0.023	0.027	0.027	0.023	0.023	0.027	0.028	0.027	0.021	0.027	0.030	0.014	0.021	0.027	0.028	0.025	0.034	0.028	0.032
Cap1 A	0.171	0.165	0.165	0.163	0.167	0.167	0.163	0.162	0.165	0.162	0.162	0.165	0.169	0.169	0.169	0.171	0.165	0.167	0.165	0.163	0.165	0.165
Cap1 B	0.171	0.165	0.165	0.163	0.163	0.167	0.163	0.162	0.165	0.162	0.162	0.165	0.169	0.169	0.169	0.171	0.165	0.167	0.165	0.163	0.165	0.165
Cap2 A	0.192	0.187	0.188	0.187	0.187	0.192	0.188	0.188	0.188	0.192	0.187	0.190	0.194	0.194	0.190	0.190	0.190	0.192	0.192	0.187	0.190	0.190

Appendix 3.2. cont.d

	23	24	25	26	27	28	29	30	31	A	B	C	D	E	F	G	Cap1 A	Cap1 B	Cap2 A
1	0.016	0.012	0.016	0.016	0.014	0.012	0.017	0.014	0.019	0.017	0.023	0.016	0.016	0.014	0.023	0.024	0.228	0.227	0.281
2	0.017	0.014	0.017	0.017	0.016	0.014	0.019	0.016	0.017	0.016	0.021	0.010	0.014	0.012	0.024	0.026	0.219	0.219	0.271
3	0.017	0.014	0.017	0.017	0.016	0.014	0.019	0.016	0.021	0.016	0.021	0.012	0.014	0.012	0.021	0.023	0.219	0.219	0.274
4	0.014	0.010	0.014	0.014	0.012	0.010	0.016	0.012	0.017	0.012	0.017	0.010	0.010	0.009	0.021	0.023	0.216	0.216	0.271
5	0.014	0.010	0.014	0.014	0.012	0.010	0.016	0.012	0.017	0.016	0.021	0.014	0.014	0.012	0.024	0.026	0.223	0.216	0.271
6	0.017	0.014	0.017	0.017	0.016	0.014	0.019	0.016	0.021	0.016	0.021	0.014	0.014	0.012	0.024	0.026	0.222	0.222	0.281
7	0.017	0.012	0.016	0.016	0.014	0.012	0.017	0.014	0.019	0.014	0.019	0.009	0.012	0.010	0.021	0.023	0.215	0.215	0.273
8	0.014	0.009	0.012	0.009	0.010	0.009	0.014	0.010	0.016	0.012	0.017	0.010	0.009	0.007	0.021	0.023	0.213	0.212	0.274
9	0.014	0.010	0.014	0.014	0.012	0.010	0.016	0.012	0.017	0.016	0.021	0.014	0.010	0.009	0.025	0.026	0.220	0.220	0.273
10	0.017	0.014	0.017	0.017	0.016	0.014	0.019	0.016	0.021	0.019	0.025	0.016	0.014	0.012	0.026	0.028	0.212	0.212	0.280
11	0.014	0.010	0.014	0.014	0.012	0.010	0.016	0.012	0.017	0.016	0.017	0.014	0.010	0.009	0.024	0.026	0.213	0.213	0.271
12	0.014	0.009	0.012	0.012	0.010	0.009	0.014	0.010	0.016	0.007	0.012	0.009	0.009	0.007	0.019	0.021	0.219	0.218	0.277
13	0.019	0.014	0.017	0.017	0.016	0.014	0.019	0.016	0.021	0.012	0.017	0.014	0.014	0.012	0.024	0.026	0.225	0.225	0.285
14	0.017	0.014	0.017	0.017	0.016	0.014	0.019	0.016	0.021	0.019	0.021	0.017	0.014	0.012	0.028	0.030	0.225	0.225	0.285
15	0.017	0.012	0.016	0.016	0.014	0.012	0.017	0.014	0.019	0.014	0.017	0.012	0.012	0.010	0.012	0.014	0.225	0.224	0.278
16	0.025	0.019	0.023	0.019	0.021	0.019	0.024	0.021	0.026	0.024	0.028	0.023	0.023	0.021	0.019	0.021	0.228	0.228	0.278
17	0.017	0.014	0.017	0.017	0.016	0.014	0.019	0.016	0.017	0.016	0.021	0.014	0.014	0.012	0.024	0.026	0.219	0.218	0.277
18	0.019	0.016	0.019	0.019	0.017	0.016	0.021	0.017	0.019	0.017	0.023	0.016	0.016	0.014	0.026	0.028	0.222	0.221	0.280
19	0.019	0.016	0.016	0.019	0.017	0.016	0.021	0.017	0.019	0.017	0.023	0.016	0.016	0.014	0.023	0.024	0.219	0.218	0.281
20	0.014	0.010	0.010	0.012	0.009	0.007	0.012	0.009	0.017	0.019	0.025	0.021	0.012	0.014	0.032	0.034	0.217	0.216	0.271
21	0.012	0.009	0.012	0.014	0.010	0.009	0.014	0.010	0.016	0.017	0.023	0.016	0.010	0.009	0.026	0.028	0.219	0.219	0.278
22	0.016	0.012	0.016	0.017	0.014	0.012	0.017	0.014	0.019	0.021	0.026	0.019	0.014	0.012	0.030	0.032	0.220	0.219	0.279
23		0.009	0.012	0.014	0.010	0.009	0.014	0.010	0.016	0.021	0.023	0.019	0.014	0.012	0.030	0.032	0.233	0.233	0.278
24	0.009		0.007	0.009	0.005	0.003	0.009	0.005	0.010	0.016	0.021	0.014	0.009	0.007	0.024	0.026	0.222	0.222	0.278
25	0.012	0.007		0.005	0.005	0.003	0.009	0.005	0.010	0.016	0.025	0.017	0.009	0.007	0.024	0.026	0.226	0.225	0.286
26	0.014	0.009	0.005		0.007	0.005	0.010	0.007	0.012	0.016	0.025	0.017	0.007	0.005	0.028	0.030	0.226	0.225	0.278
27	0.011	0.005	0.005	0.007		0.002	0.007	0.003	0.012	0.017	0.023	0.016	0.010	0.009	0.026	0.028	0.225	0.225	0.281
28	0.009	0.004	0.004	0.005	0.002		0.005	0.002	0.010	0.016	0.021	0.014	0.009	0.007	0.024	0.026	0.223	0.222	0.278
29	0.014	0.009	0.009	0.011	0.007	0.005		0.007	0.016	0.021	0.026	0.019	0.014	0.012	0.030	0.032	0.226	0.225	0.275
30	0.011	0.005	0.005	0.007	0.004	0.002	0.007		0.012	0.017	0.023	0.016	0.010	0.009	0.026	0.028	0.226	0.225	0.275
31	0.016	0.011	0.011	0.012	0.012	0.011	0.016	0.012		0.019	0.028	0.017	0.012	0.010	0.032	0.034	0.226	0.226	0.272
A	0.021	0.016	0.016	0.016	0.018	0.016	0.021	0.018	0.020		0.009	0.016	0.009	0.010	0.026	0.028	0.225	0.224	0.270
B	0.023	0.021	0.025	0.025	0.023	0.021	0.027	0.023	0.028	0.009		0.021	0.017	0.019	0.030	0.032	0.229	0.228	0.275
C	0.020	0.014	0.018	0.018	0.016	0.014	0.020	0.016	0.018	0.016	0.021		0.014	0.012	0.023	0.025	0.219	0.218	0.263
D	0.014	0.009	0.009	0.007	0.011	0.009	0.014	0.011	0.012	0.009	0.018	0.014		0.002	0.024	0.026	0.216	0.216	0.278
E	0.012	0.007	0.007	0.005	0.009	0.007	0.012	0.009	0.011	0.011	0.020	0.012	0.002		0.023	0.024	0.219	0.219	0.282
F	0.030	0.025	0.025	0.028	0.027	0.025	0.030	0.027	0.032	0.027	0.030	0.023	0.025	0.023		0.002	0.237	0.237	0.296
G	0.032	0.027	0.027	0.030	0.028	0.027	0.032	0.028	0.034	0.028	0.032	0.025	0.027	0.025	0.002		0.234	0.233	0.292
Cap1 A	0.172	0.167	0.169	0.169	0.169	0.167	0.169	0.169	0.169	0.169	0.171	0.165	0.163	0.165	0.176	0.174		0.007	0.313
Cap1 B	0.172	0.167	0.169	0.169	0.169	0.167	0.169	0.169	0.169	0.169	0.171	0.165	0.163	0.165	0.176	0.174	0.007		0.320
Cap2 A	0.190	0.190	0.194	0.190	0.192	0.190	0.188	0.188	0.187	0.187	0.188	0.183	0.190	0.192	0.199	0.197	0.206	0.210	

Appendix 3.3.

Sample number (n), haplotype diversity (h), and nucleotide diversity (π) for COI sequences of *Caprella mutica*. Site codes correspond to Table 3-1.

Site	n	h (± SD)	π (± SD)
Jap1	19	0.621 (0.121)	0.009 (0.005)
Jap2	4	1.000 (0.272)	0.011 (0.009)
Jap3	10	0.867 (0.085)	0.012 (0.007)
Jap4	16	1.000 (0.022)	0.014 (0.008)
Fra	22	0.000 (0.000)	0.000 (0.000)
Bel	10	0.356 (0.159)	0.003 (0.002)
Can1	17	0.677 (0.075)	0.009 (0.005)
Can2	11	0.436 (0.133)	0.004 (0.003)
Ire1	17	0.000 (0.000)	0.000 (0.000)
Ire2	8	0.000 (0.000)	0.000 (0.000)
Eng	2	1.000 (0.000)	0.008 (0.010)
Sco	103	0.642 (0.022)	0.008 (0.004)
Ger	20	0.000 (0.000)	0.000 (0.000)
Nor	19	0.199 (0.112)	0.003 (0.002)
SaF	20	0.190 (0.108)	0.000 (0.000)

Appendix 3.4.

Pairwise comparisons of F_{ST} values for COI sequences of *Caprella mutica* from fifteen sites in the native and non-native range. Bold text indicates comparisons within the native range. Site codes correspond to Table 3-1.

Site	**Jap1**	**Jap2**	**Jap3**	**Jap4**	Fra	Bel	Can1	Can2	Ire1	Ire2	Eng	Sco	Ger	Nor	SaF
Jap1															
Jap2	**0.180**														
Jap3	**0.259****	**-0.015**													
Jap4	**0.234****	**-0.000**	**0.079***												
Fra	0.771**	0.922**	0.772**	0.633**											
Bel	0.688**	0.743*	0.666**	0.555**	0.856**										
Can1	0.549**	0.452**	0.496**	0.410**	0.379**	0.085									
Can2	0.640**	0.664**	0.599**	0.484**	0.315*	0.376*	0.034								
Ire1	0.796**	0.932**	0.809**	0.692**	1**	0.189	0.350	0.755**							
Ire2	0.673**	0.819**	0.635**	0.498**	0	0.755**	0.242	0.154	1**						
Eng	0.556*	0.427	0.455**	0.338**	0.839	-0.094	-0.320	-0.234	0.799	0.628					
Sco	0.533**	0.417*	0.457**	0.391**	0.269**	0.363**	0.157	0.179*	0.498**	0.221*	0.065				
Ger	0.761**	0.916**	0.760**	0.619**	0	0.847**	0.364	0.298*	1**	0	0.825	0.264**			
Nor	0.660**	0.688**	0.625**	0.508**	0.067	0.627**	0.228	0.125	0.836**	-0.012	0.264	0.210**	0.059		
SaF	0.829**	0.940*	0.843**	0.754**	0.994**	0.959**	0.857**	0.946**	0.994**	0.991**	0.973**	0.783**	0.994**	0.938**	

***** Significant at $P < 0.05$, ****** Significant at $P < 0.001$; statistical probabilities derived from 1023 permutations

Appendix 3.5.

Pairwise comparisons of F_{ST} values for COI sequences of *Caprella mutica* from eleven sites on the west coast of Scotland. Site codes correspond to Table 3-1.

	Lis	Cal	Che	Car	Eri	Kis	Mel	Roa	Sea	Boi	Mad
Lis											
Cal	0.462*										
Che	0.802*	0.234									
Car	0.647*	0.058	-0.055								
Eri	0.709*	0.053	-0.055	-0.105							
Kis	0.234*	0.282*	0.470*	0.298	0.400*						
Mel	0.401*	0.610*	0.817*	0.659*	0.749*	0.064					
Roa	0.802*	0.234	0	-0.055	-0.055	0.470*	0.817*				
Sea	0.831*	0.319*	0	0.038	0.038	0.543*	0.856*	0			
Boi	0.276*	0.551*	0.779*	0.615*	0.706*	0.033	-0.087	0.779*	0.825*		
Mad	0.825*	0.301	0	0.020	0.020	0.528*	0.848*	0	0	0.816*	

***** Significant at $P<0.05$; statistical probabilities derived from 1023 permutations

Chapter 4

Secondary dispersal of *Caprella mutica* on the west coast of Scotland

4.1 Introduction

Following introduction, secondary dispersal is a key mechanism for the extension of exotic species to other suitable habitats (Geller 1994, Sakai et al. 2001). Dispersal is a component of life-history strategies and has been linked to colonising ability (Ross 2001). Ultimately, dispersal will determine the ecological impact of an introduced species (Lodge et al. 1998). Understanding the secondary dispersal mechanisms of an introduced species provides the basis for predicting its future spread (Ludwig & Leitch 1996, Schneider et al. 1998), and may allow effective management procedures to be implemented (Ricciardi et al. 2000). Human-aided dispersal is a major contributor to the observed global distribution of the marine amphipod *Caprella mutica*, with shipping (ballast water and hull fouling) and the aquaculture industry highlighted as important transportation vectors (Chapter 2). Over shorter distances, natural dispersal may play a more important role (Havel & Schurin 2004). At the local scale, on the west coast of Scotland, the relative roles of natural and human-aided mechanisms in the dispersal of *C. mutica* are unknown.

On the west coast of Scotland, *C. mutica* has only been identified on artificial habitats and the distribution could be described as discrete suitable habitat patches connected by the dispersal of individuals, i.e. metapopulations (Baguette 2003). Dispersal distances are essential features in the functioning of metapopulation systems, determining the range of recolonisation after local extinction and thus the spatial scale of the metapopulation (Hanski 1999). Dispersal mechanisms of caprellid species have been shown to include crawling, swimming and rafting, but varies between species, for example, *Caprella laeviuscula* disperses by all three mechanisms, whilst *Deutella californica* disperses predominantly by rafting (Caine 1980). *Caprella mutica*, like all amphipods, does not have a planktonic larval stage (Buschbaum & Gutow 2005); free-swimming (natural) dispersal is, therefore, most likely through short-distance swimming and current-driven distribution following disturbance from the substrate. In order to successfully establish a new population, individuals of both sexes (or an ovigerous female successfully producing individuals of both sexes) are necessary. Sexual dimorphism in dispersal is common (Lambin et al. 2001) and can act to prevent close inbreeding or may be the result of sexual asymmetries in social interactions, for example, reproductive behaviour

and competition (Greenwood 1980, Perrin & Mazalov 2000, Stevens et al. 2006). It may also reflect the natural sex ratio of the population. Sexual dimorphism in dispersal has not been previously noted for Caprellidae, however, populations of *C. mutica* have been shown to be female-biased (Chapter 6, Fedotov 1991) and this may also be reflected in dispersing individuals.

Metapopulation dynamics are considered to be dominated by short-distance movements and were previously assumed to follow a negative exponential distribution (Hanski 1999). However, this distribution strongly underestimates the probability of long-distance movements and might lead to misinterpretation of the scale of the population (Baguette 2003). More recent studies have shown that the inverse power function gives a better fit to empirical dispersal data (Hill et al. 1996, Thomas & Hanski 1997, Baguette et al. 2000, Baguette 2003). Both functions can be parameterised with field data to generate a dispersal kernel describing the probability of an individual travelling a given distance (e.g. Kot et al. 1996, Clark et al. 2001). The question remains about the choice of theoretical distribution function and is most likely species- and habitat-specific (Baguette 2003). Migration rate, habitat quality, habitat area and distances between habitats can all be important (Baguette et al. 2000).

Caprella mutica has been found amongst drifting seaweed communities in its native habitat (Sano et al. 2003) and this is another, potentially important, natural vector for this species. Driftweed, or free-drifting conglomerations of macrophytic algae detached from the substrate (Dwyer 2002), is a common occurrence in many near-shore areas of the world's oceans. It has been suggested that this mechanism is responsible for the long-distance dispersal (> 1000 km) of several planktonic species, including reef corals, bivalves and other macrobenthic species (Jokiel 1984, Helmuth et al. 1994). Rafting on driftweed enables non-planktonic species which brood their young to broaden their distributions and undergo genetic exchange with geographically distant populations (Helmuth et al. 1994, Thiel & Gutow 2005). A recent study of drifting kelp rafts (*Macrocystis pyrifera*) found 72 macrofaunal species (Hobday 2000), while a review of rafting organisms identified a total of 1205 species (fauna and flora), including several Caprellidae (Thiel & Gutow 2005). Caprellids favour highly branched substrata (Caine 1978) and, therefore, may have preferences for specific growth forms of drifting algae. There is strong spatial and temporal variation in abundance of floating macroalgae; a higher abundance is found in coastal waters than in open-ocean waters and in spring and summer months at temperate latitudes (Thieltges et al. 2004, Thiel & Haye 2006).

Dispersal dominated by short-distance movements (characteristic of metapopulations) decreases the risks associated with primary introduction, but may be counterbalanced by human-mediated, long-distance dispersal (Minchin & Gollasch 2002, Wolff & Reise 2002).

Secondary dispersal of exotic species is often tightly linked to human activities: for example in association with aquaculture transfers (Naylor et al. 2001); through transportation with recreational boats (e.g. *Dreissena polymorpha*, Minchin et al. 2003); in ballast water (e.g. fishes, Wonham et al. 2001) or hull-fouling (e.g. *Undaria pinnatifida*, Hay 1990); and on plastic debris (e.g. *Elminius modestus*, Barnes & Milner 2005).

Over very large distances, human-mediated transport may be far more important for dispersal than natural methods (Carlton 1987). However, quantitative comparisons of the relative importance of various methods of dispersal are lacking (Watts et al. 1998). On the west coast of Scotland, recreational boating is common and could be an important vector for dispersal of *C. mutica*. Fouling assemblages develop on the submerged surfaces of boats and *C. mutica* has been observed amongst these assemblages on local marinas and boats hoisted from the water (pers. obs.). Although recreational boats are often cleaned at regular intervals, travel at relatively fast speeds and spend only a few days in one port (Carlton & Hodder 1995), recreational boating has been implicated in the introduction and spread of a number of other marine species (Floerl et al. 2005). These species include the zebra mussel *Dreissena polymorpha*, the Caribbean tubeworm, *Hydroides sanctaecrucis*, the cladoceran zooplankter *Daphnia lumholtzi* and several ascidian species (Lambert & Lambert 1998, Field 1999, Lützen 1999, Minchin et al. 2003, Havel & Medley 2006).

Analysis of dispersal over several spatial scales allows a better understanding of the mechanisms governing the introduction process. The global distribution and introduction pathways of *C. mutica* have suggested that shipping and aquaculture are the most important vectors over long distances (>100 km, Chapters 2 & 3). At the local scale, natural dispersal (both independent and rafting) and recreational boating are likely to be important in the distribution of *C. mutica*. Previous studies of natural dispersal dynamics have implemented mark-release-recapture techniques (MRR, e.g. Hill et al. 1996, Baguette et al. 2000, Baguette 2003) or used an historical data set to estimate a dispersal rate (Crisp 1958, Herborg et al. 2003). However, in the case of *C. mutica*, MRR techniques were unfeasible. The location of suitable habitat patches in the local area was not fully understood and there are few historic records of the distribution of *C. mutica* in Scotland. Therefore, suitable habitat patches were artificially installed around a known source (Jenkins & Buikema 1998). To parameterise the functions, dispersal was assumed to be density dependent (Hanski 1999), i.e. the number of individuals dispersing would be a function of the abundance at the source.

Rafting on drifting seaweed and recreational boats may also be important in the local dispersal of *C. mutica*. In order to demonstrate that a species is dispersed by rafting or floating it is necessary to show that sufficient numbers of an existing population drift away from an

inhabited site and arrive at a new, uninhabited site and that the species is able to persist at the new location (Highsmith 1985). To investigate the importance of recreational boating as a vector for the dispersal of *C. mutica*, arrival of individuals at a marina site was compared with arrivals in the neighbouring area.

The aim of this study was to parameterise the free-swimming dispersal kernel of *C. mutica* and to test the hypotheses that driftweed and recreational boating are dispersal vectors of *C. mutica.*

4.2 Methods

All the experiments were completed in 2005, during the spring (April-June) when populations in the area were known to be most abundant (Chapter 6).

Free-swimming dispersal

Suitable habitat patches were installed around Dunstaffnage fish farm (see Chapter 6 for site description). Abundance of *C. mutica* at the fish farm has been recorded in a parallel study (Chapter 6). On 13-14th April 2005, an array of experimental panels were deployed at increasing distances of 20 m, 150 m and 1000 m from the edge of fish-farm cages (Kinlan & Gaines 2003), at a water depth of 6 m below the surface (Figure 4-1) using GPS (Furuno GP-50 Mark 2 GPS Navigator). *C. mutica* have been previously sampled at approximately this depth (Willis et al. 2004, Cook et al. 2006, Vassilenko 2006). The positions were chosen parallel to the expected direction of the residual current in the area (northeast, Cook et al. 2006), giving the maximum likelihood of caprellids arriving on the meshes. To confirm the effect of current, two Aanderaa RCM8 current meters (Aanderaa Instruments, Bergen, Norway) measuring hourly current direction were suspended in the water column at a water depth of 6 m below the surface for the duration of the experiment.

Experimental panels consisted of 4 replicate meshes (25 x 10 cm, mesh size 5 mm, Clean-ups, EGL Homecare) attached to a frame, suspended at a water depth of 6 m on a shot and buoy system (Figure 4-1). Each deployment was separated from the next by at least 20 m.

After 8 weeks (sufficient for recruitment, Cook et al. 2004), the panels were collected. On 22 - 23rd June, abundance of *C. mutica* individuals on the mesh panels were recorded; where fewer than 10 individuals were present, sex and presence of ovigerous females was also recorded.

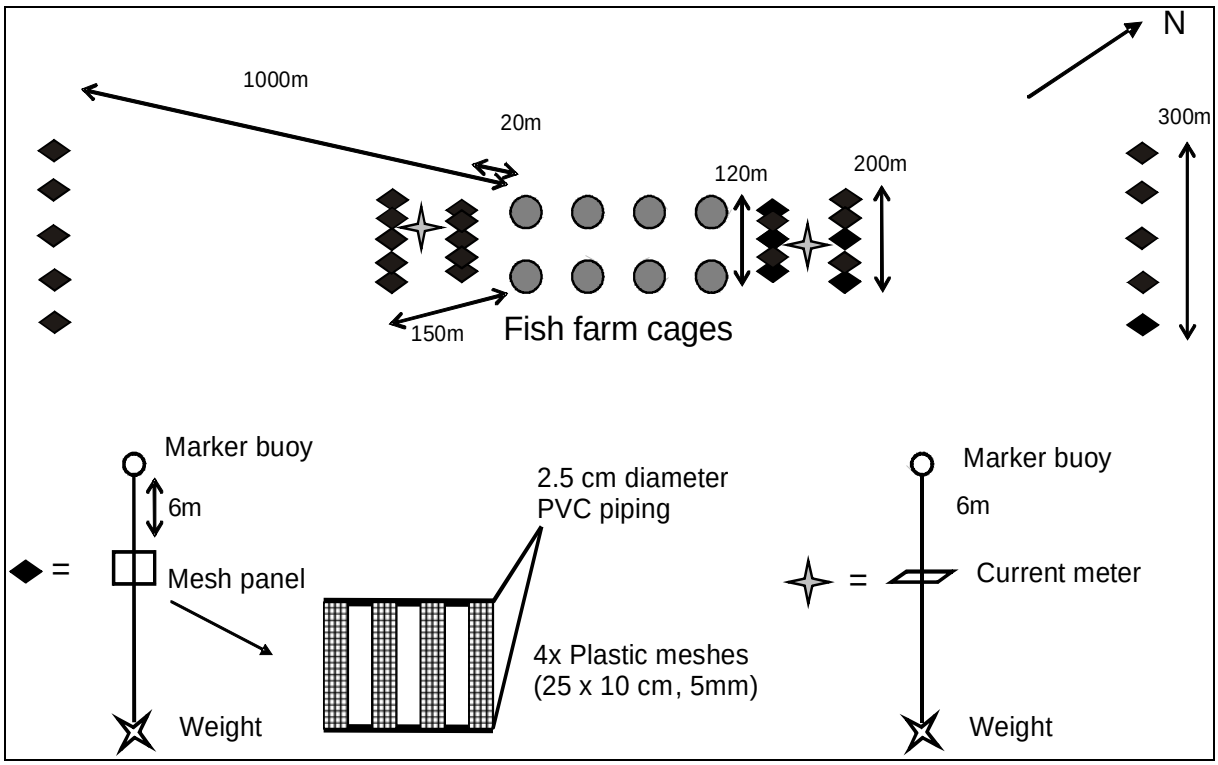


Figure 4-1. Layout of apparatus used to suspend experimental panels.

Driftweed-associated dispersal

The area east of Lismore Island, where Loch Linnhe meets the Lynne of Lorne, was used to study the drift-weed associated dispersal of *C. mutica* (Figure 4-2). This area encompasses several aquaculture and other man-made sites (e.g. Dunstaffnage Marina and buoy deployments above an artificial reef) where *C. mutica* has been found previously (Figure 4-2). The maximum depth of the area is 47 m (*C. D.*). Residual current flow is southwest (Wilding & Sayer 2002), with water flowing out of Lochs Linnhe and Etive, parallel to Lismore.

During the summer of 2005, three sampling trips were taken on the RV Seol Mara (SAMS) following the same approximate course over a distance of 40 kilometres (Figure 4-2). *C. mutica* is known to inhabit several fish farms in the area and also several independent buoy deployments (Figure 4-2). During the trips, driftweed was collected opportunistically from within 25 m of the boat (a total area of approximately 1 km^2, i.e. 40 km × 25 m^2) and placed in plastic bags for inspection on return to the laboratory. Where clumps were too large for collection and analysis, a sub-sample, including all types of algae present, was collected. On return to the laboratory, the algae were weighed (wet weight in kg) and inspected; presence/ absence of *C. mutica* was noted and the algae present in the clumps were identified (according to: Hiscock 1979, Hiscock 1986, Sears 2002). Where individuals of *C. mutica* were present, abundance, sex and presence of ovigerous females was also noted.

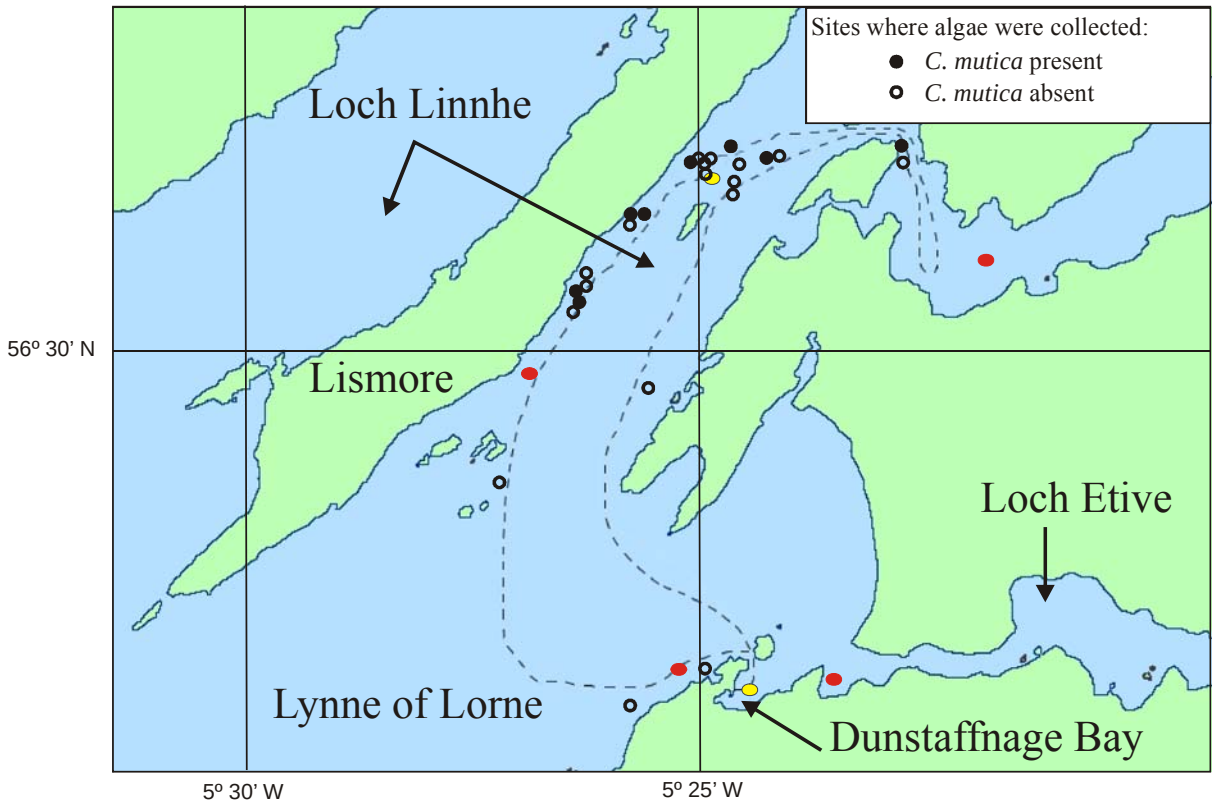


Figure 4-2. Approximate course taken by Seol Mara RV during sampling trips for collection of driftweed (dashed line).

Recreational boating - associated dispersal

The Dunstaffnage Marina was used as a site with recreational boating activity (see Chapter 6 for site description), the open water adjacent to the Marina was used as a site without recreational boating activity. *Caprella mutica* has been present at the Marina in previous years but was not recorded at this site for 8 months prior to this study (Chapter 6).

Weekly sampling began on the 2nd June 2005 and continued for 9 weeks (approximately the same time period as the free-swimming dispersal experiments). Every week, sampling meshes (25 x 10 cm, mesh size 5 mm) were deployed at the positions shown in Figure 4-3. Each cluster of 3 deployments was more than 50 m away from any other, each deployment was separated by 5 m. Deployments consisted of a weighted rope with one mesh attached at a water depth of 0.1 m (surface) and one mesh at 3 m. At the Marina, the floating pontoons were used to suspend the weighted ropes, whilst in open water a buoy was used. After 7 days, the meshes were observed for presence of *C. mutica* and replaced. When caprellids were present, abundance and sex (including juveniles and whether females were ovigerous) were recorded.

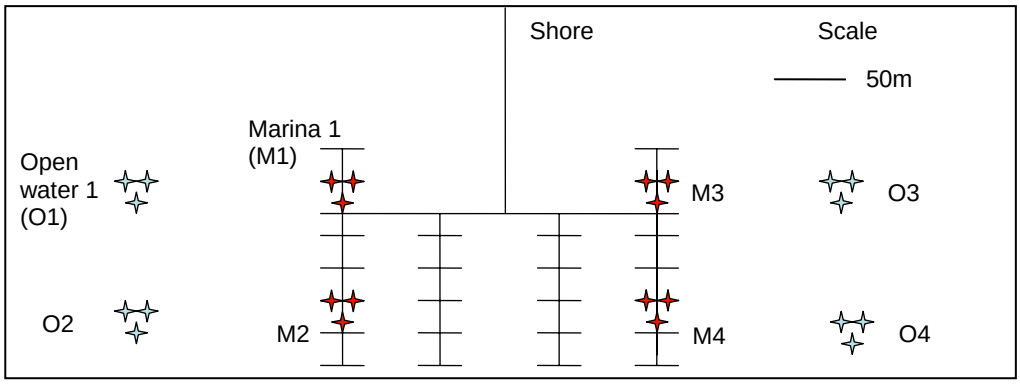


Figure 4-3. Position of mesh deployments at Dunstaffnage Marina (✧), near Oban; see text for description of deployments. (M- Marina, red symbols; O- open water, blue symbols)

Statistical analysis

The probability of free-swimming dispersal was calculated as the abundance (individuals m^{-2}) at a given distance (20 m, 150 m, 1000 m) as a proportion of the average abundance recorded at the fish farm. Both the negative exponential function and inverse power function were fitted to the observed distributions of proportions dispersing (Baguette et al. 2000). Using the negative exponential function, the probability (P) of an individual moving a certain distance (D) is:

$$P = a\, e^{-k\,(D)} \qquad \text{or} \qquad \ln(P) = \ln(a) - k\,(D)$$

where *k* is the dispersal constant describing the shape of the particular exponential curve and *a* is a scaling constant. Using the inverse power function, the probability (P) of an individual moving a certain distance (D) is:

$$P = a\, D^{-n} \qquad \text{or} \qquad \ln(P) = \ln(a) - n \ln (D)$$

where *a* is a scaling constant and *n* is a variable determining the effect of distance on dispersal. Linear transformed data were analysed using regression analysis (Baguette et al. 2000).

The functions were used to predict the proportion of individuals dispersing over 10 m intervals up to 1 km. These were applied to the abundance of individuals at the source population to predict the abundance of individuals at increasing distances from the source population. These were presented with the actual observed abundance. A *t*-test (or its non-parametric equivalent, Mann-Whitney test) was used to test if the sex ratio of dispersing individuals was biased.

The relationship between wet weight of algae (kg) and abundance of dispersing *C. mutica* was tested with Spearman's rank correlation. A *t*-test (or its non-parametric equivalent, Mann-Whitney test) was used to test if sex ratio of dispersing individuals was biased. Algae were classified into growth form (broad, strap-like, filamentous) and colour (brown, green, red).

Chi-squared tests were used to test significance between the presence of an algal species or class and presence of *C. mutica*.

The abundance of individuals dispersing in association with recreational boating consisted of a large number of zero values, therefore, the data from both depths were combined. The probability of dispersal was calculated as the number of meshes with *C. mutica* as a proportion of the total number of meshes (calculated independently for open water and Marina; n = 12). A Chi-squared test was used to analyse if caprellids were equally likely to be found on the Marina and open water meshes (presence/absence data). A further Chi-squared test was used to analyse differences in numbers of males and females found on the meshes.

4.3 Results

Free-swimming dispersal

The two current meter deployments confirmed that the predominant direction of currents around the fish farm was parallel to the fish farm (Figure 4-4). The difference between the two figures is a result of the fish farm obstructing the current reaching the northeast current meter on the flood tide and the southwest current meter on the ebb tide.

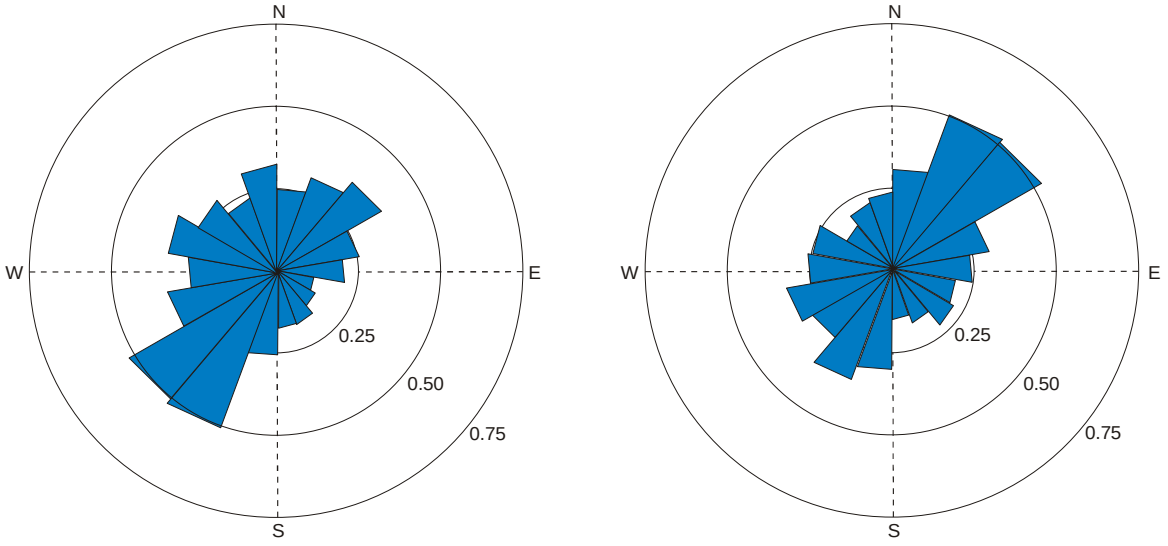


Figure 4-4. Direction of current flow as proportion of time when the current was flowing within 20° angles, measured from the two current meters (figure on right refers to current meter southwest of the fish farm). Compass directions are outside of the circles. The fish farm lies approximately parallel to the northeast-southwest current flow.

Abundance declined rapidly as the distance from the source population at the fish farm increased (Figure 4-5). There was no directional bias in the dispersal, indicating that natural

dispersal was responsible for the observed pattern and vector driven dispersal (e.g. the fish farm boat operating to the south of the site) was not influencing the results.

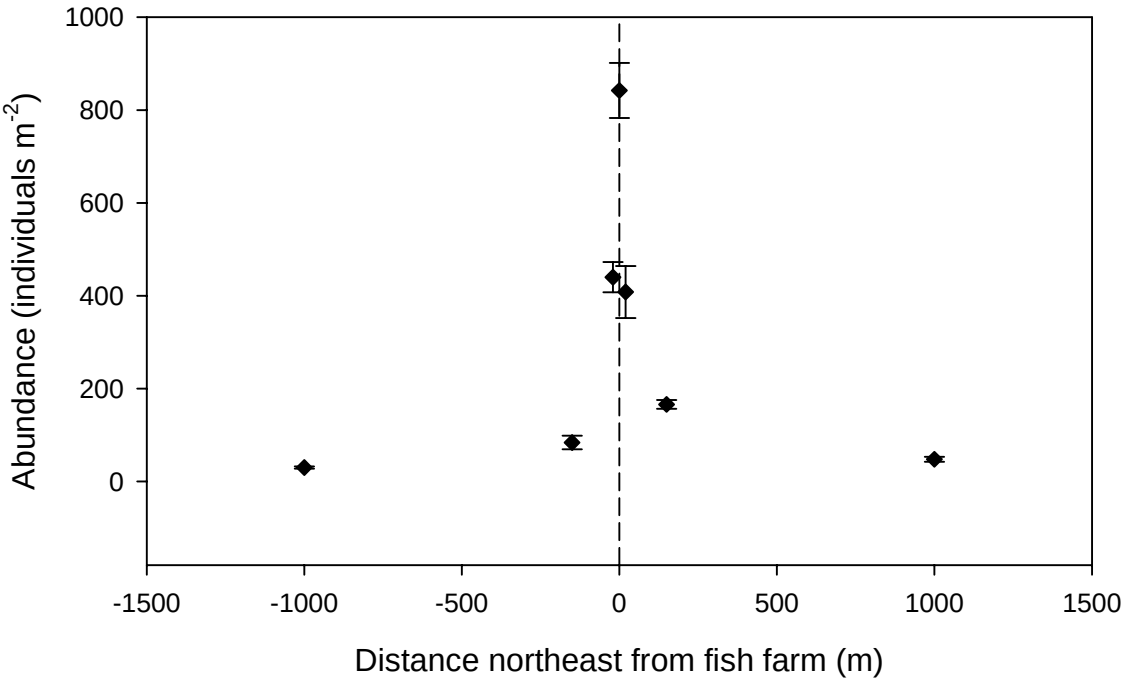


Figure 4-5. Abundance of *Caprella mutica* dispersing from a fish farm source population. Data are mean ± SE (n = 5 except for distance = 0, where n = 3).

For the negative exponential function, ln (P) was regressed upon distance in metres:

$$\ln (P) = \ln (a) - k (D)$$

$$P = a\, e^{-k (D)}$$

$$P = 0.3777\, e^{-0.0023(D)} \quad (R^2 = 0.69)$$

For the inverse power function, ln(P) was regressed upon ln(D) in metres:

$$\ln (P) = \ln (a) - n \ln (D)$$

$$P = a\, D^{-n}$$

$$P = 1.5739\, (D)^{-0.5002} \quad (R^2 = 0.79)$$

The negative exponential function predicted a gradual but continuous decline to 40.96 individuals m^{-2} at 1000 m (Figure 4-6). The inverse power function predicted an initially steep decline, which flattened to a fatter tail (56.62 individuals m^{-2} at 1000 m). The inverse power function was better at predicting the observed abundance ($R^2 = 0.79$ compared to 0.69 with the negative exponential function).

On the meshes where sex ratio was recorded, males were significantly more abundant than females (Mann-Whitney test, n = 13, $P < 0.001$).

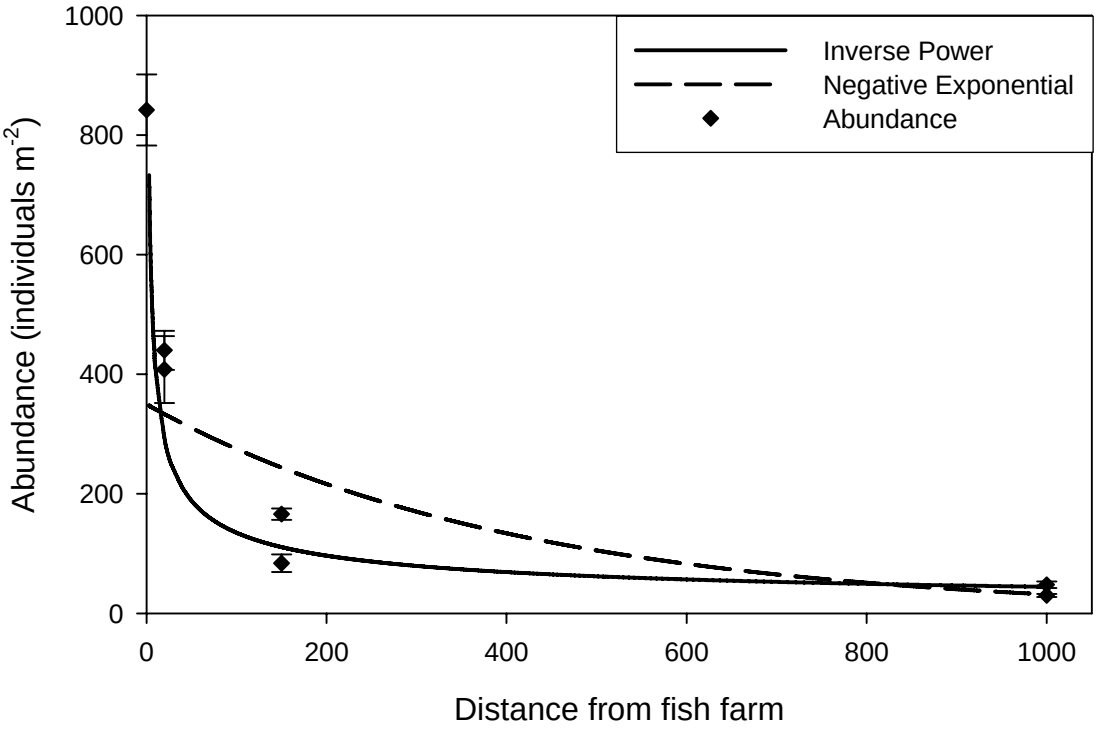


Figure 4-6. Dispersal kernels generated by the inverse power and negative exponential function on the proportion of individuals dispersing from the source (fish farm) population. Recorded abundance is also presented (mean ± SE).

Driftweed-associated dispersal

Twenty-six clumps of driftweed were collected over the three sampling trips (Figure 4-7); seven clumps (26.9%) had *C. mutica* present in varying, but frequently low abundance (< 10 individuals per clump, Figure 4-8). The maximum number of *C. mutica* on a single clump was 71, including 26 males and 37 females, 10 of which were ovigerous; this was the only sample to have ovigerous females present. There was no significant difference between female and male presence or abundance on the algal clumps (Mann-Whitney test, n = 26 for presence, n = 7 for abundance, $P > 0.05$). The greatest number of algal clumps was collected on the first trip (n = 14), which also had the highest number of clumps containing *C. mutica* (n = 6). There was a positive, but non-significant relationship between the wet weight of the clump and the number of *C. mutica* present (Spearman's rank correlation $r = 0.316$, P = 0.116). Most of the clumps of algae were found within 200 m of the shore.

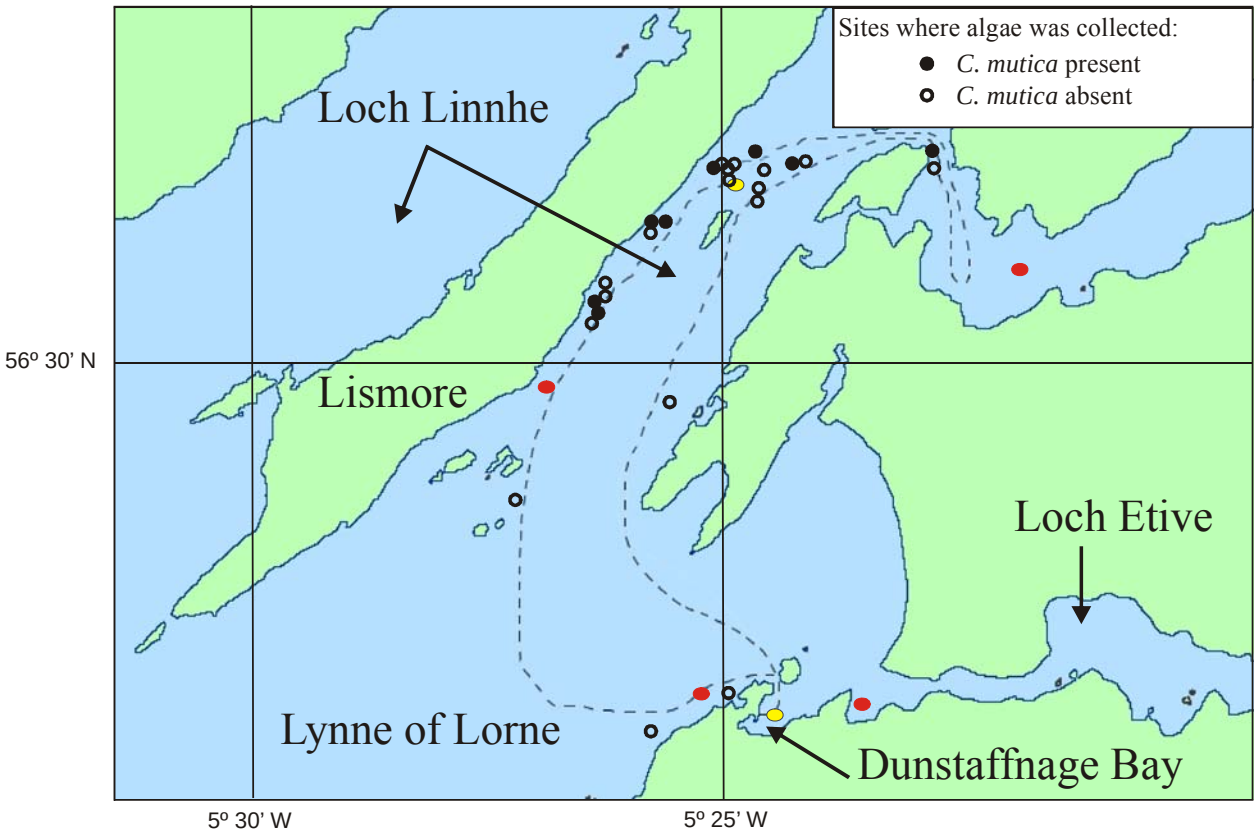


Figure 4-7. Position of algal clumps collected during sampling trips. Circles indicate position of algae; closed circles indicate presence of *C. mutica* on the algae.

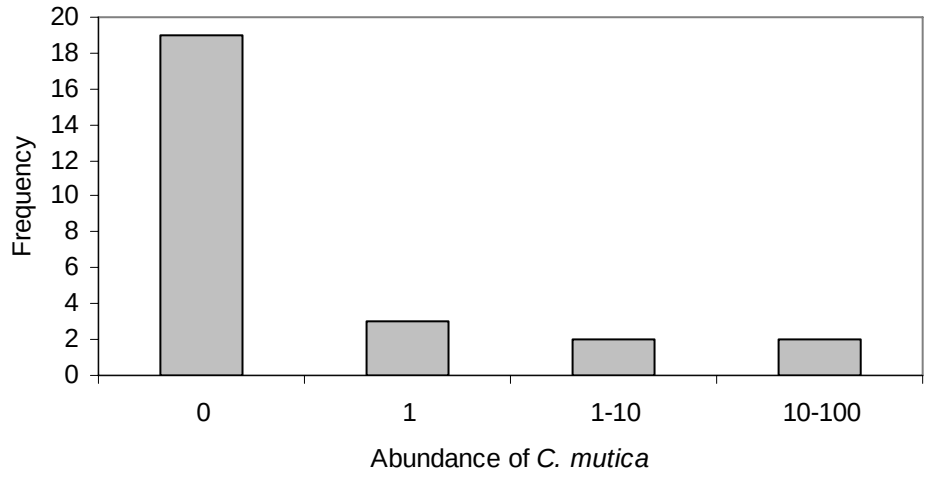


Figure 4-8. Frequency of abundance categories (number of individuals per clump) of *C. mutica* collected on drifting seaweed patches.

16 classes of algae were identified from the clumps (Figure 4-9). *Fucus vesiculosus* was present in the most clumps, with *Ascophyllum nodosum, Chorda filum* and *Enteromorpha* spp. also being found frequently (Figure 4-9). *C. mutica* was found most often in association with *Chorda filum*. *Fucus vesiculosus*, *Enteromorpha* spp. and *Pylaiella* spp. were also frequently found in clumps with *C. mutica* present. The species of algae present in clumps did not, however, predict the presence of *C. mutica* ($\chi^2 = 7.548$, $P > 0.05$, df = 14). Clumps categorised by growth form or class were also not significant ($\chi^2 = 0.048$ and 0.142 respectively, both $P > 0.05$, df = 1).

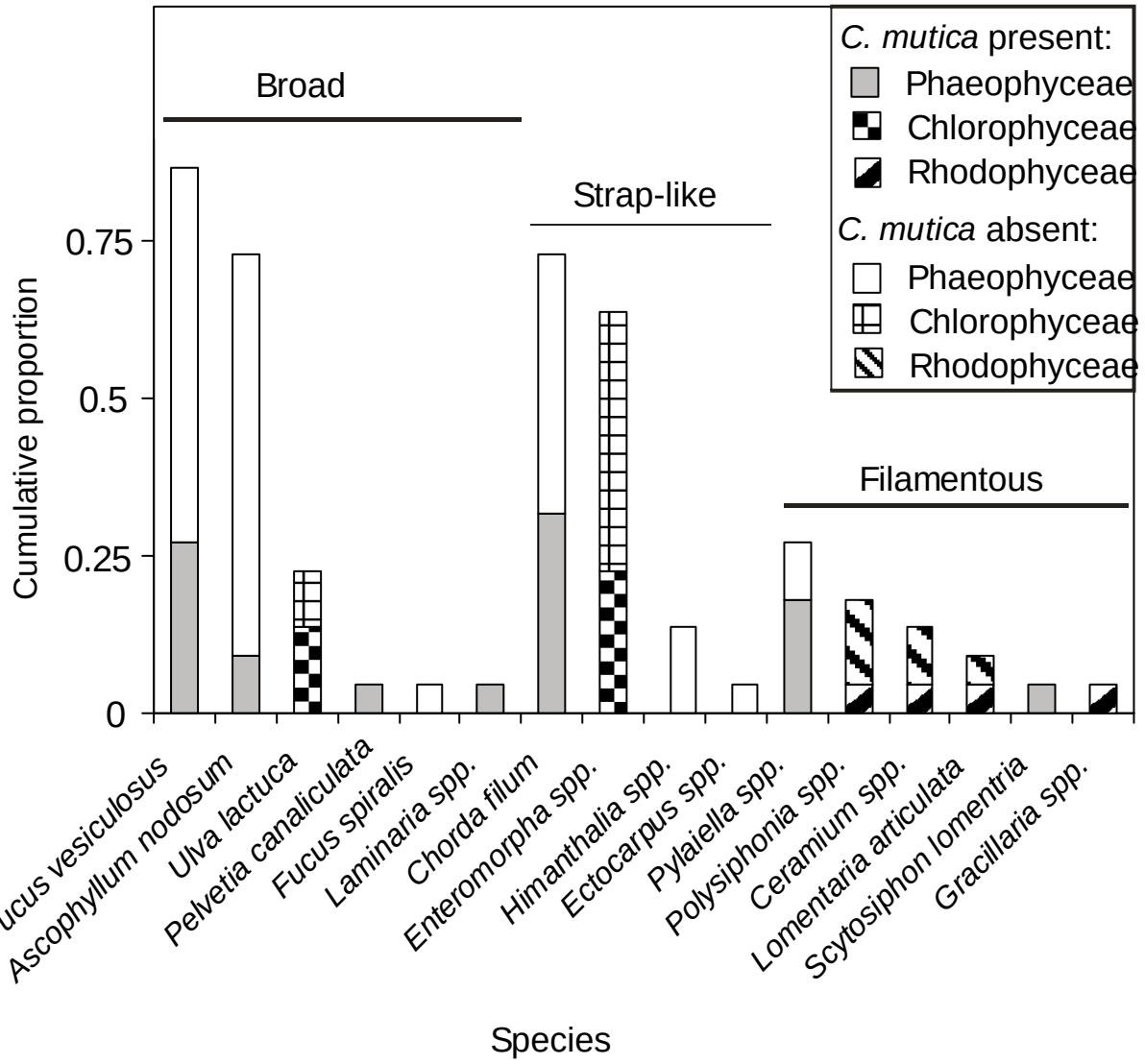


Figure 4-9. Presence of algal classes in drifting algal clumps. Algae are categorised by growth form (broad, strap-like, filamentous) and column fill indicates Class (Phaeophyceae, Chlorophyceae and Rhodophyceae).

Recreational boating - associated dispersal

The probability of finding *C. mutica* on the meshes was low throughout the experiment (≤ 0.35, Figure 4-10). *Caprella mutica* were present on the meshes attached to the Marina on 7 sampling occasions and those in open water on 4 occasions. *Caprella mutica* were present at the Marina 2 weeks before the open water meshes. After the first sighting (16th June 2005), caprellids were present at the Marina throughout the study and significantly more likely to be present on the meshes at this location compared with the open water ($\chi^2 = 4.054$, $P = 0.044$, Table 4-1a). Probabilities of finding *C. mutica* both on the Marina and in open water increased through the experiment (Figure 4-10). For this reason general linear modelling of probabilities was used to compare the two sites. 'Site' was used as a random variable to predict the probability of *C. mutica* being present, with time after the start of the experiment as a covariate. Both variables were significant ($P < 0.05$, df = 8 for time and 1 for site). *Caprella mutica* were more likely to be found on the Marina than the open water meshes.

There was no significant difference in numbers of males and females arriving on the meshes ($\chi^2 = 0.394$, $P > 0.05$, Table 4-1b); juveniles were only present on two meshes (0.4% of all meshes).

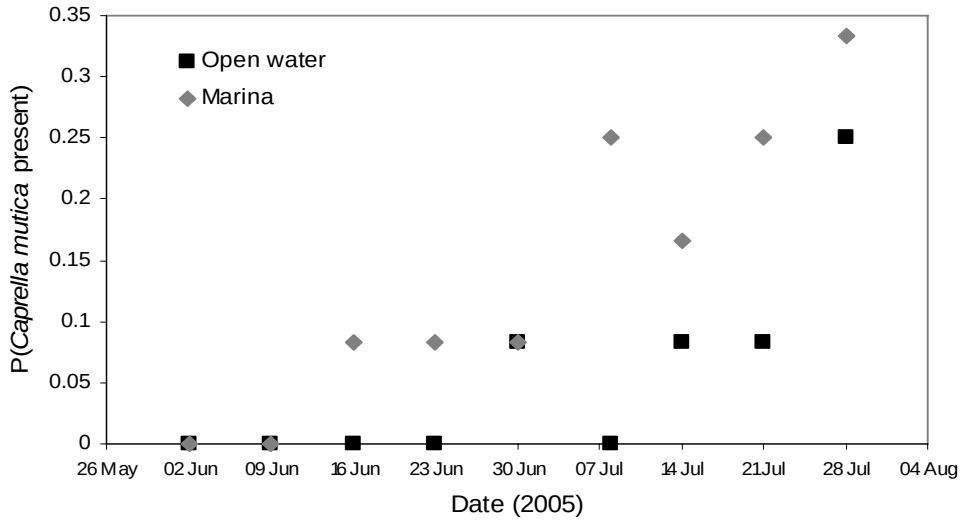


Figure 4-10. Probability of presence of *Caprella mutica* on Marina and open water meshes (n = 12)

Table 4-1. Contingency tables for chi-squared analysis. a) presence/absence data ($\chi^2 = 4.054$, $P = 0.044$); b) number of males and females ($\chi^2 = 0.8402$, $P = 0.394$)

a)

	Present	Absent
Marina	15	201
Open Water	6	210

b)

	Males	Females
Marina	8	11
Open Water	6	4

4.4 Discussion

Dispersal is a central trait in understanding the ecology and evolution of a species, affecting the abundance, distribution and dispersion of individuals, populations and communities. In a uniform environment, the frequency of dispersing individuals follows a monotonic dispersal-distance function (Wiens 2001), often described using a dispersal kernel. The dispersal kernel which best predicted the observed dispersal of *Caprella mutica*, was the inverse power function, with the value of the power being approximately -0.5. The value of the power reflects the 2-dimensional nature of the dispersal. If the dispersal was equal in all directions (i.e. 3-dimensional), an exponent of -2 would be expected, i.e. doubling the distance from the source would quadruple the area over which individuals were being dispersed, bringing about a four-fold decline in abundance ($P = a / D^2$ or $P = a D^{-2}$). If dispersal were 2-dimensional, an exponent of -1 would be expected, i.e. the abundance declines uniformly with the distance from the source. Dispersal of *C. mutica* is close to 2-dimensional, as individuals can only spread from the source within the water column and cannot travel in 3-dimensions. However, the experiment was designed parallel to the direction of the current (confirmed with current meters) and *C. mutica* were more likely to arrive on the meshes than if the panels were randomly spaced around the fish farm, and the value of the power is less than 1. (1-dimensional dispersal occurs in a unidirectional flow e.g. a river system, when there is little decline in dispersing individuals with distance, and the value of the power would be close to 0.) The better fit of the inverse power function supports the findings of previous studies (Hill et al. 1996, Thomas & Hanski 1997, Baguette et al. 2000, Baguette 2003) and indicates that long-distance movements are important in the dispersal of *C. mutica*.

The fit of the exponential function and the interpretation of the exponent suggest that the data are representative of natural dispersal and not a result of human-aided vectors. Marine vessels are minimal in the area due to the presence of the fish farm and a boat servicing the fish farm was known to enter from the south daily; the influence of this vessel was not apparent from the pattern of dispersal. Small numbers (1 – 5 individuals per 150 m depth haul of a 45 cm radius, 315 µ mesh plankton net) of caprellid amphipods have previously been identified in planktonic samples from marginal seas (Takeuchi & Sawamoto 1998) and have been observed swimming short distances in the laboratory (pers. obs.). Passive planktonic dispersal is most likely an effective mechanism for *C. mutica*. However, the experiments of Takeuchi and Sawamoto (1998) and those carried out around Dunstaffnage fish farm can not distinguish between free-swimming *C. mutica* and those attached to other suspended matter (biotic or abiotic). Anecdotally, when disturbed in a laboratory tank *C. mutica* will swim a short distance before extending all pereopods and sinking to the bottom. Further experiments,

for example video monitoring of the water column or plankton tows in the area, would be needed to confirm true free-swimming dispersal of *C. mutica*.

Estimates of dispersal from genetic studies of marine organisms that lack both a planktonic larval phase and an adult dispersal mechanism are generally less than 1 km, whilst those which have dispersal mechanisms in the adult phase (including rafting) range up to approximately 10 km (Kinlan & Gaines 2003). A review of the dispersal abilities of marine benthic organisms found estimates of dispersal distances to range from <1 m to 4400 km, with that for Crustacea ranging from 30 m to over 200 m (Shanks et al. 2003). The current study found individuals of *C. mutica* to disperse around an established population to distances of up to 1km. The inverse power function predicts a fatter tail to the dispersal kernel, the better fit of this model supports the suggestion of the importance of long-distance dispersal processes in marine systems (Kinlan & Gaines 2003). Furthermore, direct estimates of propagule dispersal often underestimate dispersal scale, but seldom overestimate it (Cain et al. 2000, Nathan & Muller-Landau 2000); indicating individuals of *C. mutica* may be capable of dispersing much further. However, over longer distances, other processes are likely to be involved; for example, vector driven transport (Chapter 2).

Analysis of caprellid amphipods in plankton samples from north-east Asia (including the open Pacific Ocean) found that caprellids were limited to marginal seas (Takeuchi & Sawamoto 1998). Dispersal by marine currents has the potential to move individuals over distances greater than physical processes for terrestrial species (Grosholz 1996). Despite the increased likelihood of long-distance dispersal events, however, the predicted rate of range expansion of marine taxa is similar that that of terrestrial taxa (Grosholz 1996). Grosholz (1996) also found predictions of dispersal for marine taxa to consistently exceed observations, and it was suggested that the long-distance dispersers were failing to establish. After 8 weeks, 80% of the substrata at 1 km from the fish farm had up to 5 individuals of *C. mutica*. The results show that individuals can disperse over relatively long distances but do not give an indication of whether numbers dispersing over these distances can establish viable populations in the recipient environment. Factors limiting the successful establishment of *C. mutica* may include: environmental conditions, propagule pressure and biotic interactions (Williamson 1996). The subject of propagule pressure and the number of individuals necessary to establish a population demands further attention (Lockwood et al. 2005).

Rafting on floating material can greatly enhance natural dispersal in the marine environment (Watts et al. 1998, Kinlan et al. 2005, Thiel & Gutow 2005, Thiel & Haye 2006). *C. mutica* was found on 27% of the algal clumps collected; dispersal by rafting can enhance the free-swimming dispersal of *C. mutica*. Rafting may contribute to population connectivity over a

wide range of geographic (< 100 km up to > 5000 km) and time scales (frequent, intermittent and episodic; Thiel & Haye 2006). Dispersal by rafting is a stochastic event and it is difficult to quantify its significance in the marine environment (Thiel & Haye 2006). The correlation between the wet weight of the drift algae and number of individuals present, although not significant in this study, has also been described in other studies (Gore et al. 1981, Stoner & Greening 1984). Dispersal of *C. mutica* by rafting is likely to be most frequent during spring and summer months, when large quantities of algae are produced along the continental shelf (Thurston 1997, Thiel & Haye 2006). Rafting is only important if rafters can subsequently establish in a new area. Success of an introduction is positively related to the number of propagules introduced (Williamson 1996, Lockwood et al. 2005). The presence of 71 individuals of mixed sexes, including ovigerous females, on a single algal clump suggests that *C. mutica* has the potential to successfully establish a population following dispersal by rafting on driftweed. Another important implication of the presence of *C. mutica* on seaweeds native to the UK, is the potential for them to become established in more natural habitats, extending beyond the disturbed habitats where they have been found to date (Chapter 2).

While oceanographic and climatic conditions probably determine the direction of free-swimming or rafting routes in the ocean (Gaines et al. 2003, Muñiz-Salazar et al. 2005), transport associated with human vectors can make these factors irrelevant (Petersen et al. 1992). *C. mutica* individuals arrived on the meshes attached to the Marina before those in the open water, and were opportunistically collected from boat hulls lifted from the Marina. The association of *C. mutica* with recreational boating vessels not only increases the frequency of arrival in natural currents but also allows dispersal against the direction of current flow and the crossing of physical barriers in time and space (Carlton 2003b). Localised recruitment through natural dispersal may be punctuated by brief episodes of long-distance dispersal by human-aided vectors (Thresher et al. 2003).

The presence of *C. mutica* on the meshes attached to the Marina was followed two weeks later by individuals arriving on the meshes deployed off the Marina. One explanation for this is the establishment of a population on the Marina (brought in by recreational yachts), which then spread to the open-water meshes. This is supported by Chapter 2 (in 2006, *C. mutica* individuals first arrived on the Marina on the 9th June) and would also explain the increase in the likelihood of presence of *C. mutica*, both on the Marina and in the open water, with time. An alternative explanation is the presence of a permanent population on the Marina, which lies dormant over winter. However, environmental conditions at the Marina are very similar to those at the fish farm, and it is thought that if the population was lying dormant, it would most likely have been active before the onset of the study. The natural dispersal of individuals

is most likely density-dependent (Travis et al. 1999) and, therefore, more individuals disperse later in the year when abundance is greatest (Chapter 6).

C. mutica is capable of dispersing over distances of at least 1 km naturally in the water column. Care must be taken when extrapolating beyond the data range (Hill et al. 1996), however, it is expected that at the height of abundance (60,000 individuals m^{-2}; Chapter 6) *C. mutica* could naturally disperse further than 1 km, most likely over a few kilometres (the invasion front of *Elminius modestus* advanced approximately 20-30 km per year; Crisp 1958). Enhancing this natural movement, driftweed and recreational boating vessels are both vectors of secondary dispersal which are likely to be more important over longer distances (>10 km). The importance of scale has been emphasised in several recent reviews, including a special issue dedicated to the subject (Biological Invasions 8:3 2006). The data presented here indicate that several dispersal mechanisms are responsible for the dispersal of *C. mutica* over several spatial scales. Evidence of swimming, rafting and anthropogenic transfer of *C. mutica* indicates populations can interact on scales of up to 10s, 100s and over 1000s of kilometres using these dispersal mechanisms, respectively (Thiel & Haye 2006).

Dispersal, either natural or in association with human activity, is a stochastic process that is difficult to parameterise (Grosholz 1996, Kinlan & Gaines 2003, Trakhtenbrot et al. 2005, Thiel & Haye 2006). More than one function may be necessary to describe dispersal in theoretical models (Baguette 2003), for *C. mutica*, several models describing the multiple dispersal mechanisms would need to be parameterised. However, the highly adaptable nature of *C. mutica* to cling to surfaces and thus foul boat hulls and raft on native species of macroalgae, is an important factor in its success as an introduced species. Following long distance introduction, individuals can take advantage of several vectors enhancing natural dispersal and leading to the distribution patterns observed on the west coast of Scotland (Chapter 2).

Chapter 5

Environmental tolerance of *Caprella mutica*: implications for its distribution as a non-native species

5.1 Introduction

Vermeij (1996) poses the question "What factors prevent populations from spreading beyond their geographical limits?" and offers one possible answer: "physiological tolerances are evolutionarily conservative, resulting in ranges being set by physical characteristics that prevent reproduction or survival". Organisms can be limited by a number of environmental factors, including temperature, pH, salinity, stream-flow velocity and the concentration of pollutants (Begon et al. 2006). Responses of organisms to these factors fall into three ranges of interest: dangerously low, dangerously high (tolerance limits), and the range in between (Begon et al. 2006). Quantitative data on the environmental tolerances of invading species assists estimation of their dispersal rates and establishment ranges in receptor environments (Mann & Harding 2003). Temperature and salinity are the major physical factors affecting the survival and growth of marine organisms (Kinne 1970a, b), and can act singly or in combination to influence distribution patterns (Wiederholm 1987, Stauffer & Boltz 1994).

The physical tolerance of non-native species will determine their success in a new environment and therefore, the potential geographic distribution of a species (the fundamental niche, Begon et al. 2006). For example, the distribution of the invasive amphipod *Gmelinoides fasciatus* in the Baltic Sea is limited by its upper salinity tolerance, in particular the low salinities necessary for successful reproduction (Berezina & Panov 2004); both salinity and temperature tolerances were determined to limit the distribution of *Carcinus maenas* in San Francisco Bay (Cohen et al. 1995). Information concerning physical tolerances can help predict range expansions of non-native species. For example, the wide salinity tolerance of the rapa whelk, *Rapana venosa*, was used to predict its widespread distribution on the west coast of North America (Mann & Harding 2003). Physical tolerance has also been shown to influence unintentional transport by humans (Bruijs et al. 2001, Fofonoff et al. 2003) and the resultant geographic distribution of a species (the realised niche, Begon et al. 2006). Tolerances can also inform decisions concerning control mechanisms, such as heat treatment, which is commonly used to control biofouling organisms in power stations (Rajagopal et al. 2005).

Temperature is considered as the most important abiotic stress affecting ectothermic animals (Davenport & Davenport 2005), having influences from molecular to population levels (e.g. Hochachka & Somero 2002). Individuals respond to temperature with impaired function and ultimately death at the upper and lower extremes (Begon et al. 2006), while over the intermediate range, metabolic reaction rates increase with temperature. Typically, reproduction in ectotherms occurs over a more restricted range of temperatures than growth, which occurs over a more restricted range of temperatures than mere survival. Ultimately, through controlling the rate of all processes, external (environmental) temperature will control the success and survival of a non-native species.

Temperature is the major factor limiting the distribution of *Caprella* spp. in North America (Laubitz 1970), Australasia and Southern Chile (Thiel et al. 2003). In its native habitat, *Caprella mutica* experiences a temperature range of -1.8 to 25 °C (Schevchenko et al. 2004), although, the tolerance range is expected to extend beyond this (Sprague 1963). The high temperature tolerance of *Caprella laeviuscula* allows it to survive where other, geographically sympatric, caprellids are excluded; especially during warmer summer months (Caine 1980). Tolerance of a wide range of environmental conditions is recognised as characteristic of successful invaders (Van der Velde et al. 1998, Strasser 1999). Therefore, does the temperature range of *C. mutica* allow it to survive transportation and introduction while other caprellids are excluded?

The salinity of the aquatic environment also influences distribution and abundance (Begon et al. 2006). This is most evident in estuarine waters where there are particularly sharp gradients between truly marine and freshwater habitats. Tolerance of a wide range of salinities is typical for many amphipod species (Kinne 1970a), and the success of *C. mutica* in sea lochs which are strongly influenced by freshwater inputs (Chapter 2) demonstrates tolerance of estuarine conditions. In the native habitat of *C. mutica* (Sea of Japan), salinities ranging from 11 to 35 have been recorded (Schevchenko et al. 2004). A wide salinity tolerance has also been reported for other caprellid species: the lower median lethal salinity (LC_{50}) of *C. danilevskii* was 13 (Takeuchi et al. 2003); the lower LC_{50} of *C. scaura* and *C. equilibria* was between 7.5 and 11.5 (Cockman & Albone 1987). The observation of *C. mutica* in open-ocean habitats (e.g. oil rigs; Chapter 2) suggests that the upper salinity tolerance is likely to be at least 35. Takeuchi et al. (2003) found the salinity tolerances of four *Caprella* species to be greater than 34 and suggested that the wide salinity tolerance of *Caprella* spp. contributes to their widespread distribution from estuarine to open ocean habitats. A combination of factors, including temperature, salinity, wave exposure, substratum and predation, were suggested as being important (Takeuchi et al. 2003).

As already stated, temperature is often regarded as the principal factor limiting species ranges due to effects on reproduction, larval survival, and growth (Hutchins, 1947; Southward, 1958; Nybakken, 2001). For population biology, the most informative experiments of a species' environmental niche involve long term experiments spanning several generations, with measurements including growth, reproduction and fecundity. In Italy, a higher tolerance of elevated temperatures and faster growth rate make the invasive crayfish, *Procambrus clarkii*, more competitive than the indigenous *Austropotamobius pallipes* (Paglianti & Gherardi 2004). The flexible reproductive processes of the invasive hydrozoan, *Moerisia lyonsi*, in different physical conditions may promote dispersal and establishment in new environments (Ma & Purcell 2005b). Wright et al. (1996) investigated the effects of salinity and temperature on survival and development of young *Dreissena polymorpha*, and found that salinities greater than 6 prevented successful fertilization and embryonic development, limiting the species' potential future distribution. However, these informative experiments were extended from preliminary tolerance experiments (e.g. Setzler-Hamilton et al. 1994, Ma & Purcell 2005a). Understanding an organism's physical tolerance limits can assist in deciding which conditions to manipulate in experiments of growth and fecundity. Tolerance limits can also be informative when considering different transport mechanisms (for example, ballast water undergoes rapid changes in salinity during exchange; Ricciardi & MacIsaac 2000, Horvath et al. 2001) and control options (e.g. heat treatment at power stations; Rajagopal et al. 2005). The following was a preliminary study to estimate median lethal temperature (LT_{50}) and salinity (LC_{50}) of *C. mutica* from a non-native source on the west coast of Scotland.

Statistical Analysis

Biological assays can be used to measure the potency of any stimulus (physical, chemical or biological, physiological or psychological) by means of the reactions which it produces in living organisms; the ultimate response being death (Finney 1971). Statistical procedures have mainly been applied to toxicity tests, calculating the median lethal dose (LD_{50}) or time response curves. However, the same methods have also been applied to tolerances of environmental conditions including temperature and salinity (e.g. Damgaard & Davenport 1993, Cowling et al. 2003, Takeuchi et al. 2003). With toxicity tests, the reactions are frequently monotonic, increasing the dose will increase the reaction. However, with environmental factors, the response can be bi-directional, for example, an animal will respond to both increasing and decreasing temperatures and thus has both a lower and upper temperature tolerance. Maximum likelihood estimates of median lethal responses can be calculated based on the Probit model (Finney, 1971) using MINITAB. This model also

generates predicted percentile mortalities. MINITAB uses a Chi-squared analysis (χ^2) to test whether the data fit the Probit model. When χ^2 rejects the Probit model ($P > 0.05$), a Trimmed Spearman Karber test (TSK) can be used to calculate a non-parametric estimate of the lethal values (Hamilton et al. 1977, U.S.E.P.A. 1990). One disadvantage of the Spearman-Karber analysis is that it only yields the median lethal response (LC_{50}) and 95% confidence limits. When the test results are not unimodal or symmetrical about the median lethal response, the confidence intervals (CI) are unreliable.

5.2 Materials and methods

In June of 2005 and 2006, *Caprella mutica* were collected attached to algal substrata from Dunstaffnage fish farm (see Chapter 6 for site description). The animals were transported, still attached to the algae, to Dunstaffnage Marine Laboratory in 10 L collecting buckets (containing sea water and algal substrate from the collection site). To allow the animals to acclimatise, the buckets were placed in a constant temperature room and maintained at 14 °C for 48 h under an 8:16 h Dark : Light regime and constant aeration. No additional food was given. Fifteen 1.5 L tanks were filled with filtered sea water (100 µm; salinity ~35) and 150 cm^2 of 5 mm mesh (three replicates for each of five experimental conditions). Larger, healthy-looking animals were isolated from the substratum using forceps (care was taken to not harm the animals) and transferred into the 1.5 L tanks. A maximum of 34 individuals, including at least 5 male and 5 female *C. mutica* were added to each tank. Starting numbers in each tank were noted and tanks were kept under an 8:16 h Dark : Light regime throughout the experiments.

Temperature tolerance

Exposure temperatures were selected pragmatically (i.e. temperatures that caused a range of mortalities between 0% and 100% were sought), to allow median lethal lower and upper temperatures to be estimated. Since temperatures below 2 °C could not be attained under natural light conditions in the laboratory, this was the lowest temperature used.

The tanks containing live animals attached to mesh substrata were placed in 60 x 30 cm water baths at 5 temperatures (approximately 0, 10, 20, 30 and 40 °C). Temperatures were maintained using either TropicMarin heaters, or a Grant chiller. Temperature loggers (Tinytag Aquatic, TG-3100) were used to monitor temperatures throughout the experiments. After 48 h, the tanks were removed from the water baths and animals were inspected for mortality

(lack of response to mechanical stimulus). To refine the initial LT_{50}, the experiment was repeated using a narrower range of temperatures around the initial LT_{50} estimate (Quinn et al. 1994).

Salinity tolerance

Exposure salinities were selected pragmatically, to allow median lethal lower and upper salinities (LC_{50}) to be estimated.

Before transferring the animals, the 1.5L tanks were filled with filtered sea water (100 µm) at 5 different salinities (approximately 0, 10, 20, 30 and 40). Salinities were achieved through addition of artificial sea salt (Tropical Marin) to water treated by reverse osmosis; confirmed using a portable refractometer (± 0.5; Atago, S/MILL). The tanks were maintained at 14 °C in a constant temperature room (8:16 h L:D regime). After 48 h, the individuals were inspected for mortality (lack of response to mechanical stimulus). Median upper and/or lower LC_{50} values were calculated in the same manner as LT_{50}. The experiment was repeated using a narrower range of salinities, around the initial lower LC_{50} estimate (Quinn et al. 1994). The upper LC_{50} was beyond the initial experimental conditions.

5.3 Results

Temperature tolerance

The initial experiments determined the upper lethal temperature for *Caprella mutica* to be between 22 and 31 ºC (Figure 5-1). The 48 h LT_{50} from the first experiment was 24.3 ºC (TSK, LT_{50} CI estimates were not reliable). Observations during the experiment indicate animals immersed in sea water at 40 ºC died almost immediately (within 5 minutes). After 48 h, individuals at 2 ºC were immobile until stimulated and responded in a slow manner, compared with the defensive response of animals at 10 and 21 ºC.

The second set of experiments included temperatures between 21 and 30 ºC. A few animals died at all temperatures but there was a marked increase in mortality above 26ºC, with 100% mortality at 30ºC (Figure 5-2). The 48 h LT_{50} from the second data set was 28.3 ± 0.41 ºC (TSK, LT_{50} ± CI). There was no significant difference between mortality of female and male *C. mutica* (t-test, df = 14, $P > 0.05$).

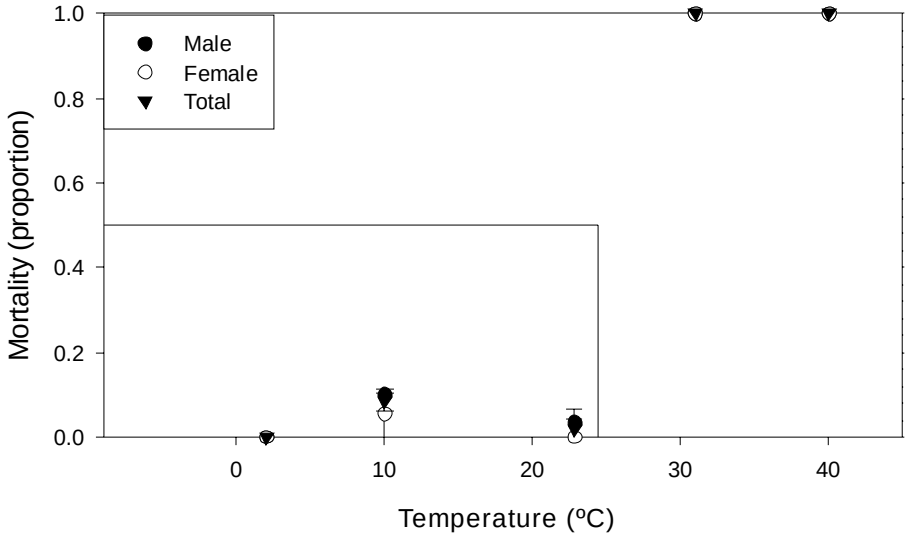


Figure 5-1. Mortality of *C. mutica* following 48 h exposure to temperatures between 0 and 40 ºC (mean ± SE, n = 3). Reference lines indicate 48 h LT_{50} (TSK, 95% CI estimates were not reliable).

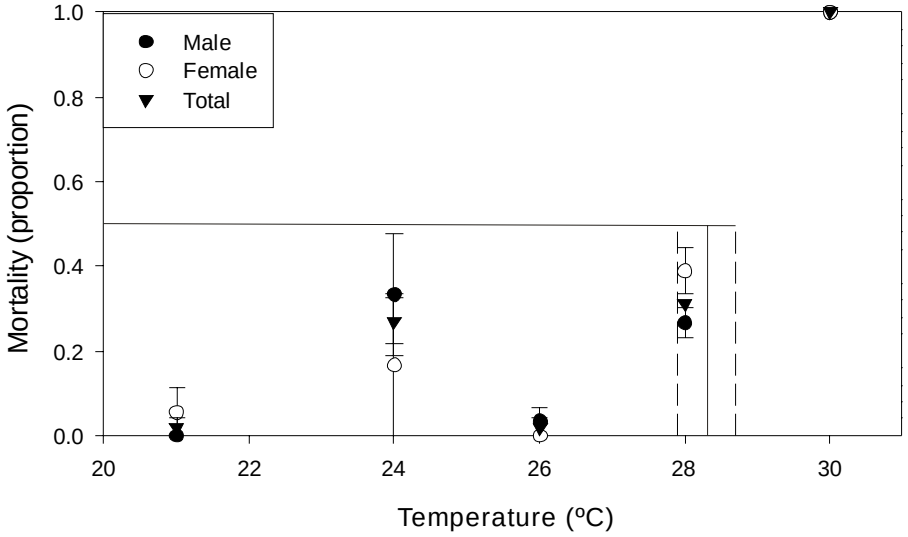


Figure 5-2. Mortality of *C. mutica* following 48 h exposure to temperatures between 21 and 30 ºC (mean ± SE, n = 3). Reference lines indicate 48 h LT_{50} ± 95% CI (TSK).

Salinity tolerance

The first set of experiments found the lower lethal salinity for *C. mutica* to be between 10 and 20 (Figure 5-3). Animals maintained at a salinity of 0 were immobile within 5 minutes of being placed in fresh water. There was no difference between mortality of female and male *C. mutica* (t-test, df = 14, P > 0.05). Total lower LC_{50} was 14.6 ± 0.35 (TSK, LC_{50} ± 95% CI), the upper LC_{50} was beyond 40.

The second set of experiments included salinities between 9 and 20. All animals were dead after 48 h at salinities lower than 15 (Figure 5-4). At a salinity of 18, individuals were less responsive to stimulation than those at 20. The lower LC_{50} of females was significantly higher than that of males (male LC_{50} = 18.0 ± 0.3; female LC_{50} = 19.3 ± 0.4; t-test, df = 14, $P < 0.05$). The total lower LC_{50} was 18.7 ± 0.24 (Probit, LC_{50} ± 95% CI).

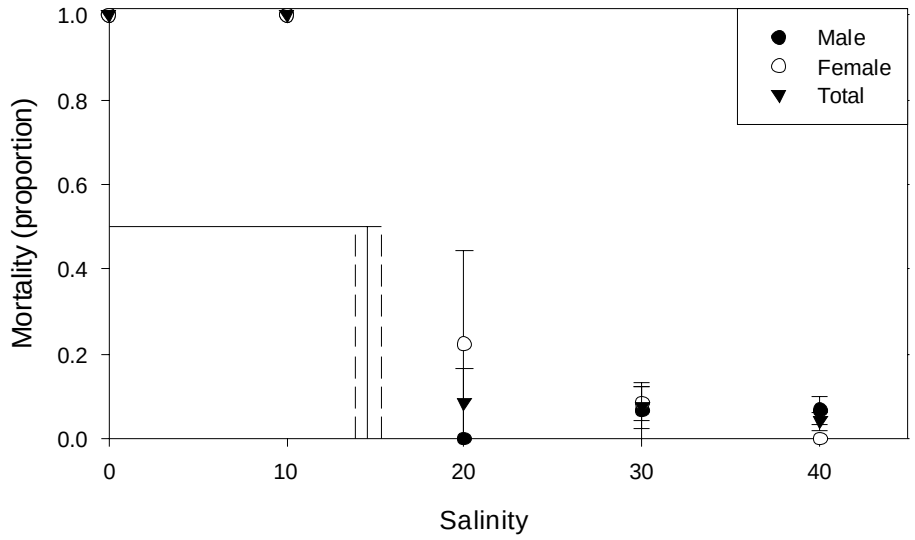


Figure 5-3. Mortality of *C. mutica* following 48 h exposure to salinities between 0 and 40 (mean ± SE, n = 3). Reference lines indicate 48 h LC_{50} ± 95% CI (TSK).

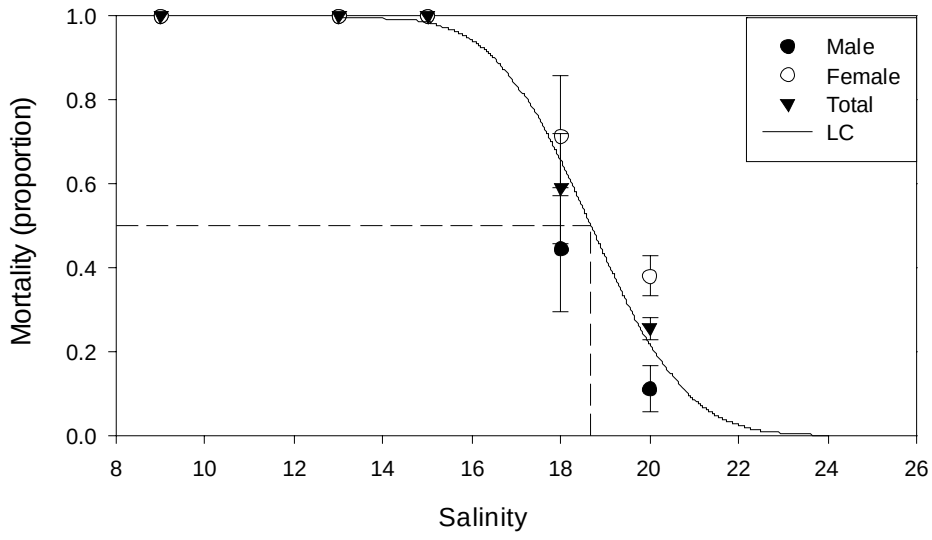


Figure 5-4. Mortality of *C. mutica* following 48 h exposure to salinities between 9 and 20 (mean ± SE, n = 3). LC indicates lethal concentration of percentile mortalities, estimated using Probit analysis; dashed reference line is 48 h LC_{50}.

5.4 Discussion

The upper lethal temperature of adult *Caprella mutica* (48 h LT_{50} 28.3 °C) is beyond the temperature range experienced in its native habitat (25 °C, Schevchenko et al. 2004). The fundamental niche of *C. mutica* is therefore beyond its current realized distribution. This is typical of marine animals and should be expected in tolerance experiments (Sprague 1963, Tait 2001). The lower lethal temperature could not be estimated in this experiment; animals kept at 2 °C for 48 h were inactive until stimulated. Thus, while *C. mutica* can survive at temperatures down to -1.8 °C, growth and reproduction is most likely severely reduced, if not suspended, at such low temperatures. Juveniles were not observed during winter months in the native habitat (Fedotov 1991), and it is not known whether juveniles can survive or if reproduction occurs at these low temperatures. Given that *C. mutica* is presently found in areas which freeze at the surface during winter (e.g. Passammaquoddy Bay, Canada, Chapter 2), it is likely that *C. mutica* can survive in any region where summer conditions allow populations to recover from low winter temperatures (i.e. temperate latitudes).

Sea surface temperatures at the equator are relatively stable at 26 to 27 °C throughout the year (Tait 2001). The survival of *C. mutica* at 26 and 28 °C in this experiment indicates that its global distribution could extend beyond that described in Chapter 2, to include tropical regions. The extent and duration of the summer increase in sea water temperatures most likely limits the distribution of *C. mutica* at higher latitudes and in polar waters. Alternatively, the distribution of *C. mutica* may reflect the frequency of introductions; generally most common in temperate waters (Minchin & Gollasch 2003, Drake & Lodge 2004) and less frequent in tropical or polar waters. The experiments indicate that if released, *C. mutica* could survive introduction in the tropics or in polar latitudes, but it is unlikely that populations would be able to successfully establish.

One hundred percent mortality was observed in adult *C. mutica* kept for 48 h at salinities less than 15. The total lower 48 h LC_{50} was 18.68 ± 0.24, which is higher than that found for several *Caprella* spp. in previous studies, for example the lower 24 h LC_{50} of *C. scaura* and *C. equilibria* was between 7.5 and 11.5 (Cockman & Albone 1987), the 48 h LC_{50} of *C. danilevski*, *C. subinermis*, *C. penantis* and *C. verrucosa* ranged from 12 to 17 (Takeuchi et al. 2003). The increased tolerance of males to low salinities is an interesting and unexpected result. It may reflect the different sizes or reproductive activity of the sexes (Hoback & Barnhart 1996). Females invest more energy in reproduction, which may divert energy from the ability to survive when environmental stress is greatest. It is surprising, however, that this trend was not observed with temperature, which would be expected to produce a greater physiological response (Davenport & Davenport 2005).

In the native habitat, individuals have been found in waters with a salinity lower than the estimated LC_{50} (Schevchenko et al. 2004). *C. mutica* most likely rely on the tidal influence in the coastal habitats and would not survive in continuously low salinities in the field if they did not regularly fluctuate into higher salinities. This theory is supported by the absence of *C. mutica* from estuarine and freshwater habitats.

Caprella mutica from different native and introduced locations may have acclimatised to different environmental conditions (Brown & Bert 1993). The west coast of Scotland experiences temperatures between 4 and 14 °C, which is less than the native range. If the same experiments were repeated with individuals from the native habitats, or collected at different times of the year, different temperature tolerance limits may be found, reflecting the in situ temperatures experienced at that time. Acclimation may also explain the genetic variation described in Chapter 3 (Koehn 1991), i.e. non-native populations have a reduced genetic diversity and experience a narrower range of environmental conditions compared to the native habitat.

When considering the physical limits to distribution ranges, Hines et al. (2004) noted the importance of investigating the temperature and salinity tolerance of larval stages. The tolerance of juvenile *C. mutica* has not been established. It is also important to consider combinations of factors, and the influence of these factors on, for example, prey and predators. Ma and Purcell (2005b) found that populations of the non-native *Moerisia lyonsi* on US coastlines were restricted to low salinity environments and attributed this to the lack of predators, rather than salinity preference. Hirayama & Kikuchi (1980) found the species composition of caprellid communities was determined by the degree of wave exposure or hydrodynamic condition rather than temperature and salinity. Referring to Vermeij (1996) once more: "physiological tolerances are evolutionarily conservative, resulting in ranges being set by physical characteristics that prevent reproduction or survival". This study has investigated physical characteristics which reduce the survival of adult *C. mutica*, future work should investigate factors limiting its growth and reproduction.

A wide range of physical tolerances are thought to be characteristic of successful crustacean invaders (Van der Velde et al. 1998). However, introduced Crustacea do not necessarily fit this criteria, as shown by the similar tolerance of native and introduced gammarids from Dutch waters to water temperature and ionic content (Wijnhoven et al. 2003). Differences in temperature or salinity tolerance of non-native gammarid species were not decisive factors in competition with indigenous species (Wijnhoven et al. 2003), although this is contrary to Caine (1980) who described the increased temperature tolerance of *C. laeviuscula* to be a competitive advantage. It would be interesting to systematically compare the environmental

tolerances of *C. mutica* to those of native amphipods, however there is limited available data from environmental tolerance tests (Gaston & Spicer 2001). The competitive implications of the physical tolerances of *C. mutica* are not known.

With regards to limiting the transport of *C. mutica* via shipping through equatorial or polar waters, changes in sea water temperature are unlikely to affect *C. mutica* in ballast or as fouling on ships' hulls. Exchange of low-salinity ballast water for highly saline oceanic water before entering the North American Great Lakes is enforced to prevent estuarine species from entering the Lakes (Ricciardi & MacIsaac 2000), similar practices are being adopted by many countries signing the International Maritime Organisation's Ballast Water Management Convention (www.imo.org). However, since the enforcement in the Great Lakes, Ponto-Caspian species have continued to be introduced (e.g. *Echinogammarus ischnus*, Witt et al. 1997), because of their euryhaline tolerances (Ricciardi & Rasmussen 1998). The euryhaline tolerance of *C. mutica* further suggests that current ballast-water management is insufficient to prevent many marine introductions (Ricciardi & MacIsaac 2000). *C. mutica* is also likely to survive the wide range of physical conditions experienced by hull-fouling species during oceanic voyages, as described by Minchin and Gollasch (2003).

Species distributional ranges are predicted to extend polewards and, for terrestrial species, to higher altitudes as a result of climate change (e.g. marine mammals, Tynan & DeMaster 1997; coral reefs, Hoegh-Guldberg 1999; pelagic ecosystems, Boyd & Doney 2002). These studies focused on the consequences of global warming. However, the complexities of interactions between atmospheric pressure systems and ice dynamics make the influences of climate change on salinity distributions difficult to predict (Tynan & DeMaster 1997). Evidence supporting the theoretical predictions of global warming is increasing (Hughes 2000), and includes large data sets from terrestrial and marine ecosystems (e.g. Southward et al. 1995, Beaugrand et al. 2002, Walther et al. 2002, Parmesan & Yohe 2003, Root et al. 2003, Hickling et al. 2006). According to these predictions, the distribution of *C. mutica* is likely to extend polewards. Predictions of rates of range extension in terrestrial environments include 6.1 km per decade (Parmesan & Yohe 2003) and 12.5 - 24.8 km per decade (Hickling et al. 2006). However, any predictions involving species' responses to climate change must incorporate measures of dispersal and species interactions (Davis et al. 1998).

Temperature and salinity tolerances combine to limit the potential distributions of marine species (Wiederholm 1987, Stauffer & Boltz 1994). *C. mutica* is limited to marine habitats experiencing a temperate climate (not exceeding 28 °C) and is tolerant of a fluctuating freshwater influence. Evidence in this thesis suggests that dispersal via human vectors is expanding the distribution of *C. mutica* (Chapter 4), and can operate at a far greater rate than climate change. Whilst human vectors continue to disperse *C. mutica* this will be the most important factor in determining its range expansion.

Chapter 6

Seasonal population dynamics of *Caprella mutica*

6.1 Introduction

Knowledge of the life history and population dynamics of a non-native species is essential to understand the invasive process and impacts on the invaded ecosystems (Garcia-Meunier et al. 2001). Population biology helps to predict ecological impacts of non-native species (Ricciardi & Rasmussen 1998, Krylov et al. 1999), development of management practices and policies (Goodwin et al. 1999, Bollens et al. 2002, Allendorf & Lundquist 2003, Townsend 2003), and to identify whether containment rather than eradication efforts would be more practical (Allendorf & Lundquist 2003). Quantitative analysis of seasonal dynamics in the population structure can greatly benefit research on the role of non-native organisms in food webs (Flores & Paula 2002). For example, species diversity and density of ichthyoplankton and zooplankton in the Black Sea was found to negatively correlate with population dynamics of the introduced American comb jelly, *Mnemiopsis leidyi* (Shiganova 1998). This correlation supported its importance as a devastating predator in the pelagic community of the Black Sea. It is critical that the basic characteristics of an invader's distribution in space and time in the new habitat are understood before many questions concerning a non-native species can be answered (Parker et al. 2003, Phillips & Shine 2006). In the early stages of an introduction, it can be argued that eradication efforts are more important than research into population biology (Simberloff 2003); however, the introduction of *Caprella mutica* is beyond the initial stages (first collected in Scotland in 1999, O'Reilly submitted 2006) and a study of its dynamics in the introduced habitat is warranted.

A small proportion of introduced species become established (Lodge 1993, Williamson 1996) and population characteristics may contribute to successful establishment (Newsome & Noble 1986, Nichols et al. 1990). Established non-native species are generally thought to be '*r*' strategists i.e. those which respond opportunistically when conditions are favourable (Lodge 1993, Van der Velde et al. 1998). Devin et al. (2004) found two characteristics of 'r' strategists, namely high fecundity and growth rates, contributed to the successful spread of *Dikerogammarus villosus* in new ecosystems. Rapid growth, production of several generations per year, early

maturity and a considerable fecundity (also 'r' characteristics) contributed to the invasive success of *Corophium curvispinum* and *Corbicula* species in the River Rhine (Den Hartog et al. 1992). As well as a long reproductive period and high fecundity, the production of several batches of eggs per year by the invasive slipper limpet, *Crepidula fornicata*, was considered a 'spreading out of the risks' to the broods (Richard et al. 2006). Van der Velde et al. (1998) defined characteristics of aquatic crustacean invaders, including: short life span and generation time; rapid growth with early sexual maturity; high fecundity; and larger than most conspecifics. These criteria do not guarantee the success of an invader but indicate their potential to invade a suitable habitat. Frequently, these traits have been based on studies of successfully established species, and the field is lacking studies which include both successful and unsuccessful non-natives (Cohen 2002). The local abundance of an introduced species can also be critical in determining its successful establishment (Williamson & Fitter 1996).

According to the above, the Caprellidae should be very successful as introduced species. Indeed, *Caprella acanthogaster*, *C. californica*, C. *natalensis* and *C. scaura* have all been identified outside of their native range (Carlton 1979b, Occhipiniti-Ambrogi 2000, AMBS 2002, Ranasinghe et al. 2005). Unfortunately, the population biology of caprellid species has been little studied and it is not possible to complete a comparison of species which have and have not been introduced outside of their native ranges. Several more caprellid species may have been introduced, that are, as yet, to be recorded. Life-cycle estimates of caprellids vary between less than 8 months (*C. laeviuscula*, Caine 1979) to around 18 months (Jessen 1969). The common pattern of caprellid reproduction is continuous throughout the year, with peaks in spring and summer (Lewbel 1978, Caine 1979, Takeuchi & Hirano 1992). Fecundity of caprellids varies seasonally, being a function of female size (Vassilenko 1991). Vassilenko (1991) found the fecundity of 6 *Caprella* species to range from 4 to 322 eggs per female, *C. bispinosa* and *C. cristibrachium* were the most fecund. Female caprellids breeding in spring were much larger and more fecund than those breeding in summer (Vassilenko 1991). More specifically, Fedotov (1991) studied the population and production biology of *C. mutica* in its native habitat (Possjet Bay, Sea of Japan). Abundance ranged from 25.3 ± 5.2 (average ± SE) individuals m^{-2} (April) to 1223.3 ± 89.7 individuals m^{-2} (June). Sexual maturity of *C. mutica* occurs at 2 to 6 months with juveniles produced later in the year (June-July) taking longer to mature (Fedotov 1991). The reproductive period stretched from March to July (average water temperate of 0 and 17.4 °C, respectively), reaching a peak in March when 72.7% of the total females carried eggs. The maximum number of eggs per female was 316, recorded in spring. In another study the range was

15 - 41 eggs per female in the autumn (Vassilenko 1991). Juveniles appearing in May were found to reproduce in June-July, thus creating a population with individuals of two generations - spring and summer. Females dominated throughout the year (Fedotov 1991), this dominance being most pronounced from July to December and less so during the reproductive period of spring and early summer. Dynamics of the size-age composition of the population indicated great variations, attributable to a complex pattern of mortality and recruitment from deeper waters (Fedotov 1991).

The ecological impact of a non-native species cannot always be predicted from knowledge of its biology in the native habitat (Krylov et al. 1999, Rosecchi et al. 2001); spatial and temporal variations in the new habitat need to be considered by ecologists and conservationists attempting to understand and manage the specific system (Phillips & Shine 2006). Preliminary observations suggest that *C. mutica* has successfully established at several artificial sites in the Lynne of Lorne, Scotland. A population has been established at these sites for at least 5 years prior to the present study (Willis et al. 2004).

The aim of this study was to investigate the seasonal population dynamics of *C. mutica*, and to determine whether:

- population structure,
- sex ratio,
- fecundity, and
- size-frequency distribution

can explain its success as an introduced species. This was achieved through an 18-month field study at 4 sites with different anthropogenic disturbances on the west coast of Scotland.

6.2 Methods

Study area

The Lynne of Lorne, on the west coast of Scotland, contains a large sea loch in the north (Loch Linnhe) which opens to the Atlantic in the south (Pearson 1970; Figure 1-5). The area experiences freshwater runoff from both Lochs Linnhe and Etive, and also exchanges oceanic water through the sound of Mull to the west. The run-off fluctuates both tidally and seasonally with maximum freshwater run-off experienced in the winter and at low tide (Barnes 1955). The total freshwater run-off into the loch system is 80 x 10^7 m^3 d^{-1}, the influence of which is typically confined to surface waters (Barnes & Goodley 1958). Vertical mixing in winter (January-April; Pearson, 1970) elevates surface nutrient concentrations leading to a spring phytoplankton bloom; chlorophyll α concentration peaking annually in May (Grantham 1981). Tides in the area are semidiurnal; maximum inter-tidal range during spring tides varying throughout the year around 3.5 m. All the study sites were located on the eastern side of the Lynne of Lorne, close to the entrance to Loch Etive (Figure 6-1).

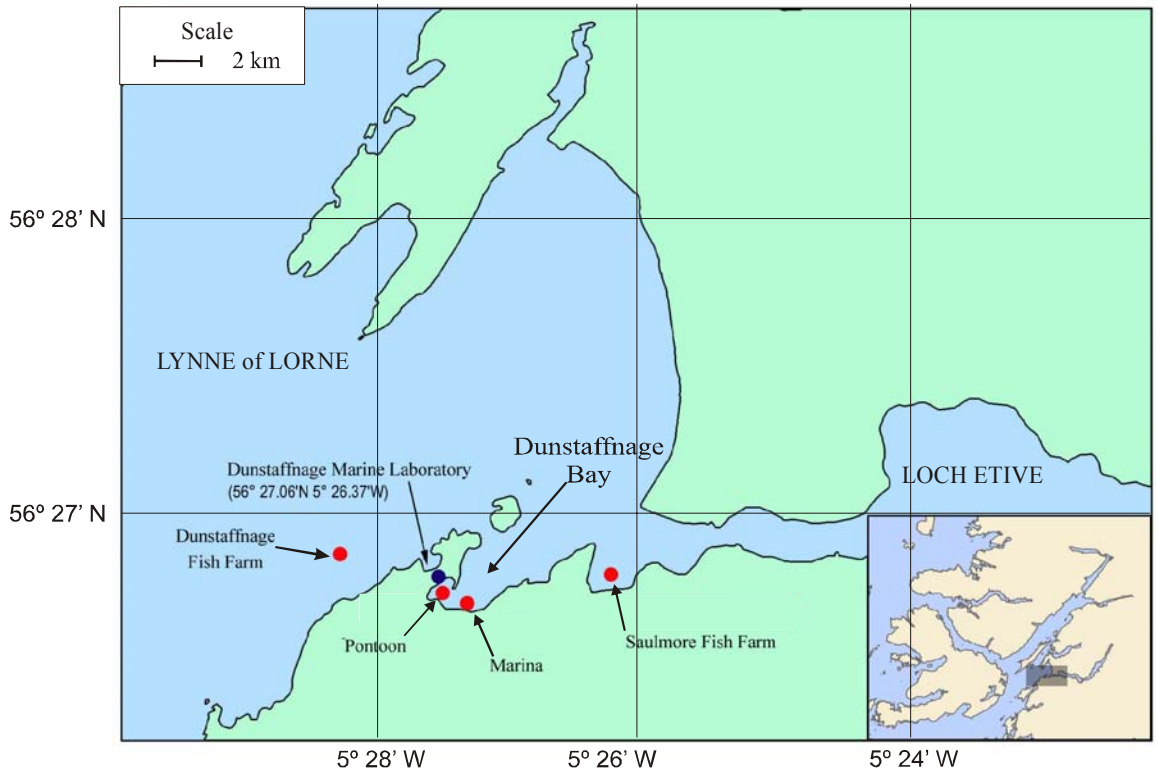


Figure 6-1. Map of east Lynne of Lorne showing position of sampling sites around Dunstaffnage Marine Laboratory.

Caprella mutica was first identified at the Dunstaffnage Fish Farm in 2000 (Willis et al. 2004). Three artificial sites, close to Dunstaffnage Fish Farm, and likely to support populations of *C. mutica* were chosen for the study: Dunstaffnage Marina, the Dunstaffnage Marine Laboratory Pontoon and Saulmore Fish Farm; henceforth referred to as Marina, Pontoon and Saulmore. *C. mutica* were at very low abundance or absent from these sites for the first three months of the study, therefore Dunstaffnage Fish Farm (henceforth referred to as Dunstaffnage) was included in the protocol.

The Dunstaffnage Marina (Figure A, Appendix 6.1, pg 137) is a recreational boating marina located in Dunstaffnage bay, a sheltered bay between the mouth of Loch Etive and the Lynne of Lorne. The Marina is operational throughout the year. Boating activity is greatest during April-October, when both the Marina and the surrounding moorings can be full (pers. obs.). The Marina provides 100 berths and 30 moorings for a combination of local boats which complete short trips within the local area and visiting boats which frequently come from the UK and occasionally from international ports. The Marina pontoons are moored in a water depth of 4 m (*C. D.*). The characteristics of the bay are similar to those experienced at the Saulmore fish farm, although the influence of Loch Etive is less marked, with the water flowing into the bay on an ebb tide, proceeding in a clockwise direction and out into the Lynne of Lorne.

The Scottish Association for Marine Science (SAMS) has a moored Pontoon on the opposite side of Dunstaffnage bay to the Marina (Figure A, Appendix 6.1, pg 137). This site is shallower than the Marina (3 m; *C. D.*) but experiences the influence of the same water currents. The Pontoon is no longer used by the laboratory but was maintained in position for the purposes of this study.

Saulmore Fish Farm (Figure B, Appendix 6.1, pg 137) is located in a bay close to the mouth of Loch Etive, raising sea trout (*Salmo trutta*). For the duration of the study there were between 95 and 234 T of trout held in square-frame cages, fed 27 to 80 T of feed monthly. At this site, a frame of metal walkways is supported by large pontoon buoys, with the nets free-hanging from the middle. The depth of the site is 7 m (*C. D.*). The site is under strong influence of freshwater run-off from Loch Etive, which plays an important role in the physical characteristics of the area. The outflow causes an eddy of water in the bay, flowing out of Loch Etive, clockwise around the bay and into the Lynne of Lorne on the ebbing tide, and in the opposite direction on the flood tide (pers. obs.).

Dunstaffnage Fish Farm is located in the Lynne of Lorne, raising Atlantic salmon (*Salmo salar*). For the duration of the study there were between 17 and 652 T of salmon held in a maximum of

16 'Polar circle' cages and fed 8 to 163 T of feed monthly. Polar circle cages are moored independently, with several plastic ring buoys supporting a free-hanging net of approximately 20 m depth (Figure C, Appendix 6.1, pg 137). The depth of the site ranges from 30 to 40 m (*Chart Datum*, *C. D.*). The residual current direction is northeast (C. J. Cromey, unpublished data), although there are also strong currents to the southwest when the tide ebbs out of Lochs Linnhe and Etive.

Experimental protocol

Fortnightly sampling started in January 2004 and continued for 18 months. This included 2 summer seasons when peaks in abundance have been observed in the native habitat (Fedotov 1992). In the native habitat *C. mutica* individuals have been recorded in the upper subtidal zone at depths of 0.7 - 13 m, mainly at depths of 2-7 m (Fedotov 1991). All sampling collection structures were positioned at a water depth of approximately 3 m. The first samples were collected from Dunstaffnage Fish Farm in July 2004 and continued for 14 months, including a complete summer and winter season. Every fortnight 3 weighted plastic meshes (25 x 10 cm, mesh size 5 mm; Clean-ups, EGL Homecare) were deployed at a distance of 2 m from one another (on separate ropes) at each site at a depth of 4 m. This allowed detection of abundance down to a minimum of 40 individuals m^{-2} (i.e. 1 individual in 250 cm^2). Eight weeks after deployment (sufficient for recruitment, Cook et al. 2004), the meshes were recovered and transported to the laboratory immersed in individual buckets of sea water. On several occasions, weather conditions made it unsafe to collect the meshes, while at other times adverse conditions led to the loss of several meshes over the study period.

On each sampling occasion, a conductivity, temperature and depth meter (Sea-Bird SBE 19 SeaCat Profiler; Sea-Bird Electronics, Inc, Washington, USA) was deployed to analyse seasonal and tidal variability in the water-column physical characteristics. To measure seasonal changes in temperature 'Tiny Tag' temperature dataloggers (Gemini Dataloggers) were used to record temperature every 30 minutes (+/- 0.1 ºC) at a water depth of 4 m from the start of the experiment. Seawater temperatures recorded at the four sites followed the same annual trend for the first 12 months; subsequently only 1 logger was deployed (at Dunstaffnage). Temperature values were averaged over the 14 d period prior to the sample date to represent the temperatures experienced since the previous sample was taken.

Individuals of *C. mutica* were removed from the meshes and preserved in 90% ethanol. To reduce sampling effects (e.g. cannibalism, predation, loss of broods due to stress) the entire process (from collection of the meshes, to preservation of individuals in alcohol) was completed in less than 8 hours.

Laboratory procedures

Individuals of *C. mutica* collected in the field were observed under an Olympus SZX9 stereomicroscope. If the sample was considered too large (approx. >300 individuals), a sub-sample of the caprellids was taken using a plankton splitter (Hiwatari & Kajihara 1984). Body length to the nearest 0.1 mm was measured from the front of the head to the end of pereonite VII, using PC-based digitising software, scaled relative to an on-screen scale object. Females were identified by the presence of oostegites, anterior position of gnathopod II and lack of setation on pereonites I and II; males by the distal position of gnathopod II and extension and setation of pereonites I and II (Willis et al. 2004). Identification of ovigerous females was through the presence of eggs in the brood pouch. Individuals smaller than 4 mm or lacking characteristics of either sex were defined as juveniles. Eggs were dislodged using a dissecting needle and fine paint brush and counted (from up to 10 randomly selected, ovigerous females from each sample).

Statistical Analysis

The significance of differences between salinity at 4 m and throughout the whole water column at the four sites were analysed using a one-way ANOVA in MINITAB. A significant result was followed by a *post-hoc* pairwise Mann-Whitney test (Zar 1996). Sample sizes were considered sufficiently large to make ANOVA tests robust to deviations from normality and homogeneity of variance (Underwood 1997).

The *C. mutica* abundance data did not show normality or homoscedacity and contained a large number of zero values. For these reasons, and in order to incorporate the seasonal nature of the data, generalised linear models of categorised (ordinal) abundance were used to test the significance of factors influencing abundance and sex ratio of *C. mutica*.

Ordinal regression models are particularly well developed in epidemic studies, toxicity assessments and social sciences, often dealing with semi-quantitative variables (Guisan & Harrell 2000). Their main use in ecology has been to predict spatial distribution of species (percentage

cover or presence/ absence; Luoto et al. 2001, Cawsey et al. 2002, Guisan 2002). Linear ordinal regression was used to predict the likelihood that the abundance of *C. mutica* would be within a defined category through an annual cycle. The continuous scale of the original abundance data was sliced in order to create ordinal data, with semi-quantitative differences between values (Guisan & Harrell 2000). Slices were logarithmically spaced to give 7 categories of abundance (Table 6-1). The minimum abundance that could be sampled was greater than 1 individual m^{-2} and, for this reason, category 1 was not included in the models but is included in Table 6-1 for completeness.

Male, female and juvenile abundance data were treated separately. Site was used as a factor in the model. 'No individuals at Dunstaffnage' was used as a reference value. The cosine and sine of the angular equivalent of the day of the year (θ; 2π * julian day/365) were used as covariates in the model to incorporate seasonality ($\cos\theta$ and $\sin\theta$ respectively). This method has been described for detecting lunar cycles in marine ecology (deBruyn & Meeuwig 2001) and has previously been used to investigate lunar periodicity in fish activity, seahorse abundance and supply and settlement of megalopae (Gibson et al. 1996, Martin-Smith & Vincent 2005, Queiroga et al. 2006).

Table 6-1. Intervals used for categorising abundance

Category (Cat)	Abundance (individuals m^{-2})	Mid-point
0	0	0
1	>0-1	0.31622
2	>1-10	3.1622
3	>10-100	31.622
4	>100-1000	316.22
5	>1000-10000	3162.2
6	>10000-100000	31622.0
7	>100000-1000000	316220

Generalised Linear Modelling (GLM) uses the combination of predictor variables (X; e.g. site and season) to produce a linear predictor *LP* which is related to the expected abundance E(Y) through a link function g() such as:

$$g(E(Y)) = LP = a + Xb$$

where *a* is the constant term to estimate and *b* is the predictor variable coefficient (more than one constant and variable can be included in a model). The distribution of Y (abundance in this study) may be any of the exponential family of distributions (where rate of increase is related to current size). The predictor variables were Site, Cos θ, Sin θ and interactions of Cos θ * Site and Sin θ * Site). Ordinal logistic regressions were done in MINITAB to calculate the cumulative standard logistic distribution function (logit). The following model was then estimated:

$$LP(\leq \text{category at site}) = a + b + c\text{Cos } \theta + d\text{Sin } \theta + e\text{Cos } \theta + f\text{Sin } \theta$$

LP= logit probability
a= category coefficient
b= site coefficient
c= Cos θ coefficient
d= Sin θ coefficient
e= site * Cos θ interaction coefficient
f= site * Sin θ interaction coefficient

The 'Goodness of fit' of the models was assessed by the: *Somers' d_{yx}*, a value between 0 and 1 where 1 indicates perfect correlation between expected and observed outcomes (Somers 1962). In MINITAB, a *t*-test was used to calculate the significance of each coefficient (with H_0 that the variable coefficient was equal to 0).

Expected abundance for an annual cycle were calculated at 7-day intervals. Ordinal logistic regression predicts logit probabilities which were back transformed to give actual probabilities:

$$P = 1 / (1 + \exp (LP))$$

MINITAB calculates a cumulative odds ratio, i.e. the probability of being in the test category or less. The probability of being in a category (n) is therefore:

$$P(n) = P (\leq \text{Cat n}) - P (\leq \text{Cat n-1})$$

Predicted probabilities were used to calculate expected abundance by summing the product of the logistic midpoint of each category multiplied by the expected probability:

$$E(Y) = \Sigma P (\text{Cat2}) * 3.1622 + \ldots + P (\text{Cat6}) * 31622$$

The expected abundance could then be graphically presented on the same graph as those observed. Four from 1170 records of abundance were in category 7, and the probability of being

in category 7 was less than 0.05 in all cases, however, because of the large value of the midpoint, the inclusion of the term in the model led to large deviations from the sampled data. For this reason, the term was excluded from the models used to predict abundance. A fourth-root scale was used on the graphs to better visualise the data. The significance of site, season and site-dependent season effects were calculated using a *t*-test (with the null hypothesis of the coefficients being equal to zero). A Chi-squared test was used to calculate the significance of differences between the site coefficients.

Another form of GLM, binary logistic modelling, was used to investigate differences between the proportion of males found at each site throughout the study (Wilson & Hardy 2002). Proportion of males was the response variable, Site was the fixed factor, and Season and Site by Season were covariates. The cosine and sine of the angular equivalent of the day of the year were used to represent season as discussed above. The significance of Site, Season and Site-Season interactions were calculated using a *t*-test (with the null hypothesis that the coefficients are equal to zero). Coefficients of the parameters in the model were used to predict sex ratios through an annual cycle.

Binary logistic modelling was also used to analyse seasonal changes in the ratio of ovigerous females to total females in the population. Seasonal differences in the number of eggs per female and the ratio of number of eggs to the length of female were analysed using general linear models. A linear relationship between female length and fecundity often characterises amphipods (Powell 1990, Sainte-Marie 1991), therefore no transformations of the data were done. Due to the lack of significance of the seasonal component, General Linear Modelling, with length as a covariate, was used to investigate between site differences in the number of eggs per female. A significant result was followed by *post-hoc* pairwise comparisons using the Mann-Whitney test (Zar 1996).

Seasonal length-frequency distributions were obtained by splitting size records into 2 mm classes (Flores & Paula 2002). Monthly samples were grouped into winter (Dec - Feb), spring (Mar - May), summer (Jun - Aug) and autumn (Sep - Nov) (Garcia & Mantelatto 2001). Lengths of males and females were compared using a *t*-test (Zar 1996). Differences in the length-frequency distribution between sites were analysed using a Kruskal-Wallis test. A significant result was followed by *post hoc* pairwise comparisons using the Mann-Whitney test (Zar 1996).

6.3 Results

Environmental data

Seawater temperatures varied from 7.4 °C (April 2004) to 14.3 °C (September 2004), with gradual changes between the late winter minima and late summer maxima (Figure 6-2).

Salinity at Dunstaffnage varied least throughout the study period. Salinity at the other sites varied inconsistently but tended to be lower and more variable when sampling was carried out close to low tide (pre-July '04 and Nov '04 - Mar '05; Figure 6-3). The state of the tide had a sub-annual periodicity of approximately 8 months (Figure 6-3). Salinities at 4 m varied between 16.47 (Saulmore, December '04) and 34.5 (all sites, March '05). There were significant differences between salinities at the four sites at 4 m (ANOVA, $F = 9.82$, $df = 3$, $P < 0.001$) and throughout the whole water column (ANOVA, $F = 8.84$, $df = 3$, $P < 0.001$) at the four sites. Salinities at both 4m and entire water column depth ranges were consistently greater at Dunstaffnage than at the Marina and Pontoon (Mann-Whitney test, $P < 0.001$); Saulmore was the least saline site (Mann-Whitney test, $P = 0.02$).

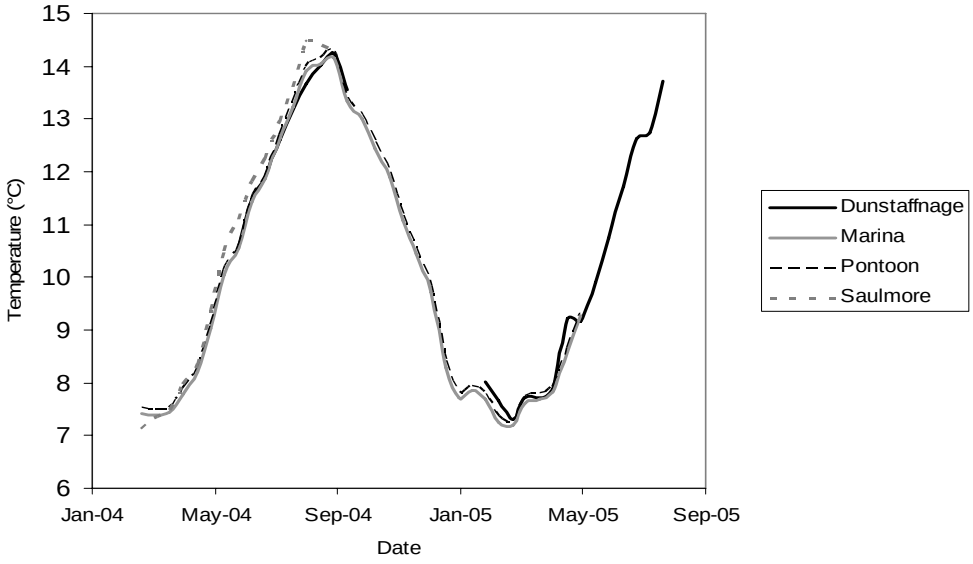


Figure 6-2. Water temperature at 4 m depth for the duration of the experiment. Data are fortnightly averages of hourly record.

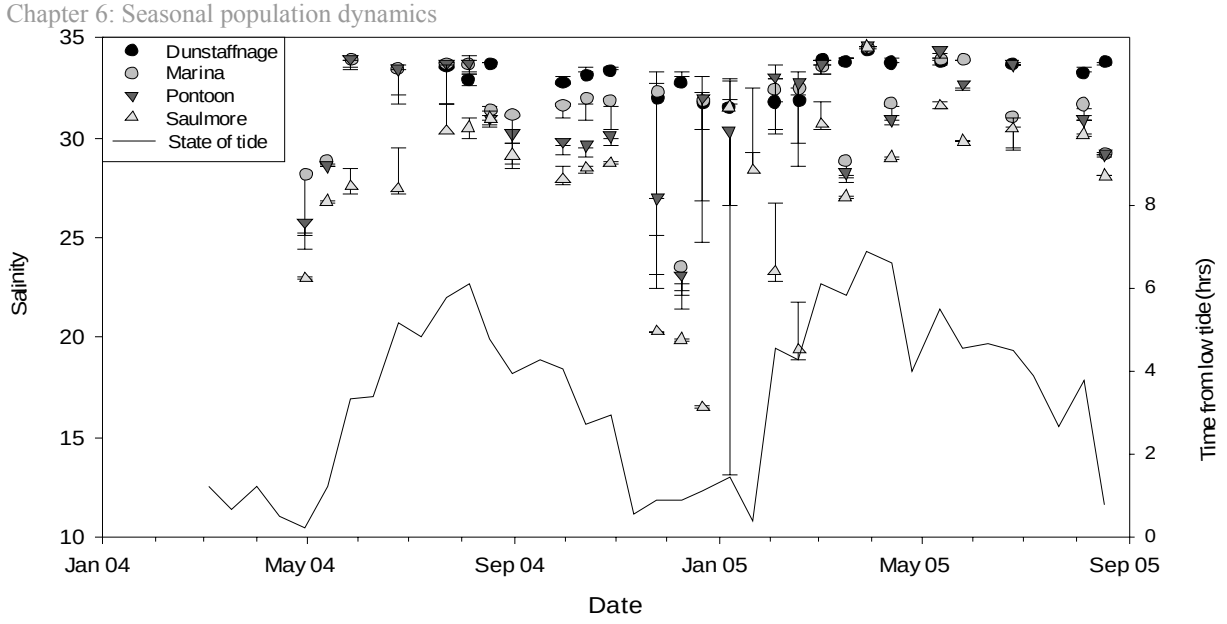


Figure 6-3. Water column salinity and state of the tide at time of sampling for the duration of the study. Data shown are salinity at 4 m water depth. Error bars indicate lower and upper quartiles of salinity over the complete water column, or down to 40 m at Dunstaffnage.

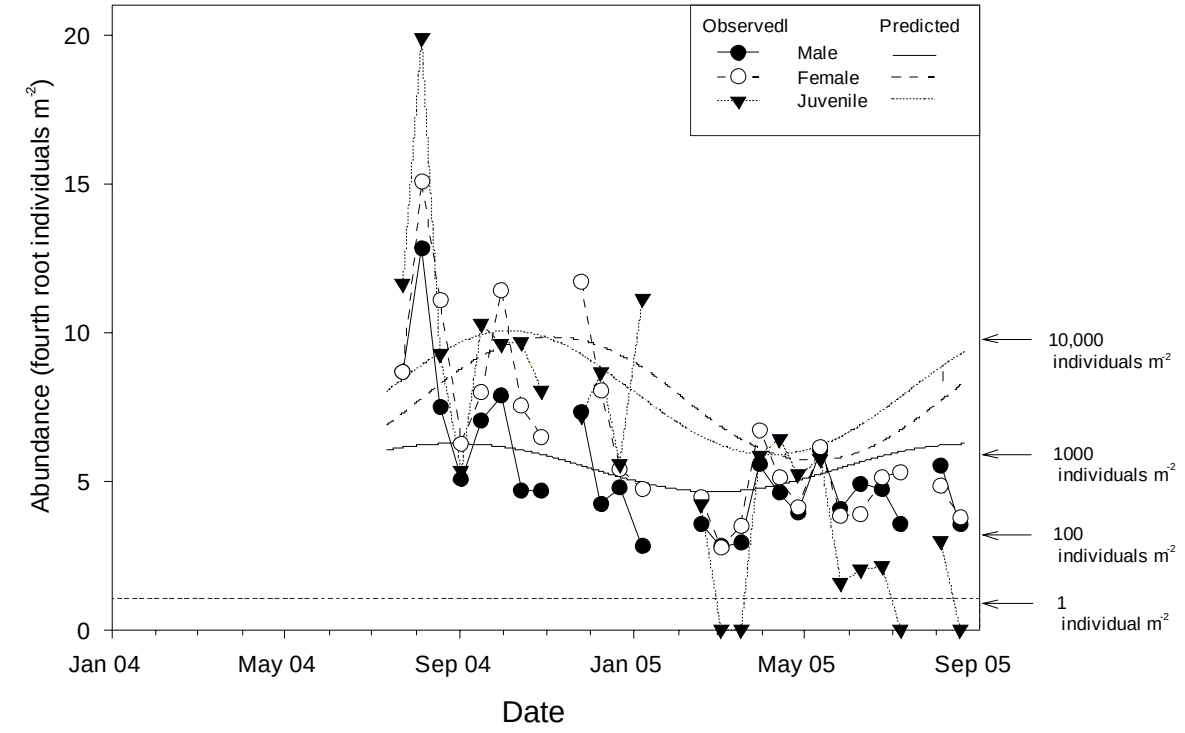
Population structure and dynamics

Abundance of *Caprella mutica* fluctuated at all sites (Figure 6-4). Dunstaffnage was the only site where individuals were found throughout the year. Adults were found on every sampling occasion at Dunstaffnage. Juveniles were absent on 4 occasions, during March, August and September 2005. Populations at the Marina and Pontoon showed clear seasonal differences, with complete absence of individuals from October to May and highest abundance during August and September (Figure 6-4). Individuals were absent from Saulmore for a shorter period of time (January to May). The highest abundance recorded from a single mesh was 319,000 individuals m^{-2}, recorded on the 5th August 2004 at Dunstaffnage; the highest abundance recorded at Saulmore was 171,000 individuals m^{-2}, recorded on the 10th June 2004; both of these samples had a high proportion of juveniles (68.9 and 98.7%, respectively). At the Marina, 127,000 individuals m^{-2} were sampled on 16th September 2004; at the Pontoon, the maximum abundance was 2,900 individuals m^{-2}, recorded on the 5th August 2004. Juveniles were more abundant than males or females, except at the Pontoon where a similar abundance of females and juveniles was observed (Table 6-2); females were more abundant than males.

Table 6-2. Abundance of male, female and juvenile *Caprella mutica* recorded at the four sites during the 18 month field study. Values are average individuals m^{-2} ± S. E..

Site \ Sex	n	Male	Female	Juvenile
Dunstaffnage	25	1269 ± 277	3416 ± 850	4588 ± 1238
Marina	35	465 ± 152	2469 ± 1224	2624 ± 1330
Pontoon	35	102 ± 32	225 ± 98	228 ± 81
Saulmore	35	532 ± 37	1710 ± 128	2140 ± 213

a)



b)

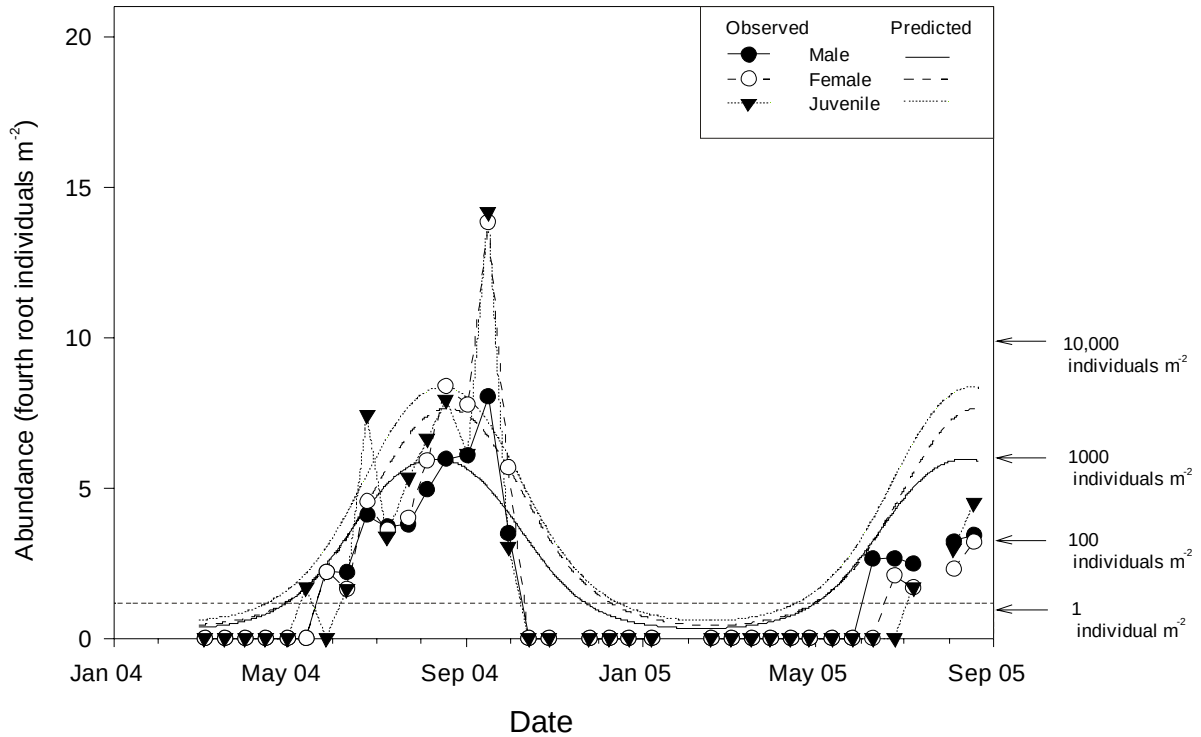
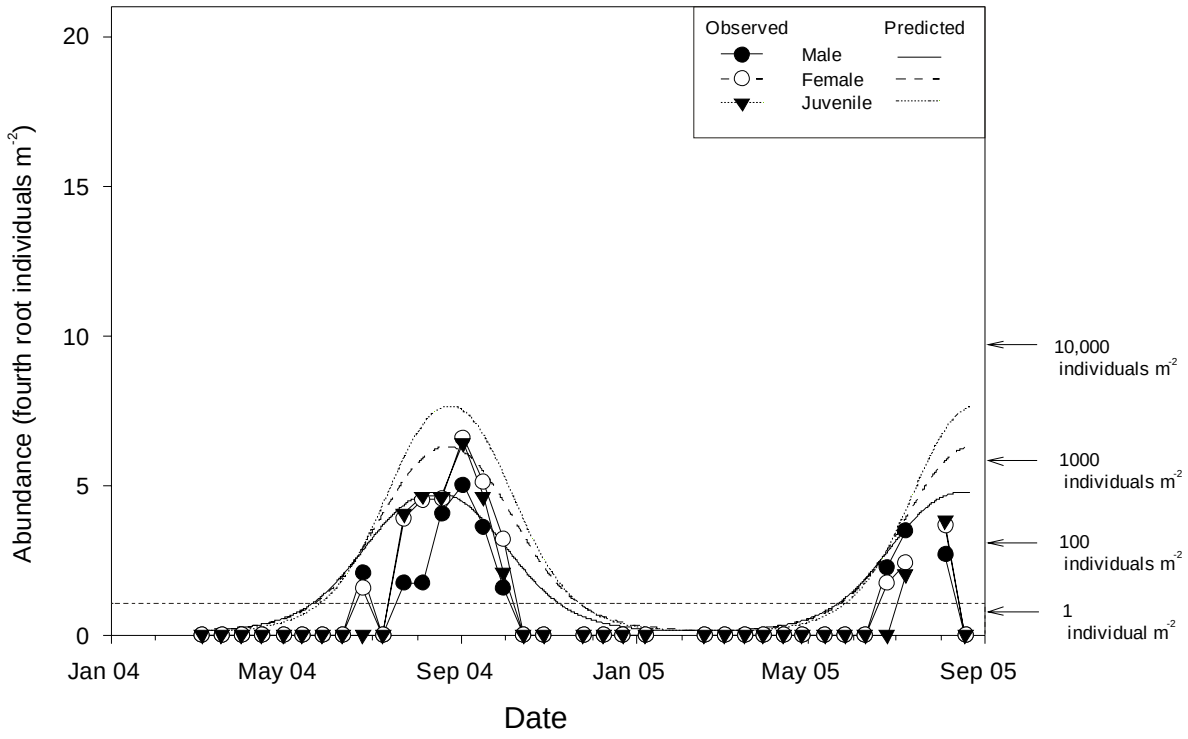


Figure 6-4. Seasonal abundance of male, female and juvenile *Caprella mutica* during the 18 month field study at a) Dunstaffnage, b) Marina, c) Pontoon and d) Saulmore. Symbols are the average fourth root (abundance m^{-2}) (n = 3). Curves are the predictions from Ordinal Logistic Regression, see methods for further details. The horizontal dashed line indicates the minimum detectable value (40 individuals m^{-2})

c)



d)

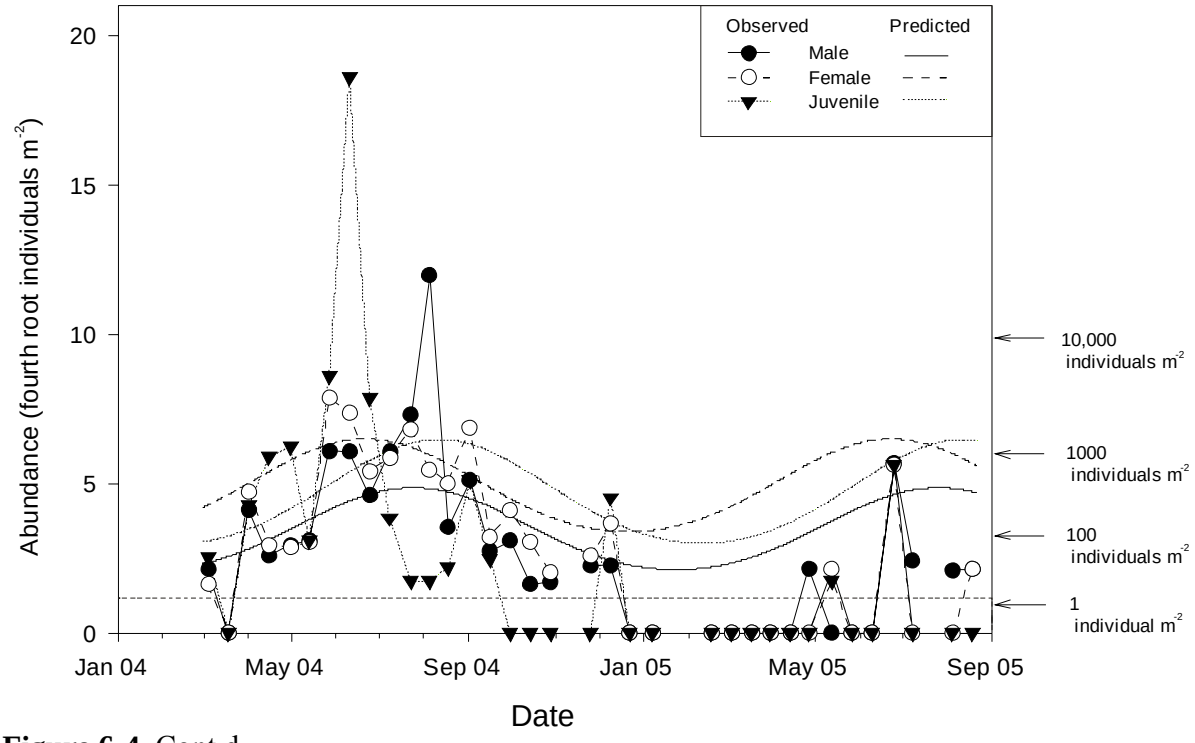


Figure 6-4. Cont.d

Likelihoods of abundance predicted using GLMs were representative of the data (D, Table 6-3). Cosine and sine parameters were significant singly or in interactions with site (Table 6-3) indicating that abundance varied significantly over the annual cycle and that the pattern of changes was not the same at each site. Caprellids were significantly more likely to be present and more abundant at Dunstaffnage; abundance values at Saulmore were also significantly likely to be greater than those at the Marina and Pontoon (Figure 6-5). The likelihood of finding *C. mutica* individuals at the Marina and Pontoon was very low for nearly 6 months over winter and spring, followed by a sharp increase to a narrow peak in abundance during September. At Saulmore and Dunstaffnage, the changes in likelihood were more gradual, with presence predicted throughout the year; the maximum predicted likelihood was in October at Dunstaffnage and July to August at Saulmore. The probability of presence of individuals of *C. mutica* varied significantly with probabilities at Dunstaffnage greater than Saulmore, greater than the Marina and Pontoon (Chi-squared, df = 3, $P < 0.05$; Figure 6-5).

Table 6-3. Significance of variables used in GLM of ordinal abundance (*t*-test, n = 276). Bold text indicates significance at the 5% level. D- *Somers' d_{yx}* (Somers 1962)

Sex	D	Site	Cos	Sin	Cos* Site	Sin* Site
Male	0.79	**<0.001**	0.071	**0.004**	**<0.001**	**<0.001**
Female	0.79	**<0.001**	0.685	**<0.001**	**<0.001**	**<0.001**
Juvenile	0.72	**<0.001**	**0.006**	**<0.001**	**<0.001**	**0.001**

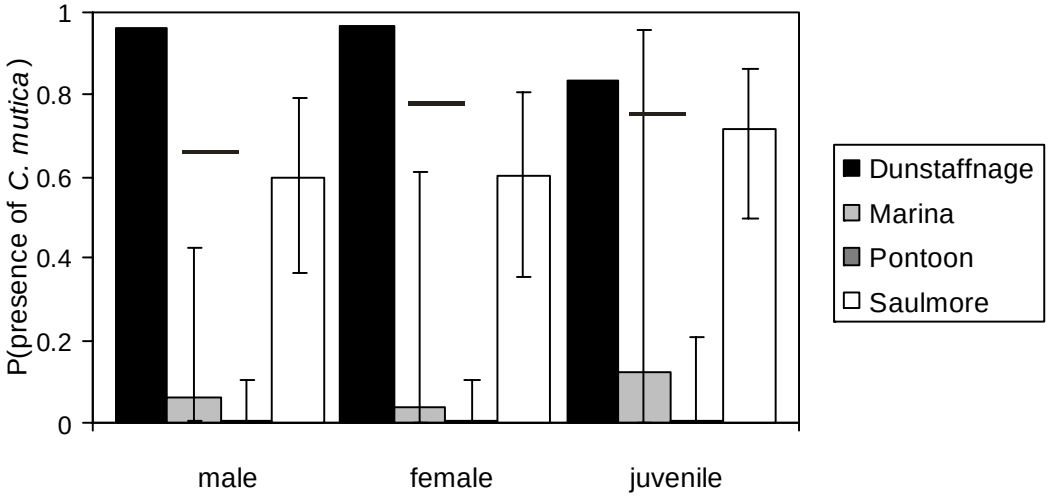


Figure 6-5. Probability of presence of *C. mutica* at each site calculated from site and category coefficients (a and b) from GLMs (± 95% CI of the site coefficient). Horizontal bars indicate no significant difference between site coefficients (chi-squared, df = 3, $P > 0.05$).

Sex Ratio

Females dominate for most of the year but their dominance is most marked in autumn and winter (Figure 6-6). The overall sex ratio was also biased towards females (males/ males + females, 0.399 ± 0.023, mean ± SE, n = 159). All parameters in the binary logistic model were significant, indicating differences between sites (*t*-test, n = 74, $P < 0.001$), seasonal fluctuations (*t*-test, n = 74, $P < 0.001$) and different seasonal patterns at the different sites (*t*-test, n = 74, $P = 0.025$). The increase in the proportion of males in spring reflects the earlier onset of the increase in abundance described previously. The predicted sex ratio at Saulmore showed less variation throughout the year than at the other sites. The greatest variations in sex ratio were predicted at the Pontoon, based on the smallest data set (n = 1245).

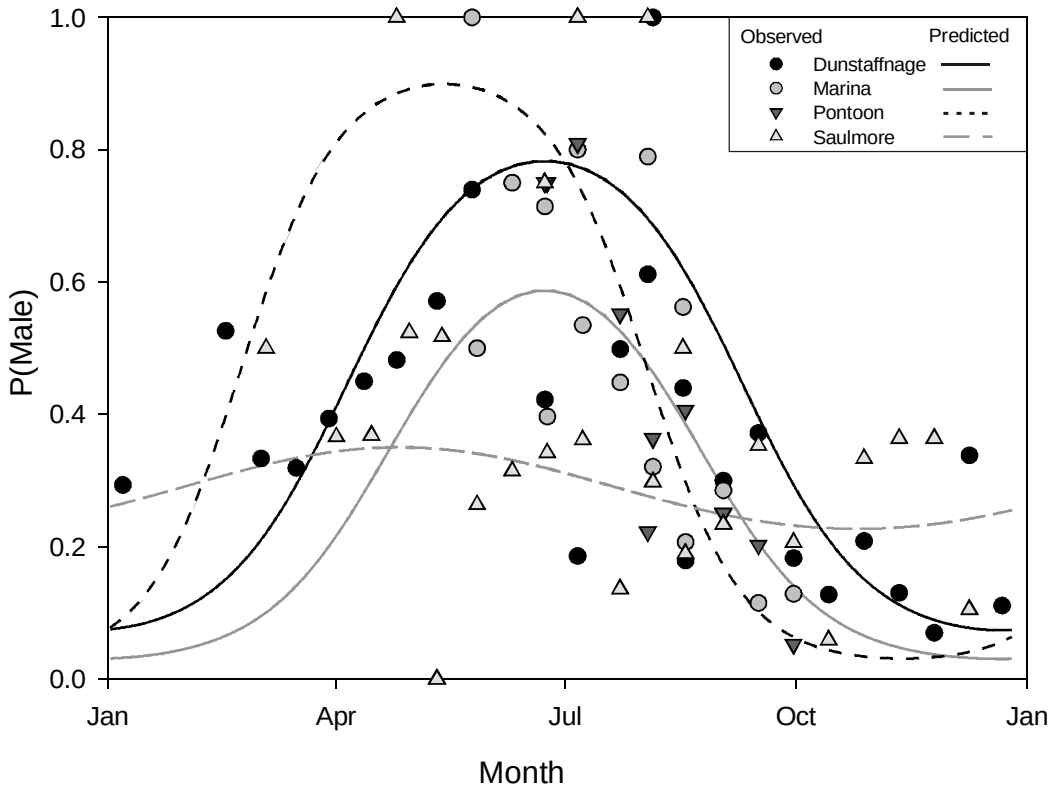


Figure 6-6. Observed and predicted values of sex ratio (male/male+female). Predicted values were calculated using Binary Logistic Models as described in the text (*Somers'* d_{yx}= 0.56).

Fecundity

Ovigerous females were found throughout the study period (Figure 6-7). All terms in the binary logistic model were significant indicating differences in site, season and different seasonal patterns in the proportions of ovigerous females at all sites (*t*-test, n = 71, $P < 0.05$ for all variables). The predicted proportion of ovigerous to non-ovigerous females was greatest in the spring and summer months (April – October). The number of eggs per female was highly variable (min = 3, max = 363, average = 74, SD = 47.7; Figure 6-8) but showed a significant positive relationship to female length (Spearman's Rank Correlation coefficient, n = 739, r = 0.469, $P < 0.01$). The Cosine and Sine coefficient were not significant in the modelling of the number of eggs per female (*t*-test, n = 739, $P > 0.05$). Apparent site variation between number of eggs per female (Figure 6-9) was explained by including length as a covariate in the General Linear Model; subsequently, there were no significant differences in fecundity estimates between sites (*t*-test, n = 739, $P > 0.05$).

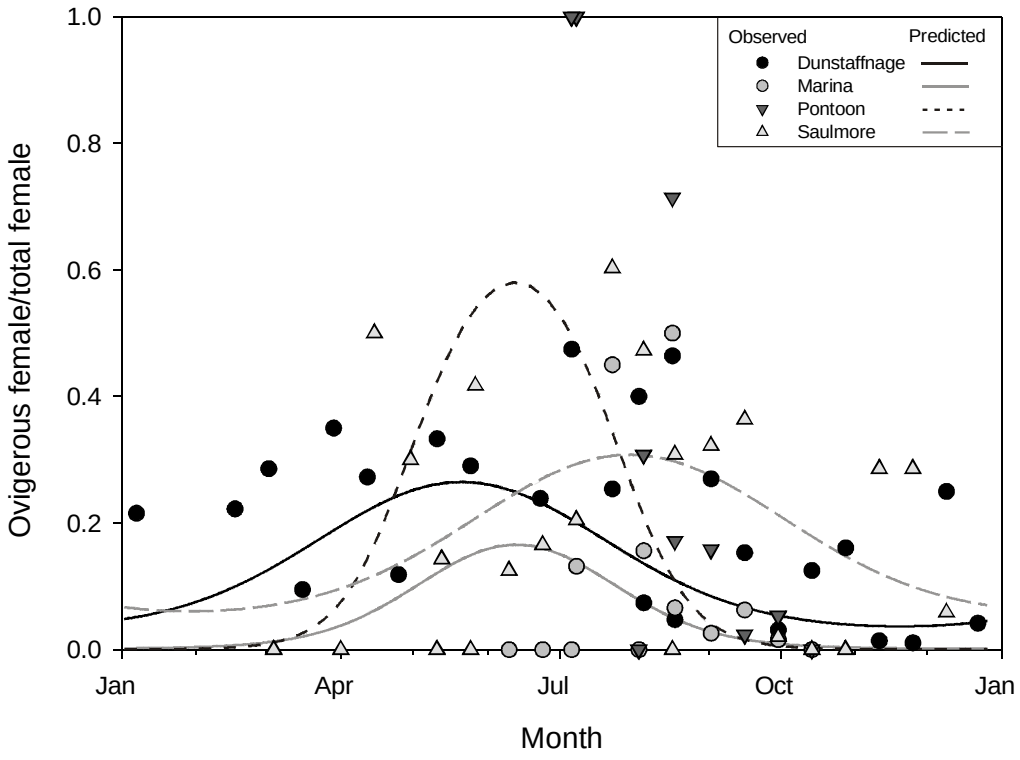


Figure 6-7. Observed and predicted ratio of ovigerous females to all females at all sites. Predicted values were calculated using Binary Logistic Models as described in the text (*Somers' d_{yx}* = 0.58).

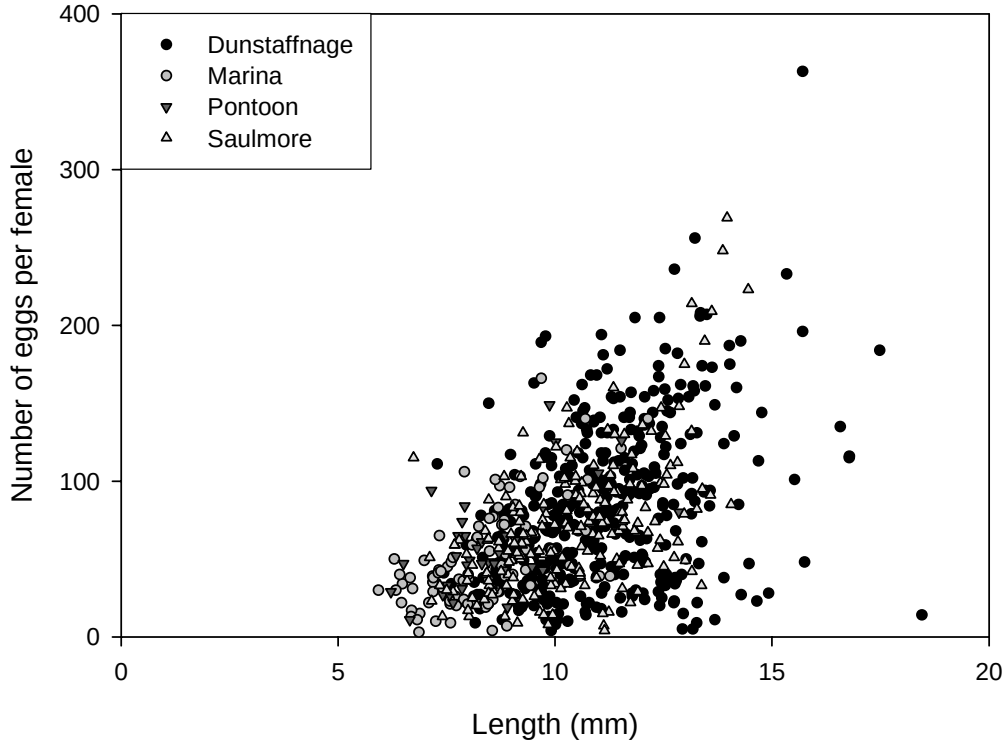


Figure 6-8. Relationship between number of eggs per female and female length of *C. mutica* at the four sampling sites.

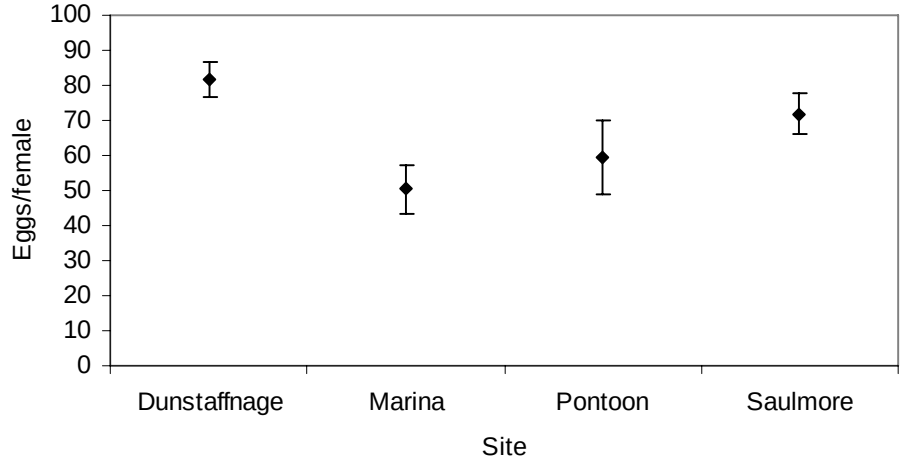


Figure 6-9. Number of eggs per female at all sites. Data are means ± 95% CI; from left to right, n = 406; 90; 36; 207.

Size-frequency distribution

The maximum total length recorded for males during the study was 47.0 mm at Dunstaffnage, whilst that for females was 29.0 mm at Saulmore (Table 6-4). Males dominated the larger size classes and were significantly longer than females (*t*-test, df = 2387, $P < 0.001$; Figure 6-10). Females had a unimodal size distribution, while males showed a polymodal or more variable distribution. The length-frequency distribution varied significantly between sites (KS = 919.75, df = 3, $P < 0.001$) with lengths at Saulmore significantly greater than Dunstaffnage ($P < 0.05$), significantly greater than Pontoon ($P < 0.05$), greater than the Marina in both males and females, the difference between median lengths at the Pontoon and Marina was not significant (Table 6-5). This trend is also seen in the size at which the sex of an individual could be identified by morphological characteristics (Figure 6-11) with adults identifiable at a smaller size at the Marina and Pontoon compared to Dunstaffnage, with juveniles growing the largest before maturing at Saulmore. The only time when adults were present and juveniles were absent was the Marina in spring (March-May), although the sample size was very small (n = 7, Figure 6-10). Juvenile, immature *C. mutica* ranged in body length from 0.81 to 7.95 mm.

Table 6-4. Summary statistics of length of sexable *C. mutica* individuals collected at all sites throughout the study period. Sites are listed in order of magnitude. Lengths are mm.

Site	Sex	Average	SD	SE	Max	Min	n
Dunstaffnage	Female	7.80	2.74	0.10	17.19	4.00	2138
	Male	12.90	6.76	0.15	47.04	4.10	828
Marina	Female	6.32	2.03	0.06	21.12	4.00	1204
	Male	8.94	5.42	0.23	44.25	4.00	547
Pontoon	Female	6.34	1.64	0.08	12.97	4.00	415
	Male	8.29	4.08	0.29	34.30	4.02	197
Saulmore	Female	8.76	2.57	0.07	29.02	4.02	1287
	Male	13.48	6.35	0.28	36.66	4.27	522
Total	Female	7.57	2.64	0.04	29.02	4.00	5043
	Male	11.58	6.46	0.14	47.04	4.00	2094

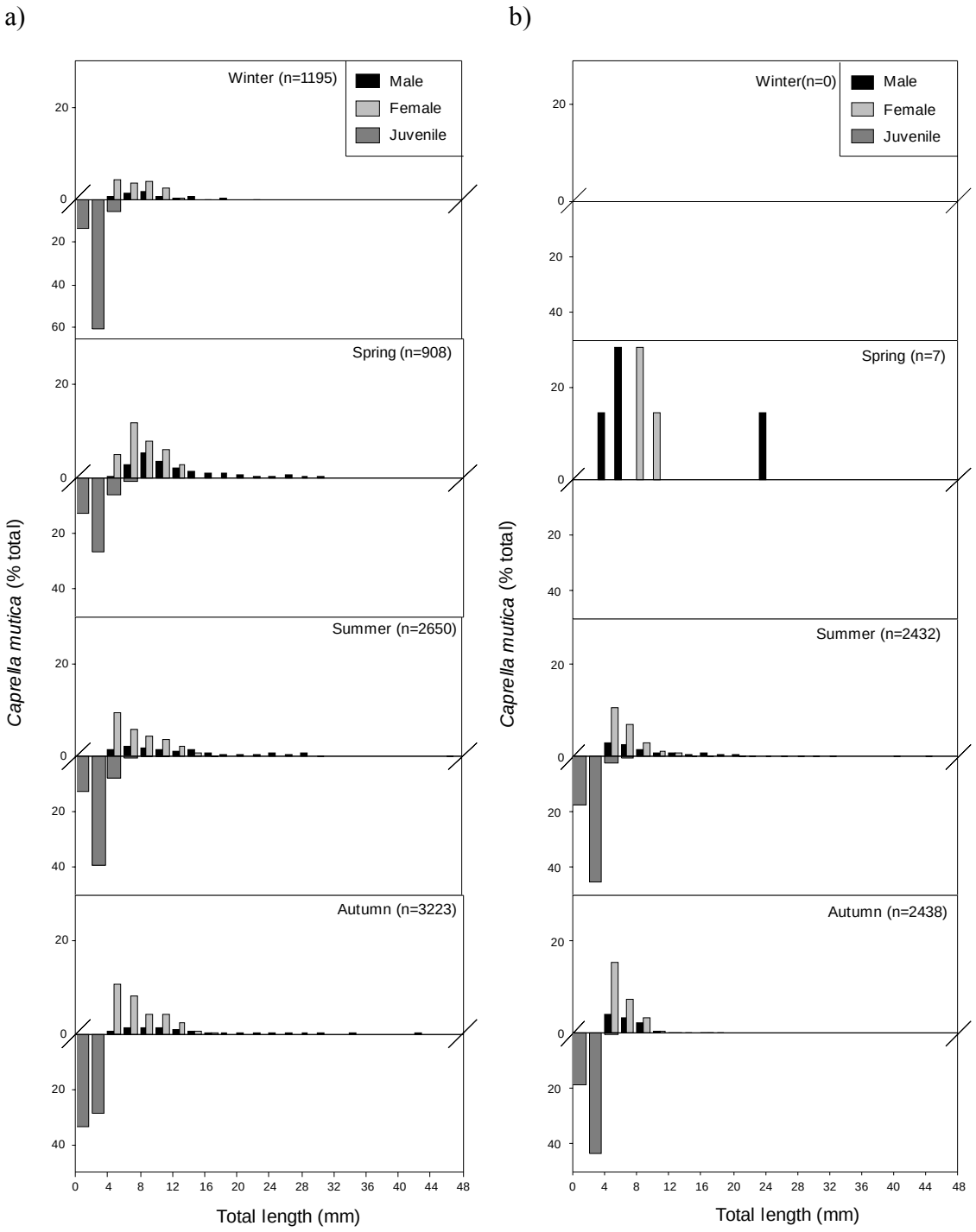


Figure 6-10. Seasonal length-frequency distribution of *C. mutica* individuals collected throughout the study period at: a) Dunstaffnage, b) Marina, c) Pontoon and d) Saulmore. Winter: Dec-Feb, spring: Mar-May, summer: Jun-Aug and autumn: Sep-Nov.

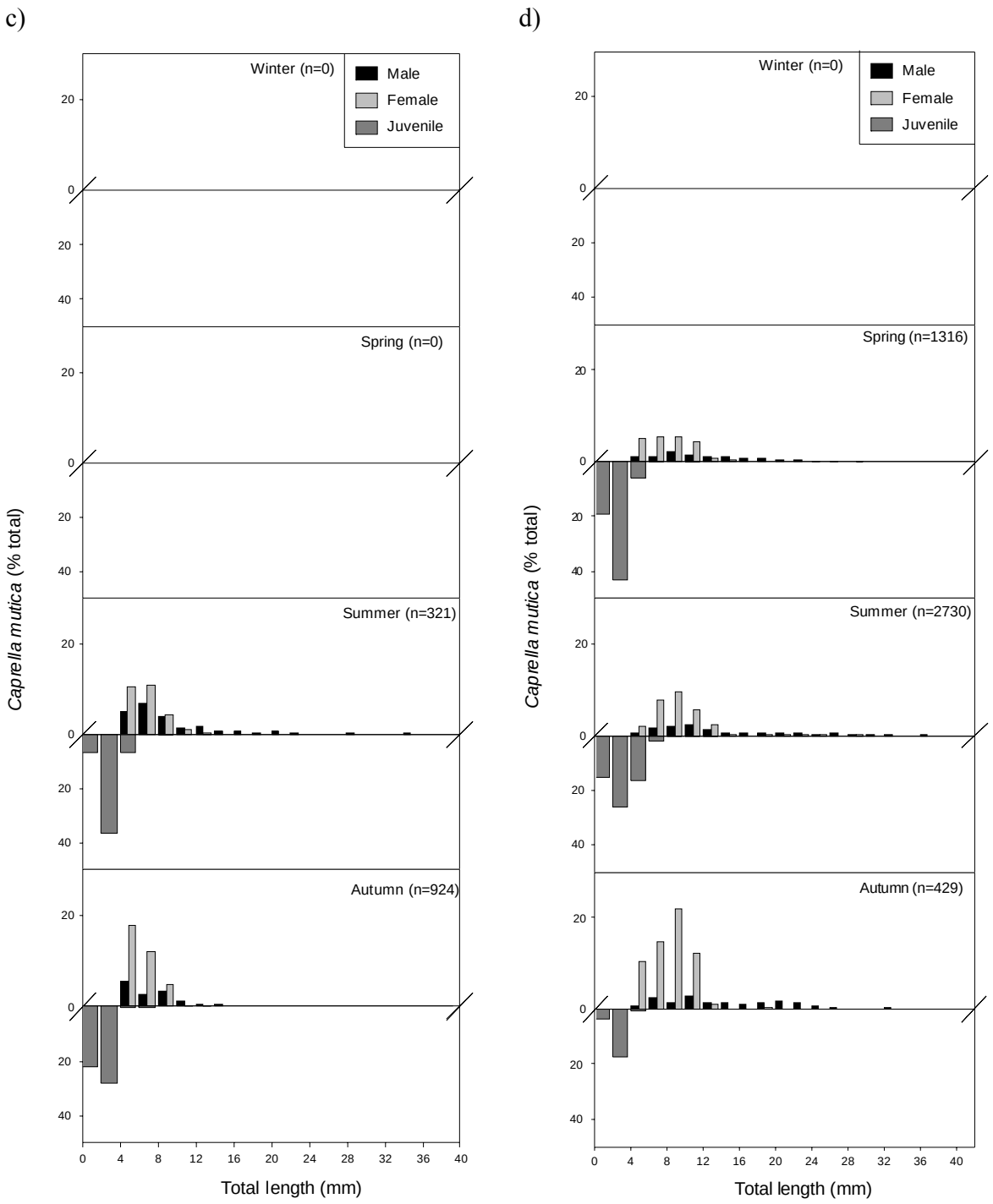


Figure 6-10. Cont.d

Table 6-5. Probabilities of differences in lengths of individuals between sites (Mann-Whitney test). Comparisons of females above the diagonal, males below. Bold text indicates significance at the 5% level. Sites are listed in order of magnitude from top to bottom and left to right.

Site	Saulmore	Dunstaffnage	Marina	Pontoon
Saulmore	*	**0.016**	**<0.001**	**<0.001**
Dunstaffnage	**<0.001**	*	**<0.001**	**<0.001**
Marina	**0.000**	**<0.001**	*	0.085
Pontoon	**<0.001**	**<0.001**	0.839	*

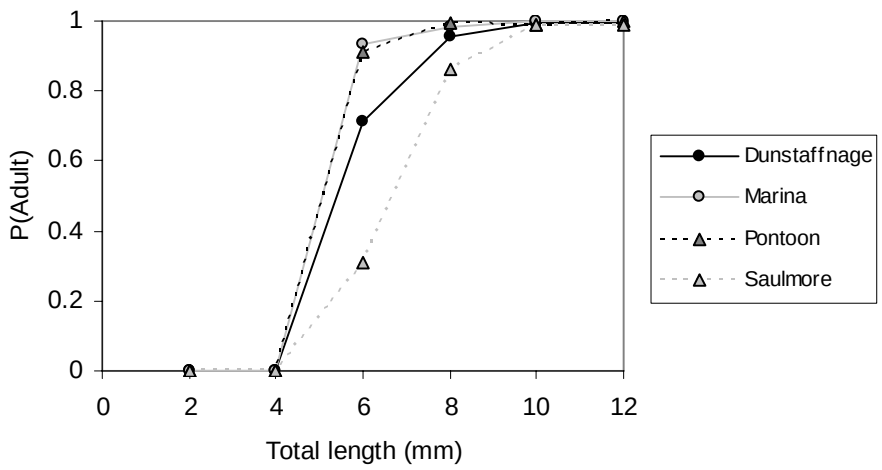


Figure 6-11. Body length of *C. mutica* individuals at sexual maturity (mm). n = 7974, 4877, 1245, 4450 for Dunstaffnage, Marina, Pontoon and Saulmore respectively.

6.4 Discussion

Population structure and dynamics

The population abundance of *Caprella mutica* showed an increase in spring to a peak in late summer (August - September), followed by a decline in population abundance during winter. This cycle was in phase with annual sea water temperatures in the area. The trend in abundance follows the seasonal population dynamics of *C. mutica* in its native habitat in the Sea of Japan (Fedotov 1991), representative of the similarity in the underlying physical environment in both areas. Populations of *C. mutica* on the west coast of Scotland are more abundant compared with those in the native habitat; abundance in excess of 10,000 individuals m^{-2} were recorded at three sites in this study (with a maximum of approximately 319,000 individuals m^{-2}), compared with a maximum of 2,600 individuals m^{-2} in the native habitat (Vassilenko 2006). A density of 3,500 – 4,800 individuals m^{-2} of the invasive amphipod, *Gmelinoides fasciatus*, and the rapid population increase from less than 500 to over 2,500 individuals m^{-2} within a period of two weeks were described to contribute to its invasive success (Berezina & Panov 2004). In comparison, the abundances of *C. mutica* are much greater and the rate of population increase is of a similar, if not greater, order. In the native habitat of *C. mutica*, *Caprella cristibrachium* has been recorded at a maximum density of 95,000 individuals m^{-2} (Vassilenko 1991). A high abundance in the native habitat is described to increase invasion potential (Ehrlich 1989, Van der Velde et al. 1998), and it may be expected that *C. cristibrachium* will be introduced in the future. The habitat preference of *C. cristibrachium*, the rocky surf intertidal zone, may render it less susceptible to human transport and therefore introduction.

Caprella mutica individuals were present at Dunstaffnage for the duration of the study, suggesting a population had successfully established at this location. Individuals were only present at Saulmore for 8 months of the year and were absent from the Marina and Pontoon sites for 6 or 7 months, between October and May or June, respectively. Crawley (1986) found abiotic factors (including weather) to be most important in determining the failure of non-native insects. Given the close proximity of the sites to each other, the weather conditions experienced would be similar. The salinity and associated water characteristics were more consistent at Dunstaffnage; the tidal decline in salinity and associated water movement may have negatively affected the population at Saulmore. Whilst inshore salinities also vary through the year, the annual variation is considered to be minimal compared to the day-to-day variations with the tide

(Craig 1959). There could be several factors contributing to the high abundance of *C. mutica* in the non-native habitats.

The year-round enhanced food supply to epibiotic communities at the fish farm sites (Cook et al. 2006) may explain the high abundance in the summer and year-round presence of *C. mutica*. Although, an abundance greater than 10,000 individuals m^{-2} in summer at the Marina and Pontoon sites, which are not artificially enriched, suggests this is not the only factor contributing to its success. Space can also be a limiting resource affecting invasion success (Stachowicz et al. 1999, Stachowicz et al. 2002); the high abundances at the sites may reflect the availability of space created by the artificial structures. A lack of native competitors, predators, diseases and/or parasites may allow uncontrolled population expansion in the introduced habitats (Van der Velde et al. 1998; the 'enemy release hypothesis', Colautti et al. 2004). However, the population abundance fluctuated at all sites, indicating high mortality or migration events which were also observed in the native habitat (Fedotov 1991).

In the Sea of Japan, *C. mutica* occurs in quiet small bays sheltered from the surf (Vassilenko 2006), hydrodynamics of an area may affect its susceptibility to the successful establishment of *C. mutica*. The models predict that *C. mutica* was likely to be present, but at very low numbers at Saulmore during the winter. The absence of individuals in the winter samples at Saulmore may reflect the inability of the sampling design to detect abundance less than 40 individuals m^{-2}. In the native habitat, it was suggested that *C. mutica* migrates to shallower waters in the Spring (Fedotov 1991); the absence of *C. mutica* from winter samples in the present study may indicate that individuals are deeper than 4 m in the winter. The dilution of the effect of the fresh-water runoff at Dunstaffnage may explain why individuals do not remain at depth to the same extent here. A migration from depth would explain the rapid increase in abundance in spring at Saulmore, which is earlier than that observed at the Marina or Pontoon.

The populations of *C. mutica* at the Marina and Pontoon showed very similar seasonality, with individuals only present on the meshes for 4 or 5 months between June and October. *C. mutica* arrived on the meshes at the Marina 4 weeks before the Pontoon. Dormancy is described as one reason for the absence of the invasive scyphozoan *Phyllorhiza punctata,* hydrozoan *Moerisia lyonsi*, and red swamp crayfish, *Procambarus clarkia,* when conditions are unfavourable (Gherardi 2002, Bolton & Graham 2004, Ma & Purcell 2005b). However, dormancy has not been previously recorded for caprellids and was not observed in the native region. Recruitment from depth at the end of spring was suggested in the native habitat (Fedotov 1991), although the

theory was not tested. If these factors were important, a similar period of absence would be expected at the fish farms. The duration of absence of *C. mutica* from the Marina and Pontoon, predictions based on seasonal abundance at these sites, and delayed onsets of population increase in spring indicate that populations were absent from the two sites during winter.

The inability of the populations to persist during winter months suggests that the populations fall below an 'Allee threshold' (the critical threshold below which a population cannot persist, Keitt et al. 2001) and decline to zero population density in winter. Recent work indicates that the combined influence of Allee dynamics and stochastic processes (random processes affecting the dynamics of virtually all populations) strongly influences the successful establishment of non-native species (Fagan et al. 2002, Petrovski et al. 2002). Inoculations may stop during winter months due to the low abundance and infrequent boating activity, or may be insufficient to bring the populations back over the Allee threshold. The fluctuating populations of *C. mutica* at all the sites make them more susceptible to extinction (Pimm 1989) and potentially open to eradication.

Assuming the populations at the Marina and Pontoon became extinct over the winter, the sites must be re-colonised in early summer when propagule supply to the areas increases above a certain threshold. Dunstaffnage and Saulmore may be acting as nutrient enriched, over-wintering refuges for *C. mutica* in the area. Recreational boating activity at the Marina is greatest between April and October and may be responsible for the supply of propagules to Dunstaffnage bay (Chapter 4), containing both the Marina and Pontoon. A high rate of population increase observed in this study is characteristic of invasive species (O'Connor 1986). This may enable *C. mutica* to rapidly re-colonise areas where populations are unable to survive the winter.

The population biology indicates that the impacts of *C. mutica* will be most apparent during summer months when abundance is greatest. No impacts have been recorded to date, although anecdotally, mussel farmers on the west coast of Scotland have noticed an abundance of *C. mutica* during the same periods that mussel spat have failed to settle, a cause and effect has not been confirmed. Management practices would be most feasible during winter when abundance of *C. mutica* is at its lowest, although absence at the surface waters (up to a depth of 4 m) may not be a good indication of complete absence from an area. The presence of *C. mutica* on all four sites sampled in the Lynne of Lorne indicates that both containment and eradication

efforts would be impractical. If considered necessary, *C. mutica* would have to be removed from all man-made structures within an area.

Sex ratio and reproductive activity

Females were dominant for most of the year, with males only dominating populations in the summer. Females were also found to be dominant throughout the year in the native habitat although their dominance decreased in the reproductive periods of spring and summer (Fedotov 1991). Reasons for the biased sex ratio are not known. Sex-ratio fluctuations can be attributed to several factors, including differential rates of production, growth, longevity or mortality (Naylor et al. 1988, Beare & Moore 1996). Deviations of sex ratio in *C. laeviuscula* have been attributed to selective predation on females (Caine 1979). Feminising bacteria can also cause a female-biased sex-ratio in amphipods (Bulnheim & Vavra 1968, Kelly et al. 2001). The influence of these factors has not been studied here, although the larger size of males could result in an increased susceptibility to predation (suggested for *Dikerogammarus villosus*; Devin et al. 2004). Advantages of a female-biased sex ratio include lessening intraspecific competition for resources (Beare & Moore 1996). In an invasion process, a female-biased sex ratio could be advantageous because it increases the reproductive capacity of the population (Devin et al. 2004). However, a female-biased sex-ratio is common in many amphipods (Costello & Myers 1989 and references therein, Powell 1990). This trait has not been suggested as correlating with invasion success in other taxa and it is unlikely to be the most important factor determining the success of *C. mutica* in a new habitat compared to other sympatric amphipods.

At Dunstaffnage, juveniles (0 – 4 mm) were present throughout the study indicating continuous reproduction or delayed growth of over-wintering juveniles. A reproductive seasonality, similar to that observed in the native region, was observed at the other three sites, where juveniles (and adults) were absent in the winter (November-January), and most abundant in Spring and Summer at Saulmore, and in Summer and Autumn at the Marina and Pontoon. Within the native range of *C. mutica*, average water temperatures can fall to 0 °C (Fedotov 1991). The minimum water temperature recorded in this study was 7.4 °C (April ’04). Warmer winter temperatures on the west coast of Scotland may enable the year-round reproduction and survival of juveniles, as recorded at Dunstaffnage. However, the absence of juveniles through the winter at the other sites indicates that temperature is not the only limiting factor. Early

developmental stages of crustaceans have a lower tolerance to changes in salinity and temperature (Maranhão et al. 2001, Berezina & Panov 2004, Tsoi et al. 2005) and the wider fluctuations in environmental conditions may have limited the presence of juveniles at the other three sites. The year-round input of feed at the fish farms most likely contributes to the maintenance of juvenile *C. mutica* individuals for longer periods at Dunstaffnage and Saulmore. However, reasons for the difference between over-winter juvenile abundance between the fish farms are not known. The widespread success of *C. mutica* may have been facilitated by phenotypic plasticity (Crawley 1987), allowing adaptation to environmental influences in the different habitats.

Fecundity

There was a positive correlation between female length and number of eggs of *C. mutica*, also found for *C. mutica* and other caprellids in previous studies (Caine 1979, Vassilenko 1991) and a common trend in amphipods (Powell 1990, Sainte-Marie 1991). Artificial feed is manufactured to increase the growth rate and quality of the aquaculture species. The high protein and lipid content of the feed has been shown to increase somatic and gonadal growth in other species found at aquaculture sites, for example, the echinoderm *Psammechinus miliaris* (Cook et al. 1998). Protein and lipid content of a diet have also been shown to influence the somatic growth of crustaceans, e.g. *Penaeus monodon* (Deering et al. 1997) and *Scylla serrata* (Sheen & Wu 1999). The artificial diet enrichment at Dunstaffnage and Saulmore is most likely responsible for the larger, more productive female *C. mutica* at these sites, but did not affect fecundity as number of eggs per female body length. The fecundity of *C. mutica* females of a similar size was quite variable. This could be due to constraints during sampling and sorting in the laboratory (e.g. variable egg loss from the marsupium; Drake & Arias 1995).

The relationship between female size and fecundity in different orders of the Crustacea has been found to be relatively constant with a ratio between brood weight and female body weight of approximately 0.16 (Ivanova & Vassilenko 1987). Vassilenko (1991) found a single equation to describe the relationship between number of eggs and female body weight of several caprellids, including *C. mutica*. It is difficult to compare the current study with these results as body weight was not measured. The fecundities as number of eggs per brood recorded in the present study are similar to those recorded in the native habitat of *C. mutica* by Fedotov (1991) although they are greater than those recorded for *C. laeviuscula* and *D. californica* (Caine 1979)

and for *C. mutica* by Vassilenko (1991). The high fecundity of *C. mutica* in the present study and that of Fedotov (1991) may have contributed to its success as a non-native species. A fecundity of 8 – 36 eggs per female in *G. fasciatus* was described as high, and contributing to its invasive success (Berezina & Panov 2004), once again the fecundity of *C. mutica* was much greater than this. Although, *C. mutica* is not the most fecund caprellid species in its native range (Vassilenko 1991) and it is therefore difficult to describe fecundity to contribute to its success in the non-native habitat when commensal caprellid species have not been introduced.

Fecundity of *C. mutica*, as a measure of the number of eggs per female, did not vary seasonally. However, the number of ovigerous females in the population was greatest at all sites during the summer months, with peaks between May and September. Only at Dunstaffnage did the peak in ovigerous females occur before the increase in abundance. This may reflect the permanence of the population here, with an increase in reproductive activity in spring followed by a peak in abundance in summer. The early increase in abundance followed by the increase in ovigerous females at the other sites could be a result of individuals arriving from another source, establishing and then commencing reproductive activity. If this were the case, the results indicate that fecund females are less likely to survive transport than non-ovigerous females, males and juveniles. This may reflect a reduced tolerance of ovigerous females to temperature or salinity conditions, also noted for the crustacean *Palaemon serratus* (Panikkar 1941) though not for *P. affinis* (Kirkpatrick & Jones 1985).

Size-frequency distribution

The maximum length of male *C. mutica* recorded during this study (47.0 mm) was similar to that recorded in Japan (50 mm; Nishimura 1995). However, lengths recorded in the present study were greater than those recorded in studies in Possjet Bay, Russia (Fedotov 1991, Vassilenko 1991). The environments in Japan may be more similar to those on the west coast of Scotland, leading to individuals of a similar size. Also, if geographically isolated populations have evolved to different size distributions, the source for the individuals introduced to Scotland may be a population of larger sized individuals, possibly from Japan.

Increased body size in the introduced range is common in marine invertebrates (Grosholz & Ruiz 2003). These increases have been attributed to increased resources, absence of predators (Keane & Crawley 2002), parasites (Torchin et al. 2001, Torchin et al. 2003) and competitors (Leger & Rice 2003, Bossdorf et al. 2004), selection of larger individuals during the invasion

process, or an effect of sampling procedures. It has recently been disputed whether larger body size implies better invasion success in marine bivalves (Roy et al. 2001, Miller et al. 2002a, Roy et al. 2002). The successful introduction of the comparatively large *C. mutica* may be another example in agreement with this theory in crustaceans.

Caprellidae native to the UK intertidal zone range in size from *Pariambus typicus* (maximum 7 mm) to *Phtisica marina* and *Pseudoprotella phasma* (maximum 25 mm) (Hayward & Ryland 2000). Introduced amphipods have been shown to out-compete smaller native species (Dick et al. 1999). The presence of *C. mutica* individuals of a large size throughout the study could give it a year-round competitive advantage over native conspecifics. No other caprellid species have been found in the same habitat where *C. mutica* has been introduced (R. Shucksmith pers. comm.). In its native habitat, however, *C. mutica* is found to be commensal with the smaller species *Caprella danilevski*, *C. penantis* and *C. eximia* (Vassilenko 2006). It may also be questioned why these commensal caprellid species have not become similarly introduced. Impacts on native caprellids would be minimal if *C. mutica* were to occupy a similar commensal niche in communities where it has been introduced. The larger size of *C. mutica* may increase its susceptibility to predation, reducing any apparent competitive advantage.

Male Caprellidae are typically larger than females. Caine (1979) suggested several explanations for this difference: adult females have a higher rate of unsuccessful moultings due to the encumbrance of a brood pouch; intraspecific aggressive behaviour among males reduces the number of smaller size males; the different sexes have different levels of camouflage, the brood pouch of ovigerous females being visually obvious. This also accounts for the pattern of size-frequency distribution of *C. mutica*, juveniles dominated the smaller size classes (0 - 4 mm) and were much more abundant than males and females, summation leads to a peak in females (6 – 10 mm), and males dominated the larger size classes (16 – 48 mm).

Individuals were significantly larger at Saulmore and Dunstaffnage than at the pontoon; this size difference is found throughout development, with juveniles at Saulmore reaching a larger stage before developing sexual diagnostic characters. The larger size of the females also explains the higher fecundity of *C. mutica* at the fish farms. The artificial feed, high in protein and lipids, has been shown to increase growth of crustacean species (Deering et al. 1997, Sheen & Wu 1999) and most likely brings about a similar response in *C. mutica* at the fish farms.

Anthropogenic influence

Anthropogenic influences are most likely responsible for the difference in population dynamics and characteristics of *C. mutica* between fish farm and non-fish farm sites (i.e. Marina and Pontoon). One influence is the enhanced food supply to epibiotic communities at caged fish farm sites (Cook et al. 2006). The feed is typically a high protein and lipid diet, also high in essential fatty acids that has been shown to increase growth rates in echinoderms and crustaceans (Deering et al. 1997, Cook et al. 1998, Sheen & Wu 1999). This input has the potential to influence all aspects of the population biology of *C. mutica*, in particular the rate of population increase and size of individuals (Stirling & Okumus 1995, Cook et al. 2006). Nutrient availability has been recorded as a factor limiting invasions of terrestrial plant communities (Huenneke et al. 1990, Burke & Grime 1996), protozoans (Miller et al. 2002b), and the mussel *Musculista senhousia* (Allen & Williams 2003). However, in a study of the zooplankton *Daphnia lumholtzi*, nutrient enhancement did not facilitate its invasion success (Lennon et al. 2003). Being most likely opportunist feeders (Keith 1969), *C. mutica* may be feeding directly on the fish feed, on the algal growth which is enhanced by dissolved organic nutrients (e.g. Rhodophyta, Troell et al. 1997), and on enhanced plankton abundance in the close vicinity of the cages (Cook et al. 2006). The high organic matter concentrations at salmon farms were found to reduce muscle reserve depletion over winter in the mussel *Mytilus edulis* (Stirling & Okumus 1995). Without the continuous input of feed, food supply at the Marina and Pontoon will be much reduced relative to the fish farms, especially in winter when productivity in the area is minimal (Grantham 1981).

Space can also be a limiting resource affecting invasion success (Stachowicz et al. 1999, Stachowicz et al. 2002); the successful establishment at the fish farms may reflect the availability of space at these sites. Space is a known limiting resource in subtidal epifaunal habitats (Dayton 1971, Sutherland & Karlson 1977, Buss & Jackson 1979) and has been reported to influence invasive success in other communities (Burke & Grime 1996, Stachowicz & Tilman 2005). Stachowicz et al. (2002) related the effect of species diversity on the invasive success of ascidians and bryozoans to the associated space availability. Space in the form of cage nets, which are held suspended in the water column, provide favourable habitats for *C. mutica*, similar to the algal beds where they are found in the native range (Vassilenko 2006). While space as a resource has been enhanced by the Marina and Pontoon structures, the quantity of space suspended in the water column is much less in comparison to the fish farms.

The rigid properties of the structures are also less favourable to Caprellidae. Caprellids favour highly branched substrata, especially those which allow encirclement by the pereopods (e.g. hydroids and filamentous algae; Caine 1978); the fibres in the nets offer such substrata. At the Pontoon and Marina, *C. mutica* will depend on epibenthic fouling organisms to provide suitable attachment sites.

At the two fish farm sites, nets are regularly lifted and exposed to the air for a period of time to limit epibiotic growth and during pharmaceutical treatments, harvesting and grading. To what degree disturbance affects invasibility and invasion success is a complex issue, confounded by differences in species properties, ecosystem properties and propagule pressure (Lonsdale 1999). Historically, the notion that disturbance promotes invasions by non-native species has been well supported (Elton 1958, Crawley 1986, Mooney & Drake 1989, Hobbs & Huenneke 1992, Davis et al. 2000, Cohen 2002, Stachowicz et al. 2002); a lack of disturbance allows the establishment of more diverse, less-invasible communities (Davis et al. 2000, Stachowicz et al. 2002). Non-native species are commonly first identified in human-impacted, or disturbed, sites. In plant communities, disturbance has been shown to promote invasions by 'weedy' non-native species (Rejmanek 1989, Hobbs & Huenneke 1992). However, it should be noted that non-natives are often sourced from human-impacted sites, released in human-impacted sites, and that these sites are the most intensively studied (Cohen 2002). A study of marine epifaunal communities also supported the theory that disturbance facilitates invasions (Clark & Johnston 2005). However, several studies have shown that over larger scales, communities which are more diverse harbour more non-natives (Levine & D'Antonio 1999, Lonsdale 1999, Simberloff & Von Holle 1999, Richardson et al. 2000, Meiners et al. 2004, Stohlgren et al. 2006), indeed factors which predict non-native species diversity are also those which predict diversity of native species (Stohlgren et al. 2006).

The intensity, timing and sequence of disturbance events is also important in determining the effect on ecological communities (Fukami 2001). The regular disturbance at the fish farms favours species which happen to be producing propagules and are within dispersal range at the time of net change. Fish farm nets are changed by partially lifting the old net, passing a new net underneath and into position around the old net; the old net is slowly lifted from one side, allowing the fish to swim into the new net (pers. obs.). Contact between the nets and dislodging of individuals from the old net is considered a likely means of introducing *C. mutica* to the new net. If the net change is completed when abundances are high, most likely a population will

establish on the new net. During the study, all the nets at Dunstaffnage were changed in August 2004 and May 2005, at both times abundance of *C. mutica* was high (over 3000 individuals m^{-2}). At Saulmore, all the nets were changed in August 2004, and April and August 2005. Half of the nets were also changed between November 2004 and February 2005, when *C. mutica* abundances were low. This disturbance may have significantly reduced population levels, which did not recover above the minimum sampling threshold until the following May.

Summary

Populations of *C. mutica* have been found on several fish farms on the west coast of Scotland, and at aquaculture sites in the northern hemisphere (Chapter 2). The increased food supply, provision of space, regular disturbance and position of the farms in areas of water movement may be responsible for this association. Crawley (1986) described that in most communities there would be times or places where a combination of available resources, scarce competitors and low densities of natural enemies, provide conditions where invaders can establish. Aquaculture sites may provide refuges for *C. mutica* and other non-native species, which would greatly enhance the probability of establishment of invasive species at these sites. On the west coast of Scotland, the physical environment is dominated by an annual cycle which drives the supply of nutrients to the water column, controlling seasonal productivity in the area (see Chapter 1, Grantham 1981). Environmental conditions experienced at the four sites were relatively similar, although, the influence of fresh water run-off from Loch Etive decreases as the distance from this Loch is increased (salinities are least variable and greatest at Dunstaffnage). The four sites experience different anthropogenic influences, which are most likely responsible for the consistent division between the population dynamics and characteristics of *C. mutica* at the fish farm and non-fish farm sites, and may influence the success of a non-native species. Biological factors described to limit invasion success include predation, competition and facilitation (Bruno et al. 2005). These can not be quantified from the present study and will not be discussed here, but may also contribute to the differences in population dynamics at the four sites.

The potential high rate of population increase (characteristic of ‘r’ strategists) and larger size may contribute to the success of *C. mutica* as an invader (O'Connor 1986, Van der Velde et al. 1998); the fluctuations in the population size make them more susceptible to extinction (Pimm 1989). The impacts of *C. mutica* will be greatest during summer months when abundance is

greatest. The commensal nature of *C. mutica* in its native habitat suggests that management practices will most likely not be necessary. However, if *C. mutica* was found to pose a threat to native communities, eradication efforts would be most economical and effective during winter months when *C. mutica* are of a low abundance or absent. It should be taken into consideration that, in the late spring, sites would be at risk of re-introduction of *C. mutica* from any neighbouring source. Apparent absence of *C. mutica* from surface waters during the winter does not necessarily denote complete absence from a site.

While the population dynamics information is of inherent interest and provides excellent background information as to how *C. mutica* might function in a new habitat, the seasonal population dynamics of *C. mutica* were not dissimilar to other caprellids or marine epifaunal Crustacea. Therefore, why is *C. mutica* a successful invader? The factors determining the success of *C. mutica* in a non-native habitat will be the same as those determining the success of any species in that particular habitat, both biotic and abiotic (Stohlgren et al. 2006). Hence, the over-riding trend in abundance of spring increase, late summer peak and decline to a winter minima, typical of marine species throughout the west coast of Scotland, and other habitats at temperate latitudes (described in Chapter 1).

Appendix 6.1

Figure A. Dunstaffnage Marina with Dunstaffnage Marine Laboratory Pontoon indicated by stars in the foreground.

Figure B. Samples being deployed from Saulmore Fish Farm, cages are suspended from a grid-like metal frame.

Figure C. Polar circle cages as used at Dunstaffnage Fish Farm

Chapter 7

Final Overview and Discussion

Distribution and dispersal of the non-native caprellid amphipod, *Caprella mutica*

7.1 Overview

Caprella mutica, the 'Japanese skeleton shrimp', has become widely introduced as a non-native species. This study has described the current distribution on a local and global scale and attempts to discern how the caprellid amphipod has established its present distribution. A combination of field studies at the local scale and phylogenetic analysis at the global scale has provided some evidence of the pathways and dispersal mechanisms of *C. mutica*. A study of the biology and physiology has given insight as to how the species might continue to spread enabling a prediction of its future distribution. Anthropogenic dispersal vectors have been central to the success of *C. mutica* as a non-native species. Finally, based on their invasion histories, several species have been identified as potential future introductions to the UK.

Distribution

At the local scale, environmental conditions have not restricted the distribution of *C. mutica* and it has now spread extensively on artificial structures on the west coast of Scotland. The UK falls within a single climatic region (cold temperate), and many native species on British coasts do not reach their geographical limits here (Lewis 1964). Amongst the species that do, it is more common to find them restricted at their northern limits than southern limits. Several species, such as *Chthamalus stellatus*, *Gibbula umbilicalis* and *Asterina gibbosa* (Lewis 1964), extend northward on the west coast of Scotland, but are scarce if not absent from the typically cooler North Sea coasts. This has implications for the distribution of other non-native species in Scotland and the UK, which might be expected to establish widely here. Species that do reach their range limits within the UK tend to be defined by thermal tolerance, either limited by winter temperatures in the north, or summer temperatures in the south (Lewis 1976).

Climate change is influencing the distribution of marine species (Southward & Crisp 1954, Southward et al. 1995, Walther et al. 2002). With climate change, an extensive restructuring of planktonic, pelagic and benthic communities is expected (Southward et al. 1995). Climate

change may facilitate non-native species adapted to warmer temperatures or tolerant of a wider temperature range (Stachowicz et al. 2002), contributing to the global homogenisation of diversity (Streftaris et al. 2005). The climate hypothesis (Chapman 2000) suggests that climate effects superimpose over all other processes controlling the distributions of non-native species diversity and geography. Chapman (2000) also suggests that the distribution of non-native species is largely independent of particular life history, taxonomic origins or dispersal pathways. With global warming, 'southern' species in the UK are predicted to increase in abundance and northward distribution while 'northern' species are predicted to decrease in abundance and retreat to the north (Hiscock et al. 2004). Other factors, such as hydrodynamic conditions, geographical barriers and species life history characteristics, may delay or prevent the responses of some species (Hiscock et al. 2004). In addition, species may soon be able to establish in habitats where they have been continuously introduced yet were unable to succeed due to environmental conditions. Species that are currently limited to areas south of the English Channel, such as *Gibbula pennanti* (Southward et al. 1995), may soon be able to establish on the south coast of England. Non-native species associated with human mediated dispersal will probably arrive before those with short-phase planktonic dispersal.

The distribution of *C. mutica* in its native range is not fully understood, but it has been described from latitudes of 40 to 50 °N (Vassilenko 2006). This area experiences a temperate climate suggesting that *C. mutica* is tolerant of a wide range of environmental conditions, as confirmed by its temperature and salinity tolerances described in Chapter 5. The widespread distribution of non-native *C. mutica* in Scotland and Europe supports the theory that a wide distribution in the native range and a wide environmental tolerance are traits that contribute to the success of non-native species (Darwin 1872, Crawley 1987, Daehler & Strong 1993, Van der Velde et al. 1998). In the UK, the distribution of *C. mutica* could, therefore, extend to encompass all coastlines with suitable habitat. Species are likely to be limited from the UK if they can not survive sustained lower temperatures of 6 °C and upper temperatures of 16 °C (Hiscock et al. 2004). Recent studies investigating the complex physical stresses of the intertidal environment suggest that the thermal stress in these habitats is much greater than that experienced in the coastal environment (Helmuth et al. 2002). The temperature range described by Hiscock et al. (2004) may not be a reliable indication of temperatures of UK inshore marine habitats. The freshwater influence in estuarine regions is also an important limiting factor determining the inland distribution of marine or estuarine species. *C. mutica* has not been found in the upper reaches of sea lochs and is most likely limited by its low tolerance to long-term freshwater influence.

Globally, *C. mutica* has been identified from all cold temperate coastlines in the northern hemisphere and two ports in New Zealand in the southern hemisphere. In North America, *C. mutica* is widely distributed on both Pacific and Atlantic coasts. A widespread distribution, similar to that found on the west coast of Scotland, is expected on all coastlines where *C. mutica* has been or may be introduced. Its distribution may be limited by the availability of suitable artificial substrata. The large number of records from North America suggests that the rapid assessments conducted on both coastlines (e.g. Cohen et al. 1998, Cohen et al. 2002, MIT. Sea Grant 2003, Pederson et al. 2003) are an effective and efficient sampling strategy and should be implemented more extensively worldwide (Ashton et al. 2006). The distribution of *C. mutica* in Europe is relatively wide, and it may be near the limits of its environmental tolerance, although the coastline of Spain in the south and Norway in the north should provide suitable environmental conditions for future introductions.

It is surprising that *C. mutica* has not been identified more widely in the southern hemisphere, where habitat assessments have been completed and specialists are working in the field (e.g. Guerra-García & Thiel 2001, Guerra-Garcia & Takeuchi 2004, Robinson et al. 2005). *C. mutica* may be less able to survive the passage across the equator from the northern to southern hemisphere, although, introduction(s) to New Zealand indicate that the long passage is not an impassable barrier. Chile, Uruguay, South Africa and parts of south Australia are climatically similar to areas where *C. mutica* has already become established (Peacock & Worner 2006). It is therefore expected that *C. mutica* may extend its distribution to these regions in the southern hemisphere, especially with the increasing speed and frequency of marine transport (Carlton 1996, Ruiz et al. 1997).

Regions where *C. mutica* has been found to date are populated and have international shipping ports (Chapter 3), suggesting that patterns of human colonization and trade are good predictors for introductions of marine species (Gaston 2003, Sax & Gaines 2006). However, this correlation may be an artefact of the intensity of surveys in these areas as rapid assessments are largely reported from human impacted habitats (e.g. Cohen et al. 2002, Pederson et al. 2003). The non-native distribution of *C. mutica* follows the diversity distribution of native caprellid amphipods in the northern hemisphere, which are most diverse at latitudes between 30 and 45 °N in waters adjacent to Japan (Takeuchi & Sawamoto 1998), and between approximately 40 and 50 °N in boreal waters of Atlantic and Arctic Canada, and the American North Pacific (Laubitz 1970, Laubitz 1972). This supports the findings of Stohlgren (1999) and Espinosa-García et al. (2004), who reported a positive correlation between native and non-native species richness. In the southern hemisphere, caprellid

amphipods are most diverse and abundant south of 30 °S, which may provide a further indication of where *C. mutica* might establish in the future.

The introduction of *C. mutica* to regions in the northern hemisphere suggests that these locations will continue to be matched donor and recipient regions for other non-native species (Gollasch 2002). Other species which have also been introduced to some of these locations in the northern hemisphere, and therefore might be expected to be introduced to the UK, are listed in Table 7-1. This list is probably an underestimate of the potential number of species (Ruiz et al. 1997), and does not include pathogens or parasites of species which have been intentionally introduced for aquaculture or other purposes (ornamental, biocontrol etc.). Attention should be given to those species that have been shown to impact species used for aquaculture purposes (e.g. through competition or predation) and may therefore cause economic and social damage in the UK. In addition, non-native species that currently have a limited distribution in the UK, such as *Styela clava, Sargassum muticum* and *Eriocheir sinensis* (Ashton et al. 2006), might be expected to extend their range in the future, especially in light of global change.

Dispersal

The wide distribution of *C. mutica* gives some indication of its robustness and ability to survive transport on a variety of vectors, both natural and anthropogenic. Its effective natural dispersal was suspected given the identification of caprellids in marginal sea plankton tows (Takeuchi & Sawamoto 1998). Indeed, *C. mutica* was found to disperse over distances up to 1 km, and may potentially disperse up to at least 5 km free-swimming (Chapter 4). It is difficult to predict the independent dispersal of *C. mutica* beyond this, and at greater distances transport vectors will play an increasingly important role in its dispersal. Rafting on drifting algae has been suggested as a vector for the long-distance dispersal (>1000 km) of several marine species (Jokiel 1984, Helmuth et al. 1994). Marine hydrozoans, bryozoans, crustaceans and gastropods were found to be the most common rafting taxa (Thiel & Gutow 2005). *C. mutica* has been found amongst drifting seaweed communities in its native habitat and in the current study (Sano et al. 2003, Chapter 4). Drifting algae is most common in near-shore areas of the world's oceans and rafting is considered to play a significant role in the coastal dispersal of *C. mutica*. Rafting on drifting algae will be most important as a vector during spring and summer months, when the maximum abundance of both drifting algae and *C. mutica* coincide (Thieltges et al. 2004, Thiel & Haye 2006; Chapter 6).

Table 7-1. Marine species which have been introduced outside their native range and are likely to be introduced to northern Europe including Scotland.

Species	Native region	Introduced to	Vector*	Impacts	Source
Homarus americanus American lobster	N America	Scandinavia	AQ	Competes with native lobsters	(van der Meeren et al. 2000)
Paralithodes camtschaticus Red king crab	Sea of Japan	N Norway	AQ	Predator on scallop beds	(ICES 2006)
Hemigrapsus sanguineus Asian shore crab	Sea of Japan, Russia	Atlantic North America, France, Netherlands, Croatia	BW	Aggressive predator with range of food preferences. Potential for significant ecological impact	(Gerard et al. 1999, Breton et al. 2002, Schubart 2003)
Hemigrapsus penicillatus Asian shore crab	China, Japan, Korea	French Atlantic coast	SF SH	Unknown	(Gollasch 1999)
Asterias amurensis Northern Pacific seastar	NE Asia	SE Australia	SH, AQ, RB	Mariculture pest	(Hewitt et al. 2002)
Rapana venosa Veined whelk	Sea of Japan	Chesapeake Bay, North Sea, New Zealand	SH, AQ	Predator on shellfish	(ICES 2004)
Corella eumyota Tunicate	Southern hemisphere	Several habitats in the southern hemisphere, France	SF	Fouling organism on artificial structures, capable of smothering other sessile species	(Lambert 2003)
Didemnum Colonial sea-squirt	Unknown	Global	AQ, SF	Fouls aquaculture cages and fishing grounds	(USGS-WHSC 2005)
Heterosiphonia japonica Red alga	North Pacific	Netherlands, Spain, France, Norway	JO	Unknown	(Husa et al. 2004)

*Vectors: AQ - aquaculture; JO – Japanese oysters; SH - shipping; BW – ballast water; SF – ship hull fouling; RB – recreational boats

Oceanographic and climatic conditions probably determine the directionality of rafting routes (Gaines et al. 2003, Muñiz-Salazar et al. 2005), and they have been described to affect the local and regional distributions of littoral caprellids (Thiel et al. 2003). However, they can be insignificant when compared with dispersal by human vectors (Thiel & Haye 2006), and it is highly unlikely that passive dispersal mechanisms are responsible for the global distribution of *C. mutica*, which has probably been established in the last 40 years (Chapters 2 & 3). Caprellids have been identified in ship ballast tanks, and *C. mutica* has been found on the hulls of recreational boats in Scottish marinas and is often found associated with aquaculture structures (Vassilenko 2006, Chapters 2 & 4). Thus, the global distribution of *C. mutica* can be attributed to the most important human-aided vectors for global introductions of marine species: shipping and aquaculture practices.

Hull fouling was considered the most likely vector responsible for the introduction of *Caprella californica* to Sydney harbour (AMBS 2002), and it is likely that *C. mutica* is also dispersed on ship hulls, especially in areas of fouling which do not experience severe drag, such as sea chests (Godwin 2001, Coutts et al. 2003). Caprellids have also been identified in ships' ballast, therefore, shipping offers an effective mechanism for the dispersal of *C. mutica*. Indeed, the areas where *C. mutica* has been introduced in the northern hemisphere are all areas of intense shipping activity (Drake & Lodge 2004, www.amver.com 2006), although, this does not explain why *C. mutica* has not been found more widely in the southern hemisphere, as Chile, South Africa and southern Australia all have high levels of shipping activity. It also does not explain the absence of *C. mutica* from the Mediterranean Sea or more tropical latitudes. However, other factors, such as productivity, competition, predation and environmental conditions, may be responsible for the absence of *C. mutica* from these regions (Thiel et al. 2003).

Transfers of oyster spat were attributed as responsible for the first long-distance introduction of *C. mutica* to the Pacific coast of North America (Carlton 1979a). Many species of shellfish, including mussels, oysters, clams and scallops, have been transported around the globe to start or boost aquaculture industries (Wolff & Reise 2002). The development of codes of conduct (e.g. ICES 1995), licence systems and quarantine measures for aquaculture imports have contributed to a decline in the number of species accidentally imported with the target species. However, within Europe, large quantities of shellfish are still being transported between culture areas, and the role of aquaculture transfers as a means of dispersal is thought to be comparable to the role of ballast water (Wolff & Reise 2002).

Having reached a new habitat, a combination of vectors are responsible for the secondary spread of *C. mutica*, including independent natural dispersal, attached to driftweed,

recreational boating and aquaculture practices (Chapters 2 & 4). Via these mechanisms, it is likely that *C. mutica* will rapidly spread from an initial introduction point. Methods of dispersal are more likely to influence the distribution of a species in the UK than environmental factors (Eno et al. 1997). A high potential dispersal (e.g. through rafting biotic or abiotic vectors) can lead to genetic homogenisation of populations or to population differentiation independent of a distance pattern (Peterson & Denno 1998, Kolbe et al. 2004, Zardus & Hadfield 2005). The low genetic diversity of Atlantic populations may result from the multiple dispersal vectors operating, and the potentially high frequency with which vessels can link geographically distant populations around the Atlantic. Other species capable of fouling biotic or abiotic material are likely to achieve similar widespread distributions. This rapid distribution reflects that described for *Elminius modestus* (Lewis 1964), which spread throughout England and Wales, Eastern Ireland and southern Scotland within 20 years of its first release on the south coast of England (Crisp 1958), probably as a result of both natural and anthropogenic dispersal. Interestingly, *E. modestus* still has a discontinuous distribution along the UK coastline (Avant 2002).

The various mechanisms of secondary dispersal, in particular hull fouling, for *C. mutica* and other species should be of wider concern. Within Europe, several mechanisms are acting to disperse marine species along the continental coastlines, and policies need to be established at an inter-governmental level (e.g. within ICES and the EU). Once a species is introduced to the UK, it will probably disperse rapidly and will most likely be found universally within as little as 20 years (e.g. *E. modestus*, Lewis 1964). While *C. mutica* has shown no negative impacts to date, other species may not be so innocuous. The most effective means of limiting the introduction of non-native species is by reducing the number of individuals released (Cassey et al. 2004). To do this, prevention measures must be implemented at or before the dispersal phase, however, this is proving difficult in an era of ever increasing international trade and travel (Levine & D'Antonio 2003).

Caprella mutica as a non-native species

Traits of *C. mutica* that have been suggested as important in determining the success of non-native species, and their progression to invasive status are presented in Table 7-2. Success as a non-native species indicates a pre-adaptation to the abiotic and biotic conditions of the habitats where it has been introduced (Darwin 1872, Prinzing et al. 2002), and a morphological, physiological and ecological capacity to overcome any potentially limiting factors (Gaston 2003). Knowledge of species' traits can assist in identifying high-risk species

and predicting likely regions where they may be introduced (Prinzing et al. 2002), but unfortunately this information is rarely available before a species invades; this is particularly true for caprellid species for which very little biological information could be found (as shown by the missing information for other *Caprella* spp. in Table 7.2). None of the 'success' traits are unique to *C. mutica* and other caprellids may be introduced to the UK in the future. Based on the similarities of traits, and the fact that they have already been identified outside their native ranges, species that are likely to be introduced include *C. scaura*, *C. acanthogaster*, *C. natalensis* and *C. californica* (Carlton 1979b, Occhipiniti-Ambrogi 2000, AMBS 2002, Ranasinghe et al. 2005). These caprellid species share the traits of being abundant and widespread in their native range, with broad habitat and food preferences, and they are commensal with human activity (Table 7-2). Whilst they are generally found in a broad range of habitats in their native range, they are often only found associated with anthropogenic activity in the non-native range. Relative size and presence of a dispersing or resting stage are less important to the introduction success of caprellids. The rapid invasion progress of *E. modestus* in the UK was most likely due to its year-round breeding and ability to use human vectors for dispersal (Crisp & Davies 1955). These are also traits that have contributed to the non-native success of *C. mutica*.

Non-native *C. mutica* populations have only been identified from disturbed sites, which may indicate its inability to survive in undisturbed habitat outside of its native range, indeed this may be why there have been no negative impacts observed to date. Fish farms provide a unique combination of high exposure and regular input of particulate organic matter, which may create a unique habitat and niche for *C. mutica*. A lag-phase prior to range expansion or rapid population growth has been described for several non-native species, including *Eriocheir sinensis* and *Styela clava* (Crooks & Soulé 1999, Byers et al. 2002, Thresher et al. 2003). *C. mutica* may be restricted to the fish farms in a lag phase, and soon move to more natural habitats when it enters the expansion phase. According to the enemy release hypothesis the fish farms may provide a habitat free of predators and competitors, allowing uncontrolled population expansion (Colautti et al. 2004). Success by exotic invaders can be explained by a lack of native competitors, predators, diseases and/or parasites, allowing uncontrolled population expansion (Van der Velde et al. 1998, Torchin et al. 2001). Specific attention should be made to the potential introductions of aggressive caprellids, *C. laeviuscula* and *C. scaura*, which are highly efficient dispersers and may out-compete native caprellids (Caine 1980, Schultz & Alexander 2001).

Table 7-2. Characteristics that may have contributed to the introduction success of *Caprella mutica* and other caprellids that have been introduced outside their native range. Characteristics taken from Van der Velde et al. (1998), Strasser (1998), Williamson (1996) and Colautti et al. (2006). Italics indicate *r*-selected traits.

Characteristic	*C. mutica* [a]	*C. scaura* [b, c]	*C. natalensis* [d, e, f]	*C. acanthogaster* [g]	*C. californica* [h, i]
Previous success as an invader	Yes	Yes	Yes	Yes	Yes
High invasion (propagule) pressure*	Yes	Yes	Yes	Yes	Yes
Abundant and widespread in native range	Yes	Yes	—	Yes	—
Tolerant of wide range of physical conditions (eurytopic)	Yes	—	—	Yes, intolerant of warm equatorial waters	—
Broad habitat preference (euryoecious)	Yes	Yes	No- prefers protected shallow water	Yes	—
Preference for disturbed habitats	Yes	—	—	—	—
Broad food preference (omnivorous)	Yes	—	Yes	—	Yes
Larger than most relatives	Yes	No	—	Yes	—
High genetic variability	In native range	—	—	—	—
Short life span and generation time	*Yes*	—	—	—	—
High fecundity, high growth rate	*Yes*	—	—	—	—
Year round reproduction	Yes	—	—	—	—
Potential to form a resting stage	No	—	—	—	—
Aggressive behaviour	Yes	Yes	—	—	—
Commensal with human activity	Yes	Yes	—	Yes	—

References for species information: a- Willis et al. 2004, b- Occhipiniti-Ambrogi 2000, c- Thiel et al. 2003, d- Ranasinghe et al. 2005, e- Caine 1980, f- Caine 1977, g- Guerra-Garcia & Takeuchi 2004, h-AMBS 2002, i- Keith 1969.
* Introduction success is sometimes considered to indicate a high invasion (propagule) pressure (Williamson 1996, Ruiz et al. 2000).

As well as affecting success in a new habitat, biological characteristics of non-native species can help to identify eradication or containment methods. The zebra mussel, *Dreissena polymorpha*, which clogs pipelines carrying raw water, is controlled using 'biobullets', microencapsulated chlorine compounds which are concentrated by the mussels (Aldridge et al. 2006). The macroalga, *Caulerpa taxifolia*, was successfully eradicated from Agua Hedionda Lagoon, California, using expertise and knowledge of its biology to design an effective eradication programme (Anderson 2005). Potential means of eradicating *C. mutica* are generally flawed due to its widespread distribution and dispersal by both natural and anthropogenic vectors. It would be necessary to remove *C. mutica* from all areas which could potentially be linked by current-driven transport as well as limiting human vectors from the area. In order to be successful, an eradication programme would have to be unified throughout the UK, a costly and unnecessary exercise given its lack of environmental impact.

7.2 Discussion

The aim of this study was to explore four questions, namely:

- The *status quo*, what is the current distribution of *C. mutica*?
- How did it get there? Using a combination of molecular techniques and field studies to establish introduction pathways and potential vectors.
- Where can we expect to see it? What does the current distribution and environmental tolerance limits tell us about the potential distribution of *C. mutica*?
- Does the population biology of *C. mutica* predispose it to be a successful non-native species?

Within the last 40 years, *Caprella mutica* has become distributed on all oceanic coastlines in the northern hemisphere and has been found at two sites in New Zealand in the southern hemisphere. Where introduced, it has dispersed effectively and achieved a wide distribution in the local habitat. A number of human vectors are responsible for this widespread distribution, including shipping at the global scale, recreational boating at the local scale, and aquaculture practices at both scales. The distribution of *C. mutica* is expected to continue to increase globally, although temperature will limit its distribution to temperate latitudes. Several other caprellids share the characteristics that may have contributed to the success of *C. mutica*, and it is expected that more caprellid species, and indeed marine amphipods, will continue to be identified from areas outside their native ranges.

While no negative impacts of *C. mutica* have been observed to date, each invader has the potential to severely threaten the biodiversity and economics of the recipient area (Gollasch 2002a). The identification of the non-native *C. mutica* on the west coast of Scotland should be taken as a warning that effective and environmentally sound biosecurity measures are needed to prevent the introduction of high risk species. The presented data indicates that many species within the cold temperate climatic region and associated with human dispersal mechanisms could be introduced to the UK in the future; with several species highlighted in Table 7-1. Once introduced, there are several effective dispersal mechanisms which can rapidly spread the species along the coastlines. Once widely distributed, eradication would most likely be unsuccessful.

The realised niche of *C. mutica* is now largely associated with human activity, rather than natural constraints that have previously limited its distribution. The reconstruction of introduction pathways has been described as critical for the design of effective management programmes (Everett 2000, Myers et al. 2000, Lodge et al. 2006). However, once in progress, the complex network of natural and human-mediated vectors forms a dynamic, non-stop process (Streftaris et al. 2005), and the movement of species from one place to another is a predominant feature of life on Earth (Elton 1958, Sax et al. 2005). Given the forecast of inevitable human globalisation (Perrings et al. 2005) and ultimate survival of the fittest (Darwin 1872), it must be considered whether attempts to control or prevent the introduction of non-native species are futile; this is almost certainly the case for *C. mutica*.

7.3 Note

This thesis is the first in a series of studies on *C. mutica* as a non-native species, and I refer the reader to the forthcoming theses of R. Shucksmith and K. Boos for further information regarding its competitive and trophic interactions respectively.

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