Habitat Composition, Sexual Conflict & Life History Evolution in *Coelopa frigida*.

Dominic Alexander Edward, MSc

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Abstract

This thesis describes an investigation into the effects of habitat composition, principally the composition of algae in a wrack bed, on the life history of the seaweed fly, *Coelopa frigida*. The mating system of *C. frigida* is dominated by intense sexual conflict characterised by frequent harassment by males leading to a vigorous pre-mating struggle. This response leads to sexual selection for large male size and sexual dimorphism. The mating behaviour of *C. frigida* is affected by their environment, with exposure to brown algae inducing harassment in males and oviposition in females. Despite more than two decades of research into coelopid reproduction little is known about how habitat composition alters the patterns and processes of sexual conflict.

Studies contained in this thesis consider environmental influences that both directly and indirectly influence sexual conflict. Direct effects of the environment are measured by conducting mating trials following culture of *C. frigida* on different species of algae and by exposing males to different species of algae. This work is accompanied by studies of larval development and adult survival on different algae. In recent years it has been observed that the distribution of European coelopids has undergone a northward range shift. An investigation into the current distribution of European coelopid species and a discussion of the effects of climate change that may have caused this change is included. Finally, the use of stable isotope analysis to determine the diet of wild coelopids and alternative statistical methods to analyse mating trials are described.

Chapter 1 - Introduction

1.1 Sexual conflict

The meeting of two unrelated individuals to share genetic information in the production of future generations is a natural phenomenon whose intricacies may too easily be taken for granted. Interactions between the sexes can provide a fascinating insight into the perpetual challenge of self preservation; giving purpose and meaning to a wide diversity of behaviours and structures seen in nature. Gone are the days of assumed cooperation between the sexes (Arnqvist & Rowe 2005). Sexual reproduction can instead be considered the acts of a single individual to maximise fitness that just happen to depend on another. As two individuals meet with independent interests, roles and resources to contribute to reproduction; mutual assistance is only one possible strategy that may satisfy individual needs.

Sexual conflict results from a divergence in the evolutionary interests of the sexes, driven by the maximisation of individual, rather than group, fitness (Parker 1979). It is now widely understood that males and females are not naturally cooperative during reproduction and conflict arises over the relative investment of each individual in the production of progeny (Trivers 1972). There is often little cost to one individual if the other can be coerced into undertaking the bulk of responsibility and investment in shared offspring. A conflict of interests is often present even before contact is made as females frequently invest more resources in the production of gametes (Bateman 1948). Asymmetrical investment at this stage of reproduction can limit the reproductive capacity of females relative to males. However, anisogamy is by no means a pre-requisite for sexual conflict, which can occur at any stage of a reproductive encounter.

Conflicts of interest may arise in a range of traits related to courtship, mating, fertilisation and parental care (Chapman 2006). At a genetic level, sexual conflict may occur over the same or different loci in males and females. Intra-locus sexual conflict is defined as conflict over a trait encoded by the same gene in males and females, inter-locus conflict being conflict over traits encoded by different genes in each sex (Chapman 2006).

Sexual conflict is of considerable interest to evolutionary biologists as it has the potential to drive rapid evolutionary change away from naturally selected optima. Sexually antagonistic coevolution can be initiated as the evolution of a trait in one sex towards its optima causes the same or other traits in the opposite sex to be moved away from their preferred optimum. The adversely affected sex may then counter-adapt in response to this conflict. The greater the difference in fitness optima the more likely that sexually antagonistic coevolution will result. Traits may continually evolve; adaptation following counter-adaptation in what can be considered an evolutionary arms race (Rice & Holland 1997; Holland & Rice 1998; Gavrilets et al. 2001; Arnqvist & Rowe 2005). Sexually antagonistic coevolution can explain observations of uncooperative reproductive behaviour and the actions of individuals that may harm or damage sexual partners (Arnqvist & Rowe 2005). A further consequence of this process is an increase in the probability of reproductive incompatibility between populations that may lead to speciation (Parker & Partridge 1998; Arnqvist et al. 2000; Gavrilets 2000; Martin & Hosken 2003a).

As sexual conflict occurs following the divergence of individual interests it should be absent only when the reproductive interests of the sexes converge. This may only occur in cases of obligate lifelong monogamy where there is no chance of future reproduction if one partner should expire (Wedell et al. 2006). Under this restriction the potential for sexual conflict can be considered the rule rather than exception. Actual sexual conflict should then arise whenever conditions allow for the evolution of conflicting traits that increase individual fitness (Tregenza et al. 2006; Parker 2006).

The incidence of actual sexual conflict is dependent upon the benefits of manipulation relative to the costs of manipulation and appropriate genetic variance providing the opportunity for conflict to evolve (Chapman 2006). De-escalation or resolution of actual conflict may arise whenever the cost-benefit ratio or opportunity for conflict is no longer present. For example, conflicts may be resolved by sex limitation of conflict traits (for intra-locus conflicts), genomic imprinting or insensitivity of female mating preferences (Rice 1984; Chapman 2006; Rowe et al. 2005).

1.2 Empirical Examples of Sexual Conflict

Between October 2004 and October 2007, during the preparation of this thesis, a total of 286 original research articles and review papers have been published that use the term “sexual conflict”. This is an increase of 122, or 74%, on the preceding three years. (Thomson ISI Science Citation Index Database search on 14/11/2007 for the phrase “sexual conflict” in title, abstract or keywords.) Though a crude measure of the amount of work carried out in this field, these figures nevertheless highlight an increasing interest in sexual conflict that coincides with the publication of a book devoted to the subject (Arnqvist & Rowe 2005). Empirical research includes the study of a number of established model species but an increasing number of novel observations are now also being attributed to sexual conflict in a wide variety of organisms. The following section is intended to provide a journey through the diversity of sexual conflict in both well established and emergent invertebrate examples, with a particular focus on more recent work.

A commonly occurring conflict occurs over the frequency of mating, males often benefiting from having a higher number of mates than females. In the damselfly, *Ischnura elegans*, males attempt to mate with many females (Cordero et al. 1997). Though males are monomorphic, females occur as one of three different colour morphs including an androchrome, or male mimic, morph (Svensson et al. 2005). One explanation for the maintenance of female polymorphism is a conflict over mating rate. As males generate a search image directed towards the most common morph, the reduction in female fitness resulting from multiple mating becomes proportionate to the frequency of each morph in a population (Svensson et al. 2005). Female polymorphism then represents an adaptation to reduce the costs of multiple mating. The frequency of female morphs fluctuates between successive years, the cost of sexual conflict effectively preventing fixation of any single polymorphism (Svensson & Abbot 2005).

Multiple mating is not necessarily of disadvantage to females. For example females may benefit from increased sperm competition, genetic diversity of offspring or receipt of nuptial gifts. In such circumstances females may be at a disadvantage from male adaptations that reduce female remating. Accessory substances passed in the seminal fluid from male house flies, *Musca domestica*, are found to induce non-receptivity of females yet simultaneously have a nutritive effect to enhance female fitness (Arnqvist & Andres 2006). In this scenario, conflict over mating rate occurs as females would benefit from multiple mating and adaptations to inhibit accessory substances are expected to evolve.

The yellow dung fly, *Scatophaga stercoraria*, is a naturally polygamous species that is now a widely studied model of sexual conflict. Larval development takes place in cow dung which also serves as the arena for mating interactions. Males quickly gather around fresh deposits and attempt to mate with females as they arrive to oviposit (Parker 1970a, b). Dependent on the density, males either establish territories or there is scramble competition for possession of females (Borgia 1980, 1982). Once formed other males may try to disrupt pairings, which is normally successful if the attacking male is larger (Parker 1970b, c). Copulation takes place prior to oviposition and the last male to mate fathers approximately 80% of offspring (Parker 1970d; Ward 2000).

When females arrive at dung the majority are non-virgin and have sufficient supplies of sperm to fertilise the clutch of eggs. The majority of copulations are therefore superfluous (Parker 1970e; Parker et al. 1993). In response to repeated male mating attempts females will often perform a rejection response, shaking from side to side and up and down to try and dislodge males. There is sexual dimorphism; males being larger than females, so that females appear to have little choice but to mate multiply (Borgia 1981; Jann et al. 2000; Parker 1970f). Sexual selection for male size results not only from a greater ability to resist female rejection, but as large males are better able to exclude other males, disrupt mating pairs and search for mates (Parker 1970e; Borgia 1981).

Multiple mating is costly for female *S. stercoraria*, evident in reduced longevity (Hosken et al. 2002a). Further, females evolving under polyandry have lower fitness and longevity than their monogamous counterparts (Hosken 2001; Martin et al. 2003). This suggests that polyandry is not the result of female choice that may have evolved through benefits of multiple mating, but a result of sexual conflict over mating rate. Adaptive female responses consistent with sexually antagonistic coevolution have been reported. For example, the arrival of females at fresh deposits of dung occurs after males and is the result of an evolutionarily stable strategy balancing the advantages of a better oviposition site and costs of increased competition for copulations (Reuter et al. 1998). In more recent work it has been shown that levels of sexual conflict in the species are dependent upon intrinsic and extrinsic factors affecting individual females. Female resistance to mate is greatest at an intermediate age and when subjected to prolonged food limitation (Blanckenhorn et al. 2007).

Multiple mating inevitably provides a greater capacity for sperm competition. Perhaps the simplest male adaptation in response to increase sperm competition is an increase in investment in sperm production. Numerical sperm competition is evident in *S. stercoraria* as lines reared under polyandrous conditions develop larger testes (Hosken et al. 2001). A prediction of sexually antagonistic coevolution is that populations reared allopatrically will develop different sets of resistance and persistence traits. Males should then be more successful in sperm competition with females from allopatric populations that have not been able to evolve resistance traits (Hosken et al. 2002b). This is distinct from mate choice theory which predicts that females will prefer males with which they have coevolved (Andersson 1994). The former has been demonstrated in *S. stercoraria* as females evolved greater resistance to sympatric but not allopatric males (Hosken et al. 2002b).

A similar mating system to that of *S. stercoraria* is observed in another dung fly, *Sepsis cynipsea*, also a comprehensively studied example of sexual conflict. Male and female *S. cynipsea* arrive soon after cow dung is deposited and females are promptly mounted by males. Copulation does not take place immediately; instead there is a period of pre-copulatory guarding (Parker 1972a, b). Males remain mounted, defending the female from other males whilst she oviposits using sperm from a previous mating. This pattern of behaviour may be explained as males transfer a spermatophore that is likely to require the bursa copulatrix to be free of eggs (Schulz 1999).

Once oviposition has taken place pairs move into the surrounding grass. Copulation ensues in 40% of cases but for the remaining 60% of pairs the male is dislodged by female shaking (Parker 1972a, b; Ward 1983; Ward et al. 1992). The rejection response is vigorous, can last up to 20 minutes and is universally observed, even among virgin females. (Parker 1972a, b). In contrast to *S. stercoraria*, male *S. cynipsea* are smaller than females (Parker 1972b). Females have a corresponding increase in control over copulation, selecting for persistent rather than large males (Ward 1983; Ward et al. 1992). Nevertheless, large males are selected as they are more likely to remain mounted during female rejection, are more likely to depose smaller paired males and are more successful at mate searching (Ward 1983; Blanckenhorn et al. 1999, 2000, 2004). Female reluctance to mate is found to increase with the density of males; indicative of convenience polyandry rather than a mechanism of female mate choice (Blanckenhorn et al. 2000).

Consistent with observations in *S. stercoraria* female reluctance to mate is driven by high costs of multiple mating. Female longevity is reduced following copulation, in part due to damage caused by spines on the male aedeagus (Blanckenhorn et al. 2002; Martin & Hosken 2004). These spines may be an adaptation to prolong copula duration (Blanckenhorn et al. 2002) which is found not to be under female control (Ward et al. 1992). In contrast, the fitness costs of repelling males are of relatively little consequence (Muhlhauser & Blanckenhorn 2002). Enforcing monogamy in this species leads to the evolution of benign males and less resistant females (Martin & Hosken 2003b). However, under monogamous conditions female fecundity is also reduced. Despite longevity costs of polyandry reproductive rates remain the same. It is suggested that females increase their reproductive rate in response to male harm, so that overall there is no net effect of multiple mating on female fitness when measured as the number of offspring produced (Martin & Hosken 2003b).

The most recently reported work on this mating system has examined factors that may influence female willingness to mate and explain variation in mating behaviour. Female receptivity is strongly dependent upon the stage in the egg-laying cycle rather than the age of the female (Teuschl & Blanckenhorn 2005). Females are more willing to mate when they have no ripe eggs; a prerequisite for spermatophore transfer. Non-virgins are also less receptive than virgins. This work highlights the need to account for such factors when conducting studies of sexual conflict.

As a model organism *S. cynipsea* has contributed to debate over the role of sexual conflict in speciation. Populations reared at high density, with a corresponding high level of sexual conflict, are found to have a greater increase in behavioural reproductive isolation when compared to populations at low density or under enforced monogamy (Martin & Hosken 2003b). This is in contrast to theory suggesting that reproductive isolation should evolve fastest between small isolated populations. Furthermore, males evolving under sexual conflict in unfamiliar populations incur greater costs to females, reinforcing reproductive isolation (Martin & Hosken 2004).

Though it is predicted that males should benefit from mating multiply it is implicit that this is with different females. The Australian scaly cricket, *Ornebius aperta*, presents a quandary to expectations as males are found to mate multiply, sometimes in excess of 50 times, with the same partner (Andrade & Mason 2000). In this species it is suggested that males benefit from a numerical advantage during sperm competition, favouring the transfer of high numbers of sperm (Laird et al. 2004). However, following the transfer of a spermatophore this is ingested by the female within just a few seconds (Andrade & Mason 2000). If the male were to transfer a large spermatophore this would not only be costly to produce but only a small proportion of sperm would be transferred before consumption. Instead, each spermatophore is small and only contains the few sperm that may realistically be passed within such a short period of time (Laired et al. 2004). By this mechanism males are able to maximise the number of sperm transferred to each female at minimal cost of spermatophore production.

Sexual conflict can be identified through the costs incurred by one sex upon the other. In contrast to the provisioning of nuptial gifts, in some invertebrate species males steal food resources from mates (Arnqvist et al. 2006). Though beneficial to males this is clearly disadvantageous to female prospects and adaptations to reduce these costs are predicted. In Zeus bugs, *Phoreticovelia* spp. females have resolved this by providing sustenance to males through a dorsal gland (Arnqvist et al. 2006). Though costly to the female this is found to reduce instances of kleptoparasitism, resulting in a net benefit.

A particularly well studied example of male imposed harm is the seed beetle *Callosobruchus maculatus*. In this species male genitalia are adorned with sclerotized spines that deliver severe damage to females during copulation (Crudgington & Siva-Jothy 2000). There is also strong evidence to suggest that seminal substances transferred by the male are toxic to females (Das et al. 1980; Eady et al. 2007). To reduce the costs of mating females perform a rejection response that reduces mating frequency (Crudgington & Siva-Jothy 2000). To add to the female's dilemma, males also transfer a substantial ejaculate that is of nutritional value. Dependent upon requirements dictated by the local environment females may benefit from multiple mating and multiple fitness peaks have been identified (Arnqvist et al. 2005).

The role of male induced harm during mating has been the subject of debate (Johnstone & Keller 2000; Morrow et al. 2003; Edvardsson & Tregenza 2005). In *C. maculatus* there is no evidence that males should benefit from harming females. Females that are harmed neither increase their oviposition rate nor delay remating (Edvardsson & Tregenza 2005). Alternatively, genital spines are more likely to have evolved as a mechanism for males to prolong copulation and the damage caused is a pleiotropic effect of this adaptation (Edvardsson & Tregenza 2005). Interestingly there does not appear to be conflict over copulation duration, most likely as females receive substantial ejaculates, but instead spiny genitalia may have evolved to prevent premature interruption of copulation by other males (Edvardsson & Canal 2006).

Sexually antagonistic coevolution in bruchid beetles has been investigated most recently in a comparative study of seven closely related species. It is shown that an increase in antagonistic adaptations by males (spinier genitalia) can be correlated with a physiological counter-adaptation in females (an increase in the amount of connective tissue in the copulatory duct; Rönn et al. 2007). Where there is an imbalance of armaments in favour of the male then costs of mating to the female and population fitness are reduced (Rönn et al. 2007). This study neatly identifies populations at different points of fluctuation in a coevolving arms race.

It is not only males that harm females through sexual conflict but the reverse is also observed. Females can indirectly harm males by mounting an immune response to sperm; which may simultaneously reduce the incidence of sexually transmitted diseases. It has been shown in the ground cricket, *Allonemobious socius*, that male sperm contents interrupt the immune response cascade of females (Fedorka & Zuk 2005). Interference with the female immune response may contribute to increased mortality and lower fecundity. Though not in the long term interest of females this is likely to benefit males that are only interested in the short term reproductive output of females shortly after copulation.

A more direct example of female induced harm, and perhaps the ultimate manifestation of sexual conflict, is sexual cannibalism (Partridge & Hurst 1998). Sexual cannibalism is most commonly observed in a number of species of spider (Elgar 1992).

Killing a male before copulation is clearly only of benefit to the female, yet killing the male after copulation could benefit the male if this increases paternal success. In an extreme example male redback spiders *Latrodectus hasselti*, actively position themselves over the female jaws. Male suicide in this case appears an adaptive response as males that are cannibalized copulate for longer, fertilise more eggs and females are subsequently less willing to remate (Andrade 1996).

If cannibalism, when compared to future mating prospects, does not benefit males then male traits to avoid consumption are predicted to evolve. For example, males of the species *Nephila fenestrata* mate opportunistically whilst females are distracted by feeding (Fromhage & Schneider 2005). Traits to avoid cannibalism may include those normally seen in the avoidance of predators. In the nuptial gift giving spider *Pisaura mirabilis* males feign death (thanatosis) to avoid the attention of a female as she approaches a nuptial gift (Bilde et al. 2006). Whilst distracted by this gift, the male will then attempt to copulate. Males using a combination of thanatosis and provision of a nuptial gift are more successful in achieving copulations. Sexual cannibalism is also observed in mantids. To avoid consumption male preying mantids, *Tenodera aridifolia sinensis*, adopt a risk avoidance strategy in their behaviour towards females (Lelito & Brown 2006). If females are hungrier, in better reach or in better view of the male then males will move more slowly and court with greater intensity.

The examples described above illustrate the wide variety of species and arenas in which sexual conflict is found. As a final example it is interesting to consider sexual conflict in a non-diploid system. In the little fire ant, *Wasmannia auropunctata*, sterile workers are produced sexually and reproductive queens clonally (Fournier et al. 2005).

This system of reproduction is clearly of benefit to females as the genome is passed to the next generation without recombination. At the same time females still benefit from a diverse workforce more resistant to disease, parasites and variable environmental conditions. It is then not surprising to discover that males also reproduce clonally, thought to be through paternal elimination of the maternal genome in the egg (Fournier et al. 2005). There is effectively no recombination between male and female gene pools - a step towards the resolution of sexual conflict or further escalation?

1.3 *C. frigida* – a model for the study of sexual conflict

Coelopa frigida (Fabricius) is a species of acalyptrate fly taxonomically located within a small family of which the majority are found in Australasia (Order: Diptera, Superfamily: Sciomyzoidea, Family: Coelopidae; Meier & Wiegmann 2002). *Coelopa frigida* is commonly found on the coastlines of Northern Europe as far north as the Arctic Ocean. A second species of coelopid, *Coelopa pilipes*, is also found on the coasts of Europe. The distribution and relative abundance of European coelopids has been widely studied (Egglishaw, 1960; Dobson, 1974a; Butlin, 1983; Phillips et al. 1995a). *Coelopa frigida* occupies higher latitudes ranging from the north coast of France as far north as Iceland and Spitzbergen. The range of *C. pilipes* extends further south down the Atlantic coastline of France and north only so far as the Scottish mainland. *Coelopa pilipes* is notably absent from the Western and Northern Isles of Scotland. On mainland Europe *C. pilipes* has been recorded as far north as the west coast of Sweden though only very rarely at greater abundances than *C. frigida*. Though both species occur sympatrically throughout much of their range within British wrack beds *C. frigida* has been described as the most abundant large Dipteran (Egglishaw 1960).

Coelopa frigida, in common with all coelopids, inhabits 'wrack beds'; accumulations of detached and decomposing seaweed deposited on the sea shore following spring tides and stormy weather. Wrack beds may remain for some time depending on local conditions, though typically they persist for 4 weeks between spring tides. The coelopid life cycle is therefore strongly associated with tidal patterns (Dobson 1974a). The presence and persistence of wrack beds may also depend on a number of other local factors such as the amount of seaweed growing offshore, the aspect and gradient of the beach and prevailing weather conditions (Dobson 1974a).

Adult *C. frigida* are attracted to wrack beds shortly after deposition, sometimes within just a few hours (Dobson 1974a). Female oviposition rate and male mating activity both increase in the presence of algae (Dunn et al. 2002). Female coelopids oviposit directly onto the decomposing seaweed; algae inducing females to lay more eggs and at a higher rate (Dunn et al. 2002). Larvae hatch within approximately 24 hours and graze on microorganisms that decompose seaweed, utilising the ability of microbes to digest algal cell walls, releasing sugars (Cullen et al. 1987). The wrack bed microfauna have been identified as derived from the marine microfauna and not introduced by adult coelopids (Cullen et al. 1987). The activity of *C. frigida* larvae have a facultative effect on bacterial numbers, rapidly increasing decomposition (Cullen et al. 1987; Leggett 1993). Decomposition creates a hot and humid microclimate within the wrack bed; temperatures in excess of 40°C being recorded and coelopids found inhabiting wrack beds despite a covering of snow (Dobson 1974a; Phillips et al. 1995a). Such rapid decomposition leads to the development of an anaerobic layer at the bottom of the wrack bed, in which larvae are not found (Dobson 1974a). Having acquired sufficient resources, coelopid larvae pupate in drier parts of the wrack bed (Dobson

1974a). The onset of pupation is affected by a combination of the development time and the size of larvae (Butlin & Day 1984). Larval and pupal life stages are completed entirely within the wrack bed with development lasting approximately two weeks from oviposition to eclosion (Dobson, 1974a). Dependence on wrack beds for reproduction dictates a littoral distribution for coelopids, nevertheless adult coelopids have been recorded to migrate over large distances and may occasionally be found many miles inland (Oldroyd 1954; Egglishaw 1961; Dobson 1974a, 1974b).

The entire life cycle of *C. frigida* may be completed within 9 days under laboratory conditions (Chapter 6), though observations in the wild show that offspring eclose 2-3 weeks following oviposition (Dobson 1974a). Adult longevity has also been estimated at 2-3 weeks under natural conditions (Dobson 1974a) though this may be extended to an excess of 7 weeks when housed in the absence of seaweed (Meader & Gilburn, in press). Within each tidal cycle it is unlikely that more than one generation of flies will develop due to temporal limitations of the life cycle and depletion of nutritional resources (Dobson 1974a). At the extremes of wrack bed longevity deposits may persist from just a few hours up to 83 days, occasionally new wrack beds being deposited above earlier remains (Dobson 1974a). Stability of local conditions has considerable effect upon the success of individual *C. frigida* (Dobson 1974a). As conditions become increasingly unpredictable flies that develop faster, have greater longevity or greater ability to migrate will be more successful.

1.4 Chromosomal inversion system

Chromosomal inversions are commonly found in insects and have provided an important focus for studying the genetic basis of evolutionary mechanisms (Hoffman et al. 2004). In *C. frigida* approximately 25% of chromosomal bands are sited within inversions. This includes a large inversion system on chromosome 1 that accounts for about 10% of the genome (Aziz 1975). This same inversion is also apparently present in it's sibling species, *Coelopa nebularum* (Dunn et al. 1999; Crean et al. 2000).

The inversion on chromosome 1 occurs in two forms, α and β, with recombination completely suppressed (Butlin et al. 1982a; Day et al. 1982). Original interest in this particular inversion arose due to widespread and relatively conserved frequencies of each form, suggesting strong selection pressures (Butlin et al. 1982a). Determination of inversion karyotype in *C. frigida* can be inferred through linkage disequilibrium with allozymes of the enzyme alcohol dehydrogenase (Adh) (Day et al. 1980; Day et al. 1982). Three Adh allozymes are commonly found in British populations, Adh-B, Adh-C and Adh-D, each present at frequencies greater than 5%. Two further alleles, Adh-A and Adh-E, also occur though at much lower frequencies (Day et al. 1980). The two most common Adh allozymes, Adh-B and Adh-D are in complete linkage disequilibrium with the α and β forms of the inversion respectively (Day et al. 1982). Adh allozymes can be identified by starch gel electrophoresis (Chapter 2).

A common observation in both Scandinavian and British populations is heterosis. This is believed to be the primary mechanism maintaining multiple forms of the inversion (Butlin et al. 1982b; Day et al. 1983; Butlin & Day 1989; Gilburn & Day 1996). Heterosis can be explained through enhanced egg to adult viability and competitive superiority (Collins 1978; Butlin et al.1984; Leggett et al. 1996). A significant difference is observed in the development times of flies dependent upon the inversion system, particularly amongst males (Day et al. 1980). *αα*-homokaryotypes have the longest development time, *ββ*-homokaryotypes the shortest and heterokaryotypes intermediate. The genotypes of newly eclosing flies consequently vary over time at each wrack bed.

Differences in development time are mirrored in adult size; *αα*-homokaryotypes being largest, *ββ*-homokaryotypes smallest and heterokaryotypes of intermediate size (Butlin et al. 1982b; Day et al. 1982; Butlin & Day 1985). Patterns of size and development time are observed in both sexes, but these differences are much more pronounced in males (Butlin & Day 1984). A positive association is found between larval density and the relative difference in size between different karyotypes (Butlin & Day 1984; Gilburn & Day 1994a) and heterokaryotype excess is greater at high larval densities (Leggett et al. 1996, Butlin & Day 1984).

The relationship between inversion karyotype and adult size is important as large size often confers an advantage in *C. frigida*. Large males have an increased mating advantage (Butlin et al. 1982a), larger flies live longer (Butlin & Day 1985; though see Chapter 8) and female fecundity increases with size (Butlin & Day 1985). In a natural population, wing lengths of *αα*-homokaryotype were 7% and 25% longer than *ββ*-homokaryotypes for females and males respectively (Butlin & Day 1985). This size advantage could equate to an increase in longevity of about 0.5 days for females and an increase in total egg production of 25% (Butlin & Day 1985). *αα*-homokaryotype males are predicted to live twice as long as *ββ*-homokaryotype males, potentially doubling their reproductive opportunities (Butlin & Day 1985). Alternatively, *ββ*-homokaryotypes may benefit from a shorter development time where wrack beds are short lived and faster development will give earlier access to females (Butlin et al. 1982b).

The *αβ* inversion system of *C. frigida* is fundamental to individual life history, though this may be dependent upon local conditions. Different patterns of development dictated by the inversion system suggest that karyotype frequencies should vary geographically according to selection pressures. Contrary to predictions there is little temporal or spatial variation in karyotype frequencies (Day et al. 1980; Day et al. 1983; Butlin & Day 1989). Karyotype frequencies are found to be stable around the English coast despite sampling at sites anticipated to have little gene flow between them (Butlin et al. 1982b). Karyotype frequencies of Scandinavian populations are also similar to those found in Britain (Day et al. 1983; MacDonald & Brookfield 2002). In the absence of geographical barriers Scandinavian and British populations of *C. frigida* are able to interbreed, yet these populations are genetically distinct and there is thought to be little gene flow (MacDonald & Brookfield 2002). Adh-C is relatively common in Britain yet rarely identified in Scandinavian flies (Day et al. 1983). Maintaining karyotype frequencies in the absence of genetic exchange suggests similar selective pressures acting over a wide geographical area.

1.5 Reproduction in *C. frigida*

1.5.1 Mating behaviour

The mating behaviour of *Coelopa frigida* has been described in great detail by Day et al. (1990) using video recordings and ablation/masking techniques to identify the role of different body structures. Following attraction to wrack beds the presence of algae encourages males to mate (Dunn et al. 2002). When flies are more than a couple of centimetres apart there is no apparent interaction, individuals moving with no obvious recognition of each other (Crocker & Day 1987; Day et al. 1990). As flies pass in close proximity the male may approach and mount the female. Some form of sexual recognition before contact is apparent as males are 20 times more likely to mount a female than another male. On the rare occasion that another male is mounted this is quickly followed by a dismount with no apparent rejection or struggle. Male coelopids can be extremely promiscuous, attempting to mate as often as every 8.4 minutes under the right conditions (Blyth & Gilburn 2006). Once mounted the male places his prothoracic legs over the front of the females head and in contact with her antennae. Male mesothoracic and metathoracic legs are wrapped around the thorax and abdomen of the female.

Females appear reluctant to mate and elicit a variety of responses to dislodge the male. Day et al. (1990) proposed that female rejection may be a method of distinguishing a mate from the myriad of other encounters made with the environment. Rejecting all advances until the correct stimulus is received would increase the chance of mating with the correct species whilst avoiding other costly encounters. Female rejection of the male involves three principle actions – downward curling of the abdomen to prevent genital contact, kicking of the metathoracic legs (sometimes accompanied by flicking of the wings) and shaking from side to side. The presence of seaweed induces oviposition in females, but otherwise does not influence female mating behaviour, a consistent rejection response being maintained (Dunn et al. 2002). The female response varies in other coelopid species. In *C. nebularum* the majority of females adopt a single rejection response, whilst only about a third performs two or more behaviours. Shaking, kicking and downward abdominal curling are all used in similar proportions; 48% of females used shaking, 46% kicking and 40-43% downward abdominal curling (Weall & Gilburn 2000). The type of rejection response exhibited by *C. nebularum* females depends in part upon the size of the male. Kicking is more often used against small males, possibly as they are easier to dislodge, whilst downward abdominal curling is used in response to larger males (Weall & Gilburn 2000). *Coelopa pilipes* and seaweed flies of the genus *Gluma* also respond to male mating attempts with upward abdominal curling. It is suggested that this may be an adaptation to assess the size of males (Crean et al. 2000).

To confirm the role of various body structures during mating Day et al. (1990) amputated female antennae, female metathoracic legs and the wings of both males and females in addition to coating female antennae and male prothoracic legs with enamel paint. The amputation of either male or female wings had no effect on mating behaviour, suggesting that wing flicking is not an important part of the female rejection response. Removal of the female metathoracic legs increased the mating success of pairs, demonstrating that kicking with these legs is important. The position of males whilst mating indicated stimulation of the female's antennae; this suggestion is confirmed as mating success was reduced for females with either amputated or masked antennae. Masking of the males prothoracic legs had a similar effect. Electron microscopy of the male prothoracic legs shows a spoon shaped structure that may be be used to stimulate female antennae.

The outcome of a male mating attempt can be classified as either copulation, successful female rejection or voluntary dismount by the male without female rejection. Voluntary dismount by the male is rare; Dunn et al. (1999) did not observe a single male *C. frigida* reject a female in over 200 mate trials. However, it may be difficult to accurately determine if a male has voluntarily dismounted, especially where mounts are brief and the female rejection response is not immediately apparent (personal observation). Voluntary dismounts by males are most often observed after females are 'sex starved' for more than 3 days, making them more willing to mate.

During copulation the female may remain stationary or slowly walk around. The male will often continue to move his prothoracic legs against the female's antennae. Males do not appear to transfer any nutrients with their sperm (Gilburn et al 1992) or provide nuptial gifts and there are no courtship displays prior to mating or contests between males (Day et al. 1990). Though exact mechanisms of fertilisation are unknown, last male sperm precedence has been identified in *C. frigida* (Thompson 1951; Burnet 1961; Blyth & Gilburn 2005). In many dipterans mating can induce oviposition in females (Chapman et al. 1998), though this is not observed in coelopids (Dunn et al. 2002).

1.5.2 Large male mating advantage

It is consistently observed that large male *C. frigida* achieve greater mating success than small male *C. frigida* (Butlin et al. 1982a; Gilburn et al. 1992, 1993, 1996; Gilburn & Day 1994b; Pitafi et al. 1995; Crean 1997; Day & Gilburn 1997; Crean & Gilburn 1998). This is consistent with the male biased sexual size dimorphism seen in *C. frigida* (Gilburn & Day 1994a). Lack of recombination between the two forms of the inversion has likely contributed to dimorphism as size determining genes on each form of the inversion have been free to evolve independently (Day et al. 1982). A large male advantage is not restricted to *C. frigida* but is characteristic of all coelopids studied to date (Crean & Gilburn 1998; Dunn et al. 1999; Crean et al. 2000; Weall & Gilburn

2000). The positive relationship between male size and mating success is thought to plateau, so that above a certain size there is no additional advantage to be gained (Gilburn et al. 1992).

One reason for a large male mating advantage may be physical limitation if simultaneous genital contact and stimulation of female antennae is required (Day & Butlin 1987). Smaller males may be physically limited to a smaller range of female sizes. Mating success is associated with the size ratio of partners though only where differences are extreme (Pitafi et al. 1990). This effect is not sufficient to explain the majority of female mating bias. Alternatively, a large male advantage may be the result of an active female preference. Evidence suggests that mate choice may have both direct and indirect benefits to the female as fertility and progeny survival rate are increased when presented with a choice of males (Crocker & Day 1987).

The large male mating advantage has been of particular interest in *C. frigida* due to the *αβ*-inversion system. A mechanism for the mating bias was initially unclear as female preference could be directed towards either male size or karyotype (Butlin et al. 1982a). It has since been demonstrated that large size is more strongly associated with mating success than inversion karyotype and a mating bias on the basis of karyotype is an indirect consequence of sexual selection on size (Gilburn et al. 1992). Crean et al. (2000) calculated the strength of sexual selection for large male size in 5 species of coelopid: *C. nebularum*, *C. pilipes*, *C. vanduzeei*, *Gluma musgravei* and *Gluma nitida*. *Coelopa nebularum* is believed to have a similar inversion system to the *αβ* inversion system of *C. frigida*, *C. pilipes* is known not to possess the inversion system and the remaining three species do not show any evidence of possessing the inversion system either. Irrespective of the presence or absence of the inversion, all 5 species exhibited a similar female rejection response to that of *C. frigida* and all exhibited a large male mating preference. Thus the apparent preference for large males is exhibited irrespective of the inversion and is likely to have originated in coelopid ancestors before its evolution (Crean et al. 2000).

In considering mating preferences of *C. frigida* observed in the laboratory, it is important to relate this to other life history traits. Smaller males have a shorter development time so are expected to have earlier access to females in the wild (Butlin et al. 1982b). Larger males are also more likely to dismount a female (Dunn et al. 2001) and couple for shorter periods than small males (Pitafi, 1997; Dunn et al. 2001). The extent of the large male advantage observed in laboratory trials may then be reduced in natural populations.

1.5.3 Male mate choice

Male reproductive success is often determined simply as a function of the number of matings achieved. However, if supply of sperm is limited or there are significant costs associated with mating then the capacity of males to mate multiply can be reduced. It has been proposed that female counter adaptations may often be less costly to express than male adaptations to mate (Stockley 1997, Clutton-Brock & Parker 1995). If the availability of females exceeds male mating capacity then male mate choice may be an advantageous strategy to improve reproductive success (Bonduriansky 2001; Wedell et al. 2002).

It is not uncommon for a large proportion of eggs to remain unfertilised following copulation in *C. frigida* (Crocker & Day 1987; Gilburn et al. 1992; Pitafi

1997). Sperm production and mechanisms of sperm utilisation and sperm longevity are currently not understood, so it is unclear whether males may be restricting the amount of sperm passed during copulation or if females are restricting the use of sperm. Males have been found to become reproductively incompetent after repeated matings which may suggest that male mating capacity is limited (Pitafi et al. 1994). Larger males are known to couple for shorter periods, which may be an example of strategic allocation of resources (Pitafi 1997; Dunn et al. 2001). In summary, there is potential for adaptive male mate choice in *C. frigida*.

Pitafi et al. (1990, 1995) found that the frequency of male mating attempts is positively, and male dismount rate negatively, correlated with female size. Dismount rate was found to be greater for less gravid females, leading to the suggestion that males may be assessing the fecundity of females by gauging abdominal distension with their metathoracic legs (Pitafi et al. 1995). Similar assessment of fecundity is suggested in the dryomyzid fly, *Dryomyza anilis* (Otronen 1984). Mating shows a similar positive correlation with female size in another coelopid, *Gluma musgravei* (Crean et al. 2000). However, the evidence for male mate choice in *C. frigida* is far from reaching consensus. A number of studies have found no association between mating success and female size (Gilburn et al. 1992; Day et al. 1987). Following 2000 observations of five different species of coelopid no association between female size and either willingness of males to mount or time taken to mount was found (Dunn et al. 1999).

Another female trait under selection may be longevity. Rather than an association with female size, mating success has been correlated with female mortality in *Gluma musgravei* (Dunn et al. 2001). Indirect advantage may be gained by males as long lived females have a greater opportunity to find suitable oviposition sites (Dunn et al. 2001). Given high promiscuity (Blyth & Gilburn 2006) and last male sperm precedence in *C. frigida* (Blyth & Gilburn 2005) similar male mate choice on the basis of female longevity seems unlikely. Further, the mechanism of assessment of longevity is uncertain but may be through a combination of indirect indicators such as female size or struggle duration (Dunn et al. 2001). Males may instead be selecting healthier individuals of higher fecundity that also happen to live longer.

1.6 Sexual selection in *C. frigida*

Sexual dimorphism in *C. frigida* is the result of sexual selection for large size. Sexual selection can act in opposition to natural selection where the benefits of reproduction exceed those of survival. In the face of this opposition under what circumstances should a mating bias arise, how should a mating bias be maintained and what are the prospects in a changing environment?

1.6.1 Good genes

The ‘good genes’ hypothesis explains the evolution of female choice through indirect benefits gained by the female in rearing genetically fitter offspring (Zahavi 1975; Andersson 1982, 1986; Iwasa et al. 1991; Iwasa & Pomiankowski 1994; Møller & Alatalo 1999). Even if the indirect genetic benefits of mate choice are minor, as long as the costs of expressing a preference are also small then the overall selective pressure may still be significant (Alatalo et al. 1998).

A fundamental prediction of this hypothesis is that female mating preferences will be directed in favour of males possessing genes that confer greater viability. In order for this to be achieved sexually selected traits should be both an accurate and honest indicator of male quality. In order for a male trait to be a reliable indicator of fitness it should be subject to additive genetic variance. Male size in *C. frigida* is determined by both genetic and environmental factors. The inversion system has been shown to play an important role in determining male size and virtually all of the genetic variance in male size can be explained by inversion karyotype (Wilcockson et al. 1995; Day et al. 1996). Inversion karyotype is far less significant in dictating female size, which is primarily the result of environmental stochasticity (Wilcockson et al. 1995; Day et al. 1996). In a wild Scandinavian population of *C. frigida* 25% of the variance in male size can be attributed to the $\alpha\beta$ inversion system (Wilcockson et al. 1995). In a similar study of British flies 7.4% of the variance in male size could be attributed to the $\alpha\beta$ inversion system (Day et al. 1996). It is predicted that there is sufficient additive genetic variation in male size that females could gain an indirect genetic advantage from mate choice based upon this trait (Wilcockson et al. 1995; Day et al. 1996).

Despite potential for good genes sexual selection in *C. frigida* this may be limited. In locations subject to greater environmental uncertainty the contribution of environmental variance to male size is increased (Day et al. 1996). The influence of good genes in maintaining mating preferences may then be environmentally determined. Non-random mating is also observed in closely related species of coelopid that lack the inversion system (Crean et al. 2000), suggesting that good-genes selection on the basis of inversion karyotype is not necessary to maintain this mating bias. Finally, due to heterokaryotype advantage, $\alpha\alpha$-homokaryotype females are not predicted to benefit from mating with large males (Gilburn et al. 1993). To counter this disadvantage, female willingness to mate is found to co-vary with karyotype (Gilburn et al. 1999;

Gilburn & Day 1999). *αα*-homokaryotype females are more willing to mate than *ββ*-homokaryotype females, which could reduce the probability of rejecting *ββ*-karyotype males whilst maintaining a preference (Gilburn & Day 1999).

1.6.2 Fisherian selection

In addition to genetic benefits, females may benefit from the production of attractive male offspring (Fisher 1930; Pomiankowski et al. 1991; Pomiankowski & Iwasa 1998). The Fisherian mechanism for the maintenance of sexual preference is founded upon the development of a genetic association between female sexual preferences and male sexually selected traits (Lande 1981; Kirkpatrick 1982). A genetically based female preference will inevitably co-inherit with genetically based male traits as females exhibiting the preference select males bearing the trait. Though preference and trait may not be simultaneously expressed, all individuals will carry genetic information determining both preference and trait that may be expressed in subsequent generations. Individuals not inheriting the enhanced male trait will be at a reproductive disadvantage as female preferences spread within a population; leading to a self reinforcing runaway process. Only as forces of natural selection condemn costly female preferences and male traits will this process be curtailed (Fisher 1930). Once established, the original cause of non-random mating may prove to be insignificant. A positive genetic correlation between female preference and male traits is predicted by the Fisherian process. This is often difficult to confirm as female preferences and male traits are sex limited.

Because of the inherent attractiveness conferred upon a male trait by any form of female preference the production of attractive male offspring may be considered an inevitable consequence of good genes selection (Kirkpatrick & Ryan 1991; Eshel et al.

2000). Fisherian and ‘good genes’ selection can then be considered two extremes of a continuous sexual selection surface in which the relative benefits of producing attractive and fit offspring is balanced against the costs of mate choice (Kokko 2001; Kokko et al. 2002). Females benefit from mating with males of high reproductive value, whether in terms of attractiveness or survival. When female preference is cheap, a strong mating skew can develop in which the benefit of producing attractive offspring is greater than the benefit of producing offspring with greater survival prospects (Kokko et al. 2002). Though these two mechanisms are not truly independent, the justification to remove the distinction has been questioned (Andersson & Simmons 2006). Central to this debate is the collation of accurate empirical evidence in support of the alternative hypotheses.

Two facets of female mating behaviour have been considered in *C. frigida*; ‘acceptance’ rate, defined as the rate at which a female accepts males, and ‘preference’, a measure of how discriminating females are over male size. Female acceptance rates and preferences are found to be associated with inversion karyotype; *αα*-homokaryotypes are the most accepting and discriminating, *ββ*-homokaryotypes the least accepting and discriminating and heterokaryotypes intermediate (Gilburn et al. 1993; Gilburn & Day 1994b). All females still prefer large males. This demonstrates an association between female preferences and male traits in *C. frigida* as both are elevated in the *α* form of the inversion. The association may be a result of the genes controlling male size and female preference being in linkage disequilibrium as a result of co-segregating with the α form of the inversion or alternatively they may have become correlated as a result of sexual selection (Gilburn et al. 1993). It is predicted that the genes conferring these traits are likely to be found either within the inversion or closely linked to the inversion (Gilburn & Day 1994b). Genetic linkage between female preferences and male sexually selected traits provides support for Fisherian selection in *C. frigida*. Resulting from such preferences there is expected to be an increase in mating between $\alpha\alpha$-homokaryotypes, reducing the number of fit heterokaryotype offspring. The strength of Fisherian selection may then be counterbalanced by forces of natural selection if changing environmental conditions favour heterosis.

1.6.3 Convenience polyandry

Coelopa frigida are known to be highly promiscuous. Males have been observed to mount a female as often as every 8 minutes, suggesting hundreds of sexual partners within the lifetime of each female and multiple paternity of egg clutches (Day et al. 1987; Blyth & Gilburn 2006). The extent of polyandry is highly likely to exceed the optimal mating rate of females, as sufficient sperm may be obtained in only a few matings. Multiple mating is more likely to reflect maximisation of male reproductive success. Sexual conflict over mating rate in this species leads to an alternative explanation for the large male mating advantage. The female reluctance hypothesis proposes that females will attempt to reject superfluous male mating attempts in order to avoid the costs of multiple mating. Convenience polyandry can be defined as the process of accepting a male when the cost of rejection is higher than the cost of mating (Rowe 1992; Crean & Gilburn 1998; Thornhill & Alcock 1983). In species where females are subject to a high frequency of mating attempts this will carry a cost in terms of increased predation risk and reduced foraging capacity as well as the physical exertion of mating (Crean & Gilburn 1998). Any bias in a male trait that improves ability to resist rejection may indirectly be selected. The apparent rejection of males by female *C. frigida* may be an example of convenience polyandry, with passive preference of large mates occurring as these males are more capable of enduring and overcoming this response. However, distinguishing between active and passive female preferences is not straightforward (Baena & Eberhard 2007).

Significant evidence in support of convenience polyandry driving sexual selection in *C. frigida* has come from studies of other coelopids. In *C. ursina* males achieve more matings with small females, duration of struggling is correlated with male size and struggles that lead to copulation are longer than those that result in rejection (Crean & Gilburn 1998). If the female rejection response were a method of direct male assessment then shorter struggle durations would be expected for larger males and struggles ending in copulation. Having statistically removed variation in mating success due to the duration of the pre-mating struggle, there was no association between male size and mating success, suggesting that the large male advantage in *C. ursina* is due to the ability of large males to withstand the rejection response for longer (Crean & Gilburn 1998).

In *C. nebularum* mate assessment can also been discounted (Weall & Gilburn 2000). Previous female experience has no effect on subsequent rejection response or likelihood of acceptance. If females are assessing males using a 'best of *n*' approach then previous experience would be expected to influence future decisions. There is no consistent rejection response observed in *C. nebularum*, with different proportions of kicking, shaking and abdominal curling being observed. If females are assessing males according to a threshold level then similar mechanisms of assessment would be expected. Finally, copulation duration is independent of either male or female size. If females are actively choosing large males then a positive relationship between copulation duration and male size would be expected. Larger male *C. nebularum* took longer to reject than small males, further confirming that large males gain an advantage as they are able to withstand the rejection response for longer.

Analogous to the disadvantages of Fisherian selection, $\alpha\alpha$-homokaryotype males are more likely to be mated as a result of convenience polyandry. The net benefits of the female rejection response may then vary as environmental conditions favour heterosis.

1.6.4 Multiple selection processes acting in *C. frigida*

Three models are presented for maintenance of sexual selection in *C. frigida*; good genes, Fisherian and convenience polyandry. These mechanisms are by no means mutually exclusive and evidence suggests that environmental variance may influence the role of each of these processes. Convenience polyandry is likely to be an adaptive response of females to multiple mating but may have been easily reinforced by Fisherian selection as female preference and male trait co-segregated with the inversion system. However any runaway process would be limited by the production of unfit homokaryotypes, the relative fitness of which is dependent upon environmental conditions.

Tidal range is one environmental variable that may influence the relative benefits of convenience polyandry, good genes and Fisherian selection. Greater tidal range results in greater instability of the wrack bed resource and may be associated with different species of marine algae and larval densities (Gilburn & Day 1994c). The cost of producing less fit offspring will be highest where wrack beds are less consistent and $\alpha\alpha$-homokaryotypes have a reduced chance of developing to adulthood. Heterokaryotype advantage may then be increased on beaches with greater tidal range.

Where wrack beds persist for longer there may be an increased advantage in production of more attractive *αα*-homokaryotypes offspring.

In natural populations an association is found between high tidal range and fitness of *αα*-homokaryotype females, but not *ββ*-homokaryotype and heterokaryotype females (Gilburn & Day 1994c; Gilburn et al. 1996). In these populations *αα*-homokaryotype females are found to prefer small males, mate choice that tends towards a good genes model rather than Fisherian sexual selection (Gilburn & Day 1994c). A theoretical model has further shown that the strength of heterokaryotype advantage can influence the processes of either Fisherian or good genes selection (Gilburn & Day 1996). As long as strength of preference for large males is high this can balance effects of heterokaryotype advantage through production of attractive male offspring.

1.7 The water striders – a related mating system

A similar mating system to *C. frigida* is found in the water striders, gerridae. There is no prior courtship before a male grasps a female in an attempt to mate. Females appear reluctant to mate and try to dislodge males with a backward somersaulting motion (Arnqvist 1989; Rowe 1992; Weigensberg & Fairbairn 1994). Males of some species of water strider have abdominal grasping apparatus that improves grip on the female during this process (Arnqvist 1989; Rowe et al. 1994). About 80-85% of male attempts prove unsuccessful (Arnqvist 1989). For the remainder, copulation is followed by a period of mate guarding, which may be of considerable duration and during which the male rides passively on the back of the female (Arnqvist 1989, Weigensberg & Fairbairn 1994). Female *Gerris odontogaster* are capable of storing sperm for up to 10 days and there is no apparent advantage of multiple mating in this species (Arnqvist

1989). With the exception of providing sperm, paternal investment in offspring is minimal (Arnqvist 1989). The observed frequency of mating is therefore far higher than the expected female optima.

In addition to selection for male abdominal graspers there is a large male mating advantage in gerrids (Rowe et al. 1994; Danielsson 2001). As with *C. frigida*, the primary question in relation to this mating system is whether the female rejection response represents a method of adaptive mate choice or a reluctance to mate multiply that leads to a passive preference. By altering the sex ratio in gerrid populations it is possible to artificially influence the level of male harassment and test predictions of the female response. The average number of somersaults decreases as harassment levels increase, suggesting that mating and the rejection response is costly (Arnqvist 1992). If females are conducting mate assessment it would be expected that female choice should increase with the availability of males.

The extent of female reluctance to mate may be determined by the relative costs of multiple mating, resisting male advances and the possibility of not achieving sufficient copulations. The costs of multiple mating to females are varied and may include increased predation risk, time and energy costs, risk of injury and risk of disease or parasite transmission (Arnqvist 1989; Stockley 1997). The advantages of mating multiply may include replenishing stores of sperm, increasing the genetic variability of offspring, to receive nutrients or other paternal investments from the male or to receive protection from the male (Arnqvist 1989). The cost of mating to female *Aquarius remigis* has been partly quantified. Females utilise about 25% more energy carrying the weight of a male and 126% more energy struggling with a male than when conducting normal skating behaviour (Watson et al. 1998). Females kept in the presence of males were found to live for shorter periods than those kept without males (Arnqvist 1989). In contrast, the cost of not achieving copulation in this mating system appears insignificant due to the frequency of male mating attempts.

Costs of mating can be variable and the response of females is found to vary dependent upon environmental conditions. There are many environmental factors that influence the level of sexual conflict in the gerridae: food availability, habitat, population density, sex ratio and predation (Rowe et al. 1994). Local populations will be subjected to different conditions and so different regimes of sexual selection can occur on both spatial and temporal scales (Rowe et al. 1994). When food availability is high it is more likely that a female has eaten recently and can more easily locate prey in the future. Under these conditions females are found to be more willing to mate (Rowe 1992). The costs of mating appears to be reduced when prey is abundant and so a rejection response is no longer cost effective (Rowe 1992).

1.8 Interactions between *C. frigida* & *C. pilipes*

Coelopa frigida and *Coelopa pilipes* lead ostensibly identical lives within a limited resource environment and are found to be sympatric throughout much of their range. Interactions with *C. pilipes* are likely to be of significant consequence to *C. frigida*. Both conspecific and heterospecific interactions within high density populations are known to influence the success of individual coelopids (Phillips et al. 1995a; Leggett et al. 1996). The mechanism of competition is most likely purely exploitative, or ‘scramble’, competition. As a higher number of larvae compete for the same resource less food becomes available and so individual larvae find it more difficult to attain an optimum size within the same period. This results in longer development times and pupation at a smaller size (Phillips et al.. 1995a; Hodge & Arthur 1997). Whilst interference competition, either physical or chemical, has been proposed (Butlin & Day 1984) there is no supporting evidence. Predatory behaviour between larvae has never been observed and exchanging liquid extracts between cultures has no significant effect on larval development (Hodge & Arthur 1997). Interestingly, at low densities below approximately 1 larvae per gram of seaweed, there is evidence of facilitation (both inter and intra specific). The decomposition of seaweed is slower without coelopids and it is proposed that the churning action of larvae assists the increase in bacterial numbers (Cullen et al. 1987; Leggett et al. 1996; Hodge & Arthur 1997). Heterokaryotype excess is positively correlated with density; the steepest increase in number of heterokaryotypes corresponding with competitive rather facilitative interaction (Butlin & Day 1984; Leggett et al. 1996). Competition or facilitation between larvae is therefore density dependent (Leggett et al. 1996).

To maximise individual success it is predicted that different species of coelopid will evolve adaptations to minimise the effects of competition. This can be achieved by utilising resources in different ways to create either a spatial or temporal separation. *C. frigida* and *C. pilipes* adults arrive on wrack beds at about the same time, though average development times are 11-13 days and 15-17 days respectively (Dobson 1974b). This difference in development time may represent temporal separation as an adaptation to competition. This separation may be the result of slower development, delayed mating or delayed oviposition. Indeed, *C. pilipes* females are known to oviposit at a reduced frequency than *C. frigida* (Leggett et al. 1996). The longer development time of *C. pilipes* may be disadvantageous where wrack beds are short-lived, though *C.*

pilipes may benefit from increased bacterial growth after preparation of the wrack bed by *C. frigida* larvae.

C. frigida larvae are reported to be competitively superior to *C. pilipes* (Hodge & Arthur 1997). However, this interaction is dependent on a variety of environmental conditions such as temperature, algal species and larval density. When cultured together under either warmer or colder conditions, both species are more susceptible to competition when at a temperature to which it is less suited (Phillips et al. 1995a). Superficial observation reports larvae of the two species found spread evenly throughout wrack beds (Dobson 1974b), though closer examination has shown a microdistribution dependent upon temperature (Phillips et al. 1995a). *C. frigida* larvae are found to migrate to colder areas whilst *C. pilipes* are more evenly spread; which may be an indication that *C. frigida* is the more temperature dependent of the two species (Phillips et al. 1995a). This spatial separation is the result of larval movement and not different oviposition preferences, though it is not clear whether larvae have a direct temperature preference or some property of the wrack bed that may correlate with temperature, such as decomposition rate, microbial composition or algal species. Ultimately, the result of this varied distribution is that the level of interaction and thus competition between the two species is under some control and temperature gradients may contribute to stable coexistence (Phillips et al. 1995a). Alternatively spatial separation may arise due to preferences for different species of seaweed.

1.9 Coelopids and seaweed

Wrack beds around the British Isles are predominantly composed of brown algae (Phaeophyta); in particular algae of the genera *Laminaria* and *Fucus* (Dobson 1974a).

Whilst *C. frigida* is known to be able to complete development in either *Laminaria* or *Fucus* algae (Dobson 1974a; Phillips et al. 1995b) there is evidence to suggest differences in the profitability of these two resources. Dobson (1974b) reported that *C. frigida* could be bred more successfully in *Laminaria* monocultures than *C. pilipes* and also that *C. frigida* could be found in greater numbers in wrack beds that predominantly consisted of *Laminaria*. Egg to adult viability and size at eclosion are increased following development on *Laminaria* when compared to *Fucus* algae (Dobson, 1974b; Phillips et al., 1995b). This pattern of success is reflected in an oviposition preference for *Laminaria* by *C. frigida* females (Phillips et al. 1995b; Dunn 2001). In contrast, *C. pilipes* show a preference for oviposition on *Fucus* (Phillips et al. 1995b).

1.9.1 Nutritional value of different algae

Given high levels of production over a wide geographical range it is not surprising that marine algae form the basis for many food webs (Mann 1973; Duggins & Eckman 1997). In comparison to angiosperm tissues, algae constitute a profitable source of nutrition; lacking cellulose, waxes and lignins that may be hard to digest and resistant to microbial degradation (Duggins & Eckman 1997). Nitrogen availability is known to limit the growth of detritivores, however algal tissues are comparatively high in available nitrogen, making them a profitable alternative (Findlay & Tenore 1982; Duggins & Eckman 1997). Microbes on the surface of algae assist in making organic carbon and nitrogen available to consumers (Newell 1965; Mazure & Field 1980; Findlay & Tenore 1982; Crosby et al. 1990).

An increase in the population of wrack bed detritivores has been attributed to the density of the microbial population (Newell 1965) and on beaches it is estimated that bacteria are responsible for most of the secondary production (McLachlan 1985). The quality of different algae can be broadly attributed to levels of secondary metabolites and carbon-nitrogen ratio, both of which decrease with age as a result of microbial degradation, making them more palatable (Duggins & Eckman 1997; Norderhaug et al. 2003). It has further been shown that microbial degradation of algae is essential in making marine algae available to a number of amphipod grazers (Norderhaug et al. 2003).

A bacterial community is associated with the surface of algal fronds that differs to the neighbouring bacterioplankton (Shiba & Taga 1980; Bolinches et al. 1988). Fucoid algae are colonised primarily by *Flavobacterium*, but also other bacteria including species of *Escherichia*, *Pseudomonas*, *Staphylococcus* and *Achromobacter* (Chan & McManus 1969; Bolinches et al. 1988). Laminariales support a different bacterial community with greater numbers of *Pseudomonas* spp. and fewer *Flavobacterium* spp. The microflora of *L. hyperborea* is found to consist of approximately 80% *Pseudomonas & Vibrio*, with *Flavobacterium* representing only 13% of isolates (Laycock 1974). A spatial and seasonal variation in the bacterial flora of laminariales has been identified with frond tips, fronds and meristems all showing different patterns of seasonal variation in their bacterial communities (Laycock 1974; Hollohan et al. 1986). Representatives of *Pseudomonas*, *Vibrio* and *Flavobacterium* have all been found to decompose *Laminaria* fronds (Uchida et al. 1995).

Bacterial communities and their ability to metabolise algal compounds can vary seasonally. A significant factor affecting colonisation is temperature (Laycock 1974; Sieburth & Tootle 1981). Overall the bacterial flora of *L. hyperborea* is heavily influenced by temperature; a psychrophilic population being present in the winter and a mesophilic population in the summer, the changeover appearing to occur at about 7°C (Laycock 1974). The *Flavobacterium* community of fucoid algae vary in number according to a seasonal pattern; peak populations being recorded following increases in primary production in April and September. This variation can be attributed to changes in salinity and nutrient levels (Bolinches et al. 1988). The ability of the microflora to metabolise various algal compounds (glucose, proteins, mannitol, laminaran and alginate) also varies seasonally (Laycock 1974). Marine algae are capable of self regulating their bacterial epiphytes by the production of antimicrobials; derived from secondary metabolites (Sieburth & Tootle 1981). Once a selected biofilm has become established the bacteria themselves may also produce antimicrobials (Lemos et al. 1995, Steinberg & de Nys 2002).

The relationship between epiphytic bacteria of free living marine algae and bacteria that decompose the same algae in wrack beds is unclear. Bacterial communities are known to be successional and once algae are beached bacterial numbers increase rapidly (Koop et al. 1982; Hollohan et al. 1986). Non-uniform population growth combined with potential contamination from terrestrial sources may alter the relative proportions of bacterial species, though it is suggested that they remain very similar (Cullen et al. 1987). Nevertheless, the primary source of bacteria within wrack beds is the marine bacteria already present on algae, which is known to vary both interspecifically and seasonally. *C. frigida* larvae have been found to survive on bacteria not normally associated with marine algae and on killed bacterial cells. This led to the suggestion that coelopid larvae use bacteria as a food source (Cullen et al. 1987). Survival rates and larval size can vary considerably between different bacterial diets, so whilst any species of bacteria may be sufficient for larval development, some bacteria may be more nutritious than others (Cullen et al. 1987). Swift development and large male size are both of great importance to the success of individual coelopids, especially in the presence of competitors (Leggett et al. 1996). Variation in seaweed species and microbial communities may make significant contributions to successful larval development.

1.10 Aims of this thesis

Since the conception of modern sexual conflict theory by Parker (1979) this field of evolutionary ecology has been attracting increased interest (Chapman et al. 2003; Arnqvist & Rowe 2005; Chapman 2006). Rather than reaching any form of definitive conclusion, work thus far has instead given appreciation of the wide ranging effects of sexual conflict and set out new challenges to research (Tregenza et al. 2006).

Specifically, the role of sexual conflict in different populations may be vastly different as ecological differences alter the benefits of reproductive allocation. Identifying environmental influences on sexual conflict are essential if we are to fully understand how mating systems operate and evolve (Ortigosa & Rowe 2002). A number of studies have investigated the effects of external factors such as predation risk, hunger and density on mating behaviour but very few have actually investigated the effects of different life histories directly on sexual conflict (Rowe 1992; Ortigosa & Rowe 2002; Kokko & Rankin 2006; Shuker et al. 2006).

C. frigida presents an excellent model to examine the interaction between a diverse environment and evolutionary change driven by sexual conflict. There is clear evidence to suggest that different species of seaweed influence the life history of *C. frigida*.

Being a fundamental factor in coelopid life history, it is predicted that habitat composition should influence the reproductive costs and benefits of manipulating the opposite sex. Investigation of interpopulation differences in the mechanisms of sexual conflict are expected to provide a framework for understanding the underlying evolutionary processes.

The aims of this project can be divided into two general themes. First is the attempt to better define the role of habitat composition in the life history of coelopids. This is achieved through relating habitat composition to patterns of larval development, female oviposition preferences, longevity and a change in coelopid distribution. Second, mating behaviour and the costs of mating when exposed to and cultured using different algae is examined. Through a combined approach it is intended that sexual conflict in *C. frigida* should be placed within the context of life history variation.

Chapter 2 - General materials & methods

2.1 General procedures

2.1.1 Introduction

Generic methods detailing collection of biological samples, culture and manipulation of coelopids are detailed. Materials and methods specific to particular studies are given in the relevant chapters.

2.1.2 Collection of wild coelopids

Coelopids were collected from beaches along the coasts of Fife and East Lothian, Scotland. Exact locations and species collected are given for each study. Flies were collected either as larvae or adults. Larvae were collected following a methodical search and from a range of depths and locations within wrack beds. Several handfuls of larvae and associated wrack bed material were placed into 1.75 litre clear plastic containers (13 x 13 x 14cm; Stewart Plastics, Croydon, UK). Typically two to five boxfuls were collected from each wrack bed depending on the number of larvae present. On return to the laboratory contents of the 1.75 litre containers were emptied into 15 litre clear plastic culture boxes (27 x 19 x 36cm; Stewart Plastics, Croydon, UK) and stored at 25°C until adults eclosed. Collections of coelopids from natural populations were often infested with phoretic mites that are known to be detrimental to laboratory cultures (Butlin 1983). To prevent the spread of mites all wild collections were stored in water baths. Dependent on the density of larvae, additional algae was added to these boxes to maximise yield.

Wild adult flies were collected by mouth pooter either directly from wrack beds or, if swarming, from a plastic box swept over the surface of the wrack bed. Adults were returned to Stirling to create laboratory cultures.

2.1.3 Collection of algae

Algae were collected from beaches along the coasts of Fife and East Lothian. Exact locations and species collected are given for each study. Algae was picked either live from rocks or as detached fronds washed up in the intertidal zone, so that they had been deposited no earlier than the previous high tide On return to the laboratory algae was coarsely minced using a Hobart commercial mincer (Hobart, London, UK) and stored at -20°C. Mincing the algae increased surface area to encourage microbial growth and decomposition (Cullen et al. 1987). Freezing the algae ensured that any coelopid adults, eggs or larvae deposited prior to collection were killed. Frozen algae was thawed overnight at 25°C before use.

2.1.4 Culturing coelopids

Coelopids were cultured in 15 litre clear plastic boxes (27 x 19 x 36cm; Stewart Plastics, Croydon, UK). Boxes were filled approximately ¼ full with minced algae. All cultures used *Laminaria* algae, except where stated otherwise. Thirty male and thirty female adult coelopids were introduced to culture boxes and stored at 25°C on a 12h:12h light /dark regime to allow development of a further generation. Mating took place rapidly and first instar larvae were commonly observed within just a few days. Larvae were allowed to develop with an excess of algae to limit the effects of density that may have differed between cultures.

To prevent drying of the seaweed boxes were sealed with a solid plastic lid. As adults began to eclose this was exchanged for a lid fitted with an access hole and sleeve. Adults eclosing from cultures were collected twice daily to ensure virginity (Thompson 1951). Adult *C. frigida* were collected from cultures until exhausted to ensure no bias in adults that inevitably occurs as development times are linked to the *αβ* chromosomal inversion (Day et al., 1980). Flies were aspirated using a vacuum pump (Compton Compressors, Ashbourne, UK) and care was taken to ensure that all flies were removed each collection.

2.1.5 Storage and manipulation of coelopids

When not being used in experiments all flies were stored at 5°C in either 250ml flasks fitted with a foam bung or 1.75 litre clear plastic containers (13 x 13 x 14cm; Stewart Plastics, Croydon, UK). Boxes and flasks contained a ball of cotton wool soaked in 5% sucrose solution as a source of moisture. This was periodically exchanged to prevent drying out and fungal growth. Larger containers were preferred for a greater number of flies and resulted in less accumulation of moisture at the base of containers that is detrimental to survival.

Under these conditions flies are not reproductively active and could be stored for many months. Nevertheless, flies used experimentally had been stored in this way for no more than 10 days. Species identification, sexing and preparation of flies prior to experimentation were often carried out under light CO_2 anaesthesia using a porous gas pad. This procedure was used to improve efficiency and accuracy only when deemed not to have an adverse impact upon results. For example CO_2 was never used once an experiment had started. At all other times manipulation was carried out by either mouth or mechanical pooter.

After experimentation flies were killed by placing in a freezer at -20°C.

2.1.6 Determining body size

Body size was estimated by measuring wing length, which has been used an indicator of size in previous studies of coelopid behaviour (e.g. Day et al. 1990; Crean & Gilburn 1998; Crean et al. 2000; Dunn et al. 2002). Wing length was measured using a binocular microscope fitted with an eyepiece graticule (Meiji EMZ-TR, Tokyo, Japan). Flies were laid with their ventral surface uppermost and the graticule aligned from a prominent supra-alar bristle to the posterior margin of the wing (Butlin et al. 1982a). Measurements were made to the nearest 0.1mm.

2.2 Mating trials

In preparation for mating trials virgin male and female flies were placed into individual plastic containers. Males were placed into clear plastic containers measuring 55mm diameter by 75mm and females into slightly smaller clear plastic containers, 30mm diameter by 55mm. Housing males in larger containers allowed for greater preparation efficiency as males were often exposed to algae. The smaller containers housing females also doubled as mating arenas, reducing the handling time during mating trials. Container lids were pierced with several pin pricks to allow ventilation. This was sufficient for survival, whilst limiting desiccation.

Dependent upon study requirements, male containers included a small amount (approximately 2g) of minced algae. This amount of algae was in excess when compared to the size of flies and allowed flies the ability to crawl between fragments. In addition to algae, and in all female containers, a small sponge square measuring approximately 1 x 1cm soaked in 5% sucrose solution was placed in each container. Unless stated otherwise males and females used in the same mating trials had been prepared at the same time. Following preparation containers of flies were stored at 25°C on a 12h:12h light dark regime for the specified period of time prior to mating trials.

All mating trials were carried out at a constant temperature of 25°C. To start a mating trial a randomly selected male was introduced into the container of a randomly selected female. The pair was observed for a minimum of 10 minutes or until mating behaviour was observed. Males failing to mount a female within 10 minutes were scored as unwilling to mount. If mating behaviour was observed this was scored as either copulation (genital contact was observed) or rejection (the male dismounted the female before genital contact). The time taken for the male to mount a female, struggle duration and duration of copulation were also recorded. Time taken for the male to mount the female was defined as the time from introduction of the pair to the first observation of mating behaviour. Struggle duration was the time taken from the male mounting the female to either copulation or rejection. Copulation duration was measured as the time from first observation of genital contact to the male dismount. All timings were measured to the nearest second. Mating trials were stopped after the male dismounted the female.

2.3 Determination of *αβ* chromosomal inversion karyotype

2.3.1 Introduction

This section details the procedure for determining the inversion karyotype of individual flies. After numerous failed attempts at implementing this procedure it is found that appreciation of a few intricacies of the procedure contribute to an efficient and repeatable process. Included are a number of aspects that may appear trivial but are nevertheless included to ensure accurate replication.

2.3.2 Gel preparation

Starch gels were prepared as 12% w/v starch (from potato, hydrolysed for electrophoresis; Sigma-Aldrich Inc., St Louis, USA) in a 1:1 mix of TEB buffer (Table 2.1) and water. This was commonly carried out in a total volume of 200ml requiring 27.25g of starch. Starch was weighed and dissolved in 100ml of TEB buffer at room temperature in a 500ml Buchner flask. 100ml of boiling water was added to the starch buffer mixture, mixing continuously. This mixture was heated for periods of no more than 10 seconds in a microwave oven, mixing vigorously between each period, until the gel became viscous and started to boil. The gel was allowed to actively bubble but not to boil over. All mixing was carried out by swirling the flask as use of a mixing tool provided a cool surface to which the gel sets, leading to a lumpy consistency.

Table 2.1 Composition of Tris-EDTA-Boric Acid (TEB) Buffer.

Constituent	Concentration	Mass in 1000ml	Source
Tris (hydroxymethyl) aminomethane	0.17M	20.59g	Acros Organics, New Jersey, USA
Ethylenediaminetetra-acetic acid (EDTA)	0.002M	0.74g	Sigma-Aldrich Inc., St Louis, USA
Boric Acid	0.05M	to adjust pH to 8.7	Sigma-Aldrich Inc., St Louis, USA

Once heated gels were de-gassed using a tap based vacuum pump until the solution became clear. On application of a vacuum the gel continues to bubble even after fully de-gassed due to reduction of the boiling point. Periodic reduction of the vacuum was required to confirm when de-gassing was complete. During de-gassing the gel would naturally cool and solidify so it was essential to complete this procedure swiftly. Once de-gassed the gel was poured into moulds consisting of a plastic frame placed on a glass plate (18.5 by 10cm). Immediately after pouring any remaining air bubbles were removed or pushed to the end of the gel using a spatula before the gel set. Gels were left at room temperature for approximately 30 minutes then stored at 4°C to allow them to set fully. Moulds were initially overfilled, proud of the plastic border. Once set the excess gel was removed by slicing with cotton thread run over the surface of the mould. This procedure had the advantage of leaving a flat and cut surface which made the later addition of samples easier and more uniform. Gels may be stored at 4°C however best results are achieved if used the same day.

2.3.3 Homogenisation of flies

Flies were prepared for electrophoresis in 12 well spotting tiles. A drop of water was pippetted onto each fly in addition to a small amount of carborundum powder (Fisher Scientific, Loughborough, UK). Flies were homogenised using the end of a solid glass rod. If the fly was large then an additional drop of water was added, however this diluted the enzyme concentration so only the minimum amount of water required was used. Homogenate was then soaked into chads of filter paper cut with a standard hole punch (6mm diameter). A 24 tooth comb was used to cut a series of wells in the starch gel into which chads were inserted. In each row the first and last wells were left empty, being close to the edge of the gel. Up to 4 rows of samples (88 flies) could be run on a single gel, however to ensure samples did not run into each other and to leave sufficient space for electrodes only 3 rows (66 flies) were commonly used.

2.3.4 Electrophoresis

Gels, still in the original mould, were electrophoresed in a sealed box spanning two TEB buffer filled chambers. J-cloth wicks soaked in TEB buffer extended from the buffer chambers overlapping the surface of the gel by approximately 1cm. To ensure efficient conduction J-cloth wicks were folded multiple times and extended the entire length of the gel. A frozen cold pack was placed on top of the gel and the entire box placed at 4°C to prevent the gel heating and denaturing the enzyme during electrophoresis. Electrophoresis was carried out at a voltage of 300V and current of 70mA for 1.5 hours.

2.3.5 Staining

Once electrophoresis was complete the plastic mould surrounding gels and chads was removed. Gels were then sliced in half along the horizontal axis using cotton thread. The top half of the gel was discarded and the original plastic mould replaced for application of the agar overlay stain (Table 2.2). Stained gels were incubated in the dark at 37°C for a minimum of 30 minutes or until a banding pattern could be scored.

Table 2.2 Composition of agar overlay stain.

Constituent	**Volume**	**Source**
Tris-HCl buffer (0.1M Tris adjusted to pH 8.6 with 5M HCl)	10ml	Acros Organics, New Jersey, USA
Propan-2-ol	6ml	BDH Chemical Ltd, Poole, UK
3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) (10 mg ml^{-1})	1.5ml	Alfa Aesar, Heysham, UK
Phenazine methosulphate (2 mg ml^{-1})	1ml	Sigma-Aldrich Inc., St Louis, USA
Nicotinamide-adenine dinucleotide (1.5 mg ml^{-1})	10ml	Melford Laboratories Ltd, Ipswich, UK
Bacteriological agar (2% w/v)	20ml	Sigma-Aldrich Inc., St Louis, USA

Chapter 3 - Climate change and coelopids

3.1 Introduction

Global temperatures have increased by approximately 0.6ºC over the past century (IPCC, 2001; Jones et al., 1999). In Central England, the 1990s were approximately 0.5ºC warmer than the 1961-1990 average; with the greatest increase in temperature being experienced during the winter months (Hulme et al., 2002; Watkinson et al., 2004). Accompanying the air temperature increase has been a global rise in sea temperatures of about 0.5 °C over the last 70 to 100 years, with the majority of the change occurring over the last 20 years (Levitus et al., 2000; Hulme et al., 2002). In the future, all regions of the UK are expected to see a further increase in sea temperatures of anything from 1 to 3 °C by the 2080s, though accurate predictions cannot be made as they depend upon future emissions scenarios (Hulme et al. 2002). It is now increasingly apparent that climatic change will not only contribute to ecological changes in the future, but that changes are already occurring (Hughes, 2000; Root et al., 2003). One widely predicted outcome is that the ranges of many species will shift either poleward or to higher altitudes (Barry et al., 1995; Parmesan, 1996; Hughes, 2000). There is now considerable empirical evidence of such shifts on a global scale (Walther et al., 2002; Parmesan & Yohe, 2003; Hickling et al., 2005, 2006; Mieszkowska et al., 2006) and models show that these changes can be attributed to climate change (Walther et al., 2005).

Climate change models are commonly based upon knowledge of the direct effects of climate but often ignore more complex effects on interactions between species within communities and ecosystems (Jiang & Morin 2004). Due to complexities in the magnitude of climatic change upon different species and restriction on distribution imposed by other ecological and physical variables such changes are rarely, if ever, manifested as poleward shifts of entire communities or ecosystems (Barry et al. 1995). Some species may be able to adapt to climatic change or survive in suboptimal conditions, particularly where physical or ecological barriers restrict the ability to migrate (Fields et al. 1993). The effects of climate change and the poleward shift of species that occurs with increased temperature will subsequently result in significant changes to community structure (Barry et al. 1995; Schiel et al. 2004). The presence or absence of a species not only affects those species it directly interacts with but can also indirectly influence interactions between other species (Wootton 1993). Changes in species interactions can alter entire food webs making predictions about how climate change will affect a specific species highly complex (Wootton 1993; Barry et al. 1995; Jiang & Morin 2004; Schiel et al. 2004). Further, within a community or ecosystem there may be a few keystone interactions or processes that have a disproportionate influence on the function of that system (Paine 1992). Where these keystone processes, or species directly associated with these processes, are sensitive to temperature then small changes in temperature have the potential to generate disproportionate ecological impacts (Sanford 1999).

Coastal organisms may be particularly susceptible to the impacts of global warming resulting from increases in both sea temperature and rising sea levels (Lawrence & Soame, 2004; Watkinson et al., 2004). Correspondingly, a number of intertidal organisms found on rocky shores around Britain have undergone poleward range shifts associated with climate change (Mieszkowska et al. 2006). A number of warm water benthic species have begun to appear around the coast of Britain whilst some cold water species are in decline (Hiscock et al. 2004). Amongst the organisms most likely to be affected are coastal invertebrates (Beukema et al., 2001; Kendall et al., 2004; Lawrence & Soame, 2004). Changes to coastal invertebrate communities may have significant consequence for other species, particularly avifauna, that depend upon them as a food source (Kendall et al., 2004; Lawrence & Soame, 2004). One particularly understudied coastal ecosystem is the strand line community (Kendall et al., 2004).

The relative abundance and distribution of the two species of coelopid commonly found in Northern Europe, *Coelopa frigida* and *Coelopa pilipes*, has been studied on a number of occasions (Egglishaw, 1960; Dobson, 1974; Butlin, 1983; Phillips et al. 1995a). Early descriptions depict *Coelopa pilipes* as a “Mediterranean species” with a northern limit of distribution on the German coast, whilst *Coelopa frigida* has a more northerly distribution with observations north of the Arctic Circle (Remmert 1965). Whilst various factors may play a role in determining the relative abundances of *C. frigida* and *C. pilipes* the most important is almost certainly temperature (Phillips et al. 1995a). This is reflected in a greater abundance of *C. pilipes* during the summer months and a greater susceptibility of this species to the effects of freezing temperatures (Remmert, 1965; Phillips et al. 1995a). In addition, the larvae of *C. frigida* are found to prefer cooler locations within wrack beds (Phillips et al. 1995a).

Historical data on the distribution of *C. frigida* and *C. pilipes* were most recently compiled over a decade ago (Phillips et al. 1995a; Fig 3.1). Since the late 1990s it has been observed that *C. pilipes* has been increasing in prevalence throughout mainland Britain so that allopatric populations of *C. frigida* are increasingly difficult to locate

(Gilburn A. S. personal communication). The objective of this study is to update knowledge of coelopid distribution and use historical data going back nearly 40 years to consider the impacts of climate change upon the strand line community.

Considering the temperature preferences of *C. frigida* and *C. pilipes*, anecdotal evidence of a change in distribution and the likely effects of climate change, it is hypothesised that poleward range shifts have occurred in these species. In addition to direct effects of temperature; the indirect influence of temperature on seaweed distribution and physical effects of climate change on wrack bed composition may also be affecting coelopid distribution. Importance of the wrack bed community and implications of changes in community structure are discussed.

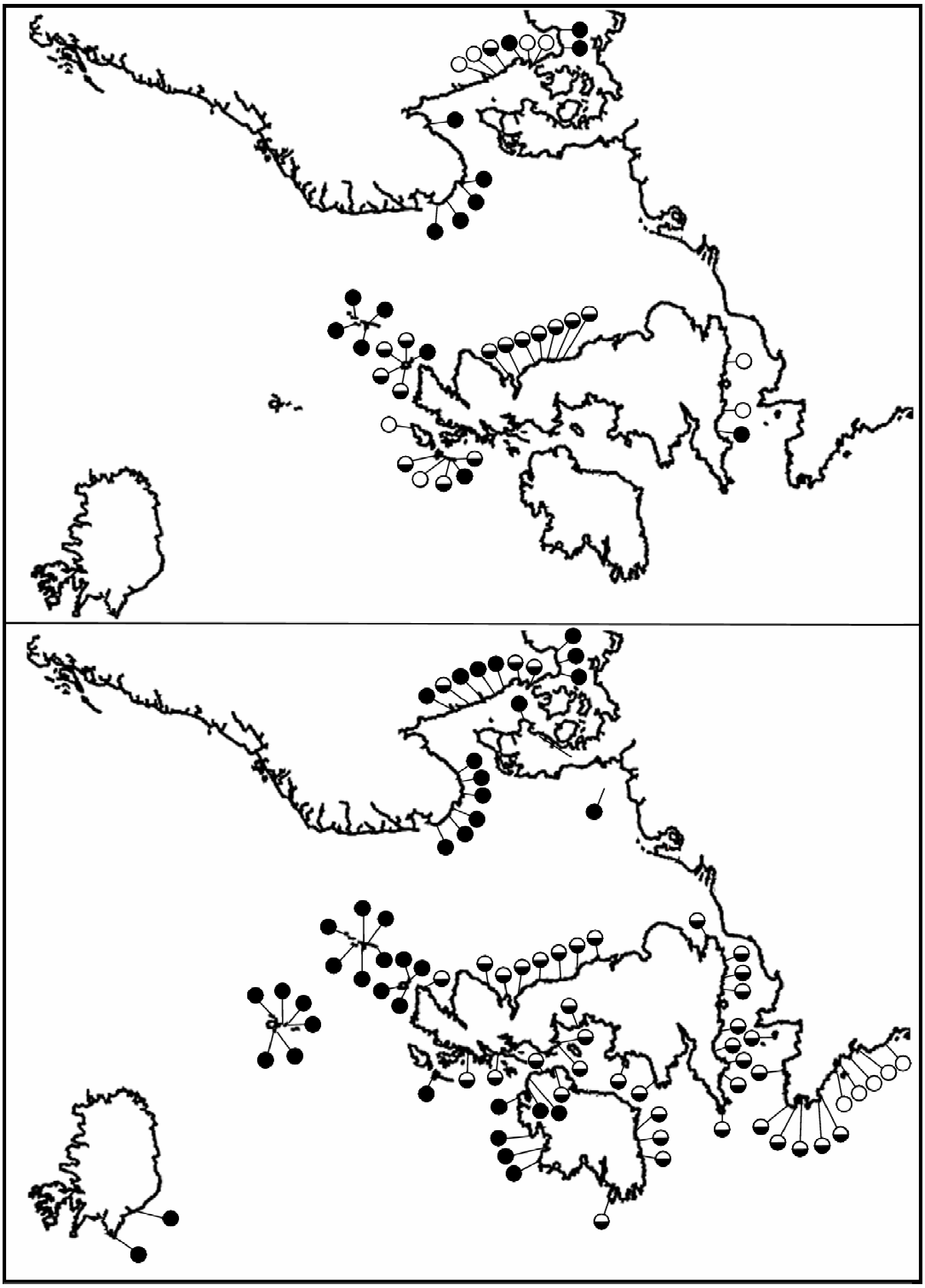


Figure 3.1 Distribution maps of *C. frigida* & *C. pilipes*, past and present. The map on top shows the current distribution of coelopids, whilst that below shows the historical distribution, adapted from Philips et al. (1995a). Filled circles represent populations of *C. frigida* (*C. pilipes* rare if present), empty circles populations of *C. pilipes* (*C. frigida* rare if present) and half filled circles mixed populations.

3.2 Methods

3.2.1 Experimental Procedure

Historical data were collated from previous collections made between February 1967 and October 1990 (Butlin, 1983; Gilburn, 1992; Phillips et al. 1995b; Day & Gilburn, unpublished data). The same locations were re-sampled between August 2004 and December 2005, where possible returning to the same beach and at the same time of year. If no wrack bed could be found at a site then another was located and sampled as close to the original wrack bed as possible. In some regions no information was available on the exact location of past collections, for example Norway and the Scottish Islands. In these cases, a number of sites were located to give a comprehensive description of coelopid distribution.

The same two collection techniques were adopted for new collections as used to collect the historical data. If sufficient adult flies were present at a site then they were collected and transported back to Stirling where the relative abundance of the two species was calculated. At sites lacking large numbers of adults, collections of larvae were made from various locations and depths within the wrack bed. Larvae were transported back to Stirling and allowed to develop within the seaweed in which they were collected. Any collections at high larval density were given additional seaweed to reduce the effects of larval competition. The relative number of *C. frigida* and *C. pilipes* adults eclosing from these samples was recorded.

3.2.2 Statistical analysis

All analyses were carried out using R 2.5.1 (R Development Core Team, 2007). A quasibinomial model of the proportion of *C. pilipes* flies at each location using year and latitude as independent variables was used as the dataset was overdispersed.

3.3 Results

3.3.1 Mainland populations

Some limited historical data were available for the south coast of Britain. During the summer of 1981 out of three sites sampled (Rustington, Portland & Beer) all comprised at least 90% *C. frigida* (Butlin, 1983). In August 2005 only one site, Beer, still contained *C. frigida*. The populations at Rustington and Osmington (a site close to Portland) were comprised entirely of *C. pilipes*. A considerable amount of historical data exists for the east coast of England. In samples taken between 1967 and 1981 the average proportion of *C. pilipes* found on east coast sites was 34% (SE ±6 %). This had increased to 55% (SE ±10%) in 2005. Precise historical data for *C. pilipes* populations in Scotland is not available, although limited records suggest that *C. pilipes* was relatively rare compared to *C. frigida*. Samples taken from around the Forth Estuary during 2004 and 2005 revealed that the mean proportion of *C. pilipes* was 81.7% (SE ±7.71%) with all sites containing a majority of *C. pilipes.*

A quasibinomial model showed that the proportion of *C. pilipes* within British mainland populations has increased over the last four decades ($F_{1,66} = 15.2$, $P < 0.001$, parameter estimate = +0.064, SE = 0.015). In both 2004 and 2005 more *C. pilipes* were collected than *C. frigida* (Fig. 3.2). Latitude was not found to affect the proportion of *C. pilipes* within mainland populations ($F_{1,66} = 0.060$, p = NS).

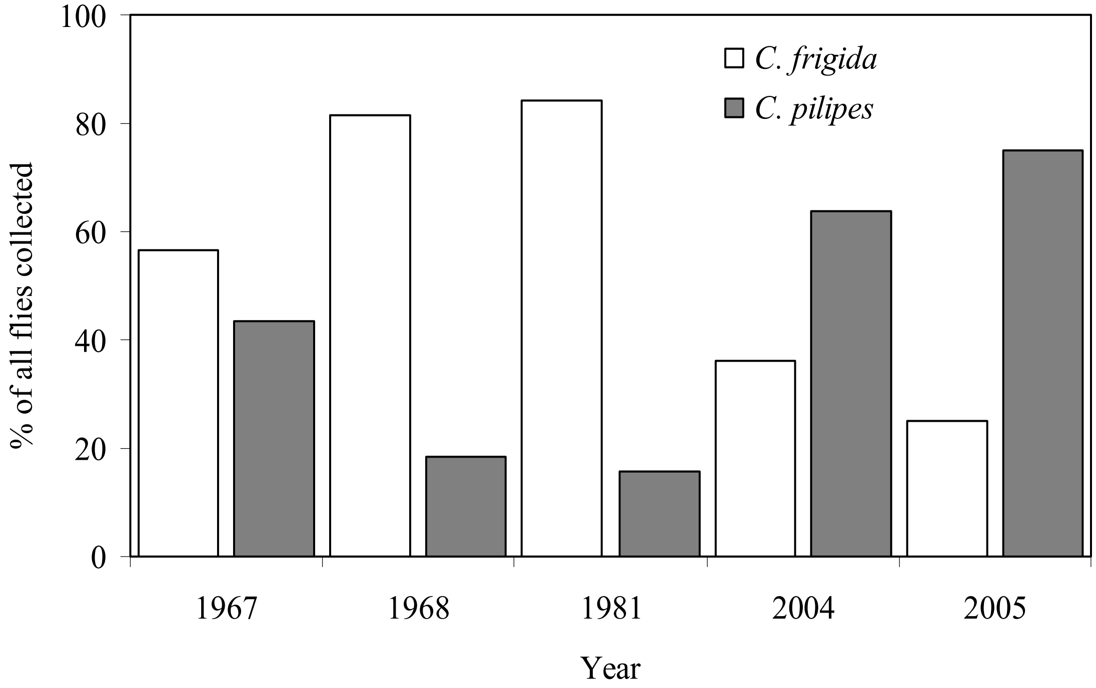


Figure 3.2 The proportion of individuals of the two common British coelopid species collected across mainland British populations in 1967, 1968, 1981, 2004 and 2005.

3.3.2 Scottish island populations

Phillips and co-workers (1995a) reported the absence of *C. pilipes* from the Western and Northern Isles of Scotland. We collected coelopids from populations on seven islands within the Western Isles in August 2004, five islands in the Orkney Isles in June 2005 and nine sites from Mainland Shetland in August 2005. *C. pilipes* was found on all seven islands (Vatersay, Barra, South Uist, Benbecula, North Uist, Harris and Lewis) within the Western Isles that hosted wrack bed communities. *C. frigida* was only found on six (absent from the one wrack bed investigated on Benbecula). *C. pilipes* was found on all five islands on which we sampled wrack beds within the Orkneys (Mainland, Burray, South Ronaldsay, Westray, Sanday). Of 15 wrack beds sampled in Orkney, five contained only *C. frigida* and three contained only *C. pilipes*.

C. frigida was the only coelopid found at all nine wrack beds on Shetland.

3.3.3 Swedish populations

Collections of coelopids were carried out on an annual basis on the West and South coasts of Sweden from 1986-1995 (Phillips et al. 1995a; Day & Gilburn, unpublished results). *C. pilipes* was found to be the commonest coelopid at Mølle, but was usually absent from other sites (Fig. 3.3). *C. pilipes* was occasionally found at Torekov, Bua and Steninge, also on the west coast, but never as the more common coelopid at these sites. *C. pilipes* was never found at any of the six sites on the South coast that were regularly found to harbour populations of *C. frigida*.

In September 2005 *C. pilipes* was absent from two wrack beds harbouring *C. frigida* populations on the south coast of Sweden. We found six wrack beds harbouring coelopid populations on the West Coast of Sweden. Of these, one consisted only of *C. frigida*, three consisted only of *C. pilipes* and two were mixed populations, one of which was 99% *C. pilipes* (Fig. 3.3). Two of the three wrack beds lacking *C. frigida* were sites not previously known to host *C. pilipes*, including the wrack bed at Träslövsläge which has previously always hosted a huge and well studied population of *C. frigida* (Gilburn et al., 1993). The wrack bed at Viken with 99% *C. pilipes* also has previously lacked any observation of *C. pilipes*. There had been a substantial shift towards *C. pilipes* as the predominant coelopid on the west coast of Sweden.

3.3.4 Norway

Much of the southern Norwegian coastline from Bergen in the West to Holmestrand in the East consists of rocky slabs descending directly into the sea, making it difficult for large amounts of marine debris to accumulate. Five locations were sampled along this coastline in December 2005 and July 2006. *C. frigida* was found exclusively at the four westernmost sites, however at the easternmost site, Stavern, a solitary male *C. pilipes* was recorded. This is the first record of *C. pilipes* occurring in Norway.

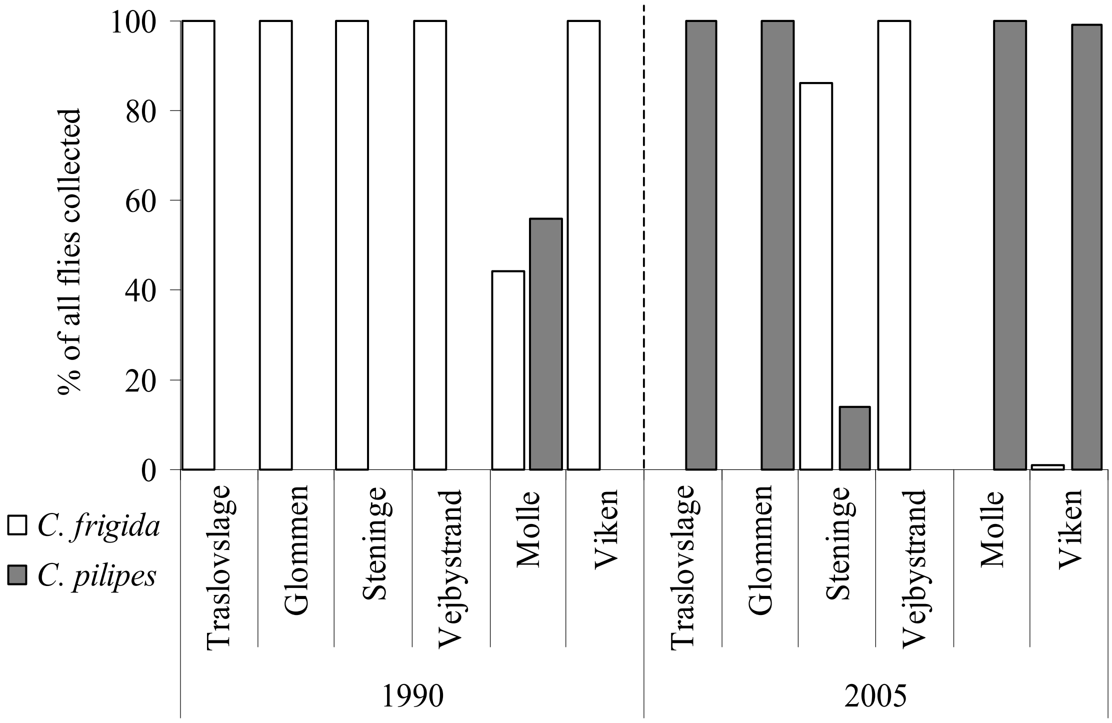


Figure 3.3 The proportion of individuals of the two common coelopid species collected from wrack beds along the West Coast of Sweden in 1990 and 2005. Sites are listed from left to right in order of decreasing latitude.

3.4 Discussion

3.4.1 Current coelopid distribution

Wrack beds are a highly stochastic resource, dependent upon weather and tidal patterns. Persistence of a wrack bed may vary from just a few days to a month or more, having a significant influence on coelopid populations that may vary from week to week (Dobson, 1974; Hodge & Arthur, 1997). This creates difficulties when comparing sites across years. Though care was taken to visit sites at a similar time of year and to collect flies in a consistent manner, few conclusions can be drawn about changes occurring at specific locations. Nevertheless, due to the numerous and wide distribution of sites studied, and considering in particular the number of invasions of *C. pilipes* at previously unrecorded locations, these results clearly demonstrate a change in coelopid distribution. The results of this study suggest a shift in the distribution of coelopid species in Northern Europe corresponding with predictions of poleward range expansions resulting from climate change.

Whilst Norway and the Shetland Isles now appear to be the new northern limit of *C. pilipes* range, this may be determined as much by physical as ecological constraints. *C. frigida* and *C. pilipes* have been reported to migrate in large numbers and over long distances; however it is predicted that the direction of migrations should follow coastlines in the direction of fresh wrack beds (Oldroyd, 1954; Egglishaw, 1961). The Western Isles of Scotland and the Orkney Islands can be reached by traversing far shorter stretches of open water than would be required to reach the Shetland Islands. Though not an absolute barrier to colonisation such distances are expected to slow the rate of range expansion.

The southern coastline of Norway was found to have reduced numbers of wrack beds suitable for the maintenance of coelopid populations due to local topography. A reduced frequency of suitable habitat along this portion of coast may limit the rate of *C. pilipes* range expansion.

The continuing absence of *C. pilipes* from the South coast of Sweden is likely to be due to the low percentage of brown algae within wrack beds along this coastline. The predominant component of wrack beds along this coast is eel grass, *Zostera marina*, which may limit the decomposition of brown algae so that these wrack beds remain relatively cold; favouring *C. frigida*. The potential effects of climate change upon *C. pilipes* range may be easily underestimated.

3.4.2 Effects of temperature change on coelopids

Phillips and co-workers (1995a) proposal that temperature is the most important factor determining the relative abundance and distribution of coelopids predicts that any changes in climate affecting temperature are likely to alter the relative abundance and distribution of these species in Northern Europe. The rise in temperatures observed in Britain over the past 30 years have been much more pronounced in winter, increasing by 1.1°C, compared to only a 0.2°C rise in summer temperatures (Watkinson et al., 2004). Furthermore there has been a substantial reduction in the number of winter days below zero (Watkinson et al., 2004). The seasonal pattern of *C. pilipes* prevalence may easily have extended during recent mild winters. It has also been shown that at increased temperatures *C. pilipes* are better able to develop when in competition with *C. frigida* (Phillips et al. 1995a). Thus a small change in temperature could exhibit a large ecological effect as the balance of competition falls in favour of *C. pilipes*.

3.4.3 Effects of temperature change on seaweed

In addition to the direct effects of increased air temperature, increase in sea temperatures may have indirect effects on coelopids. As coelopids are totally reliant upon seaweed that has been washed up in wrack beds any change in the abundance or composition of this resource may have an effect on their distribution. The distribution of the primary constituent of wrack beds harbouring coelopids, brown algae, is determined by sea temperatures (Breeman, 1988; van den Hoek et al., 1990). As a result of changes in sea temperature, it has been suggested that *Laminaria* may currently be in decline (Breeman, 1990; Barry et al.1995; Schiel et al. 2004) and that *Fucus* is predicted to decline in the future (Kendall et al., 2004). Other factors are also likely to affect marine algae distribution, such as photoperiod (Breeman 1988), longitudinal barriers (van den Hoek 1987; van den Hoek et al. 1990) and salinity (Russell 1987). However, these are less affected by climate change and are expected to have minimal impact on marine algae when compared to the effect of temperature.

Two thermal limits to seaweed distribution can be defined; lethal limits and growth/reproductive limits (Breeman 1990; van den Hoek 1982; van den Hoek et al. 1990). Lethal limits are set by a species ability to survive during seasonal extremes, either the hottest summer temperatures or coldest winter temperatures. Growth or reproductive limits to distribution are defined by the capacity of an alga to grow and reproduce during the favourable season (van den Hoek et al. 1990). The distribution of algae will vary according to changes in temperature and the thermal tolerances of individual species, which may result in local extinctions (Breeman 1990). Following an improvement in conditions re-colonisation may take place, though this will invariably be a slower process than that of extinction (Breeman 1990). In addition to extinction events along species boundaries, even minor temperature changes can affect species composition and community structure throughout a species' range (Breeman 1990).

Laminariales grow optimally between about 10°C and 15°C with maximum survival temperatures of about 20 to 23°C (Sundene 1964; Bolton & Luning 1982; Rinde & Sjøtun 2005). Fucoid algae are able to tolerate a much wider range of temperatures, growing optimally between about 10°C and 17.5°C, but withstanding temperatures over 30°C (Strömgren 1977). It has been shown that with increased temperatures warm water algae can increase their range northward (Barry et al. 1995) and that some species of *Laminaria* will decline rapidly in response to increased temperature (Schiel et al. 2004). It is predicted that with more extreme cases of temperature increase the southern boundaries of *Laminaria* spp. are expected to move north of the Iberian Peninsula, the Atlantic coast of France and the southern British Isles (Breeman 1990). It has been predicted that the amount of fucoid algae will also decrease (Kendall et al. 2004), though given the relative temperature preferences this should be later than for laminarial algae. The relative abundance of algal species within wrack beds is highly likely to have altered over recent years.

It has been suggested that *C. frigida* and *C. pilipes* have preferences for different species of seaweed (Dobson 1974; Phillips et al. 1995b; this thesis) and that the level of competitive interaction between coelopids can be dependent on the rate of wrack bed decomposition (Leggett et al. 1996; Hodge & Arthur 1997). A change in macroalgae distribution associated with rising sea temperatures represents an indirect mechanism by which climate change may influence coelopid distribution and community dynamics within wrack beds.

3.4.4 Physical effects of climate change

In addition to the physiological effects of temperature, the physical manifestation of climate change may also affect wrack-bed composition. Wrack beds are composed not of the algae growing offshore, but a subset of these algae that have been dislodged and washed onto beaches. Changes in sea temperature may influence species distributions, but it is the effects of wave action and storm events that ultimately control the deposition of wrack beds. The wave climate of the UK has become rougher in the second half of the twentieth century, with an average increase in wave height of 10-15% between the 1980s and 1990s (Hulme et al. 2002). As wind speeds and directions are expected to change in the future, wave heights are also expected to continue to change, although this has not yet been quantified (Hulme et al. 2002).

The stress of water currents and waves can put seaweed thalli under forces of tension, shear, bending and twisting that can lead to various adaptations (Luning 1990). Narrower and dissected thalli that are more ‘hydrodynamically’ shaped and more flexible can be found in more exposed locations (Luning 1990). In some species gas bladders benefit the seaweed by providing greater access to sunlight, but these also expose the algae to greater mechanical stress so may become reduced in more exposed locations (Luning 1990). *Laminaria* species flourish in strong currents and moderately to strongly exposed locations, adapting the thallus morphology to be narrower and more digitate as flow increases. *Fucus* species are less well adapted to mechanical stresses.

Changes in wave climate may not only affect the amount of algae washed ashore, but may have an asymmetric effect on different species, further influencing wrack bed composition. An increase in storm events may further influence the consistency of wrack bed formation.

3.4.5 Coelopids and the wrack bed community

Coelopids are found in a fringe habitat that is neither truly marine nor truly terrestrial, instead containing a mixture of species derived from neighbouring ecosystems. A large proportion of strand line material is composed of marine macroalgae, although other marine and terrestrial organic matter as well as anthropogenic waste is also usually present. In temperate to sub-polar coastal regions the growth of marine macroalgae can be considerable, comparable to the rainforests in terms of net productivity (Mann 1973). A large amount of this resource is deposited on beaches each year; either the result of wave action dislodging whole individuals or the erosion of aged algal material at extremities. The wrack bed is an important basis for many food chains as organic matter is recycled into both marine and terrestrial ecosystems.

Bacterial activity accounts for the vast majority of wrack bed productivity; estimated to decompose over 87% of material (Koop & Griffiths 1982; Koop et al. 1982). The macrofaunal community (amphipods, coleoptera, diptera, oligochaetes and nematodes) are relatively less abundant and account for less direct primary production (Koop & Griffiths 1982). However, rates of microbial degradation increase with decreasing particle size and so the role of macrofauna in breaking down large particles is central to maintaining the efficiency of decomposition (Harrison 1977; Robertson & Mann 1980; Koop & Griffiths 1982; Cullen et al. 1987). Nutrients derived from wrack beds can support many different food chains, although for the most part organic matter is returned to the sea at the next high tide. Decomposed wrack material can also support the establishment of terrestrial plants such as Sea Sandwort (*Hankenya peploides*), Sea Rocket (*Cakile maritima)* & Salt Wort (*Salsola kali*) that act as the precursors to sand dunes. At higher trophic levels, a number of coastal bird species feed upon wrack macrofauna and nematodes in the organically rich subsurface (Pienkowski 1982; Feare & Summers, 1985; Llewellyn & Shackley 1997; Dugan et al. 2003).

The importance of this biotope has been underestimated in the past; however the effects of its removal are measurable where the strand line is mechanically cleared for aesthetic reasons. The macrofaunal diversity of beaches becomes reduced and this can have an effect on higher trophic levels (Llewellyn & Shackley 1997; Dugan et al. 2003). A change in strand-line productivity associated with climate change has previously been suggested (Kendall et al. 2004) and it is recommended to limit the large scale harvesting of kelp as the ecological implications are not yet fully understood (Griffiths & Stenton-Dozey 1981). Coelopids are an important member of the wrack bed community; facilitating decomposition and also as a prey species. It has already become much harder to find *C. frigida* on beaches of mainland Britain, something that has been of concern since this species has been established as an important model organism for the study of sexual conflict and sexual selection (Chapter 1). It is not clear what direct effect the changes in relative abundance of *C. frigida* and *C. pilipes* will have on strand line communities. The loss of *C. frigida* may be particularly important as this is usually the first large dipteran to lay eggs in freshly deposited algae. Colonisation of wrack beds by *C. pilipes* is known to be delayed in comparison to *C. frigida*. As wrack beds are a short lived resource this delay to decomposition could have subsequent effects on other members of the wrack bed community and the recycling of nutrients. In addition to the direct effects a change in coelopid distribution may be indicative of wider ecological impacts of climate change on our beaches.

This study demonstrates that the relative abundance of coelopids in Northern Europe has changed in recent decades. The likely cause of these observations is recent changes in climate that may have affected coelopids directly, the interaction between species of coelopid or the composition of their habitat. Further work need not necessarily be focused upon coelopids, but should consider the wider implications of climate change on the strand line ecosystem.

Chapter 4 - Species of algae and male mating behaviour

4.1 Introduction

It has been established that seaweed plays an important role in coelopid reproduction, increasing male harassment of females and inducing oviposition (Dunn et al. 2002). The coelopid life cycle can be completed in wrack beds composed of a wide variety of different seaweeds though wrack beds around the UK are primarily composed of two different genera of brown algae, *Fucus* and *Laminaria*, (Dobson 1974; Cullen et al. 1987; Phillips et al. 1995b).

When females are exposed to algae and oviposition rate is increased then the chance of a female re-mating prior to oviposition is reduced. Due to last male sperm precedence found in coelopids (Thompson 1951; Burnet 1961; Blyth & Gilburn 2005) it is predicted that males should increase reproductive success by mating when exposed to algae and female oviposition rate is highest. As previous studies have reported that *C. frigida* larvae and adult females favour *Laminaria*, it may also be expected that stimulation of male harassment is algal specific. In the same way that male harassment increases in the presence of algae, it is anticipated that male *C. frigida* should demonstrate an increased willingness to mate in the presence of *Laminaria* when oviposition rate is increased in comparison to *Fucus*. By increasing mating activity in proximity to seaweed, and particularly *Laminaria,* males are more likely to benefit from last male sperm precedence (Dunn et al. 2002). *C. pilipes* females do not demonstrate an oviposition preference for *Laminaria* so an increase in harassment by *C. pilipes* males is not predicted.

An additional, and previously unstudied, factor that may affect male harassment is the duration of exposure to seaweed. *C. frigida* females are known to oviposit more readily compared to *C. pilipes* (Leggett et al. 1996). A more rapid response to the presence of seaweed in *C. frigida* males is then also predicted to increase reproductive success. Different response times following exposure to algae may be an additional factor contributing to the difference in development time between *C. frigida* and *C. pilipes*.

In this study the effects of exposure and duration of exposure to different algal genera on male mating behaviour are compared to predictions. The rate of male harassment determines the frequency at which pre-mating struggles occur. The level of sexual conflict within a population is then affected by the presence of seaweed. If male harassment is also affected by different species of seaweed then levels of sexual conflict may vary between populations dependent upon seaweed distribution and wrack bed composition. Furthermore, different responses of male coelopids to algal species and duration of exposure to algae may assist in the creation of temporal and spatial divisions in development. The implications of habitat specific male mating behaviour on sexual conflict and interspecific competition are discussed.

4.2 Methods

4.2.1 Preparation

Virgin *C. frigida* and *C. pilipes* were cultured following collection of wild larvae from the Forth Estuary in February 2004. *C. frigida* were from Whitesands, East Lothian (NT712775) and *C. pilipes* from St Monans, Fife (NO521012). Three species of algae, *Fucus serratus, Fucus vesiculosus* and *Laminaria digitata* were collected from Whitesands, East Lothian and Cellardyke Harbour, Fife (NO577038).

4.2.2 Experimental procedure

Male flies were prepared for mating trials in one of four different treatments; exposure to *F. serratus*, *F. vesiculosus*, *L. digitata* or a sucrose only control. At intervals of 1, 2 and 3 days following preparation an equal proportion of male flies from each treatment group were used in mating trials. Males and females were used in only one mating trial. Thus a 4 x 3 factorial design was adopted for each fly species to determine the effect of exposure to different algae and duration of exposure on male mating behaviour. Following mating trials the wing length of all flies was determined.

4.2.3 Statistical analysis

Binary logistic models of male harassment (whether the male mounted a female) and mating success were created separately using SPSS v12.0.2 (SPSS Inc. Chicago IL, USA). Models were further simplified to analyse differences within species and between treatments. Species, male treatment, duration of exposure to treatment, male size, female size and all two way interaction terms were initially included in models. Maximal models were selected based upon the Akaike Information Criterion calculated using R 2.5.1 (R Development Core Team 2007), non-significant terms being excluded from models. All p-values were determined using log-likelihood chi-squares.

4.3 Results

4.3.1 Male harassment

A total of 567 mating trials were completed. Harassment levels were higher for *C. frigida* than *C. pilipes* ($\chi^2_1 = 53.685$, $p < 0.001$) and were influenced by both treatment ($\chi^2_3 = 13.951$, $p = 0.003$) and the duration of exposure ($\chi^2_1 = 20.820$, $p < 0.001$; Fig. 4.1). A total of 90 out of 286 (31%) *C. pilipes* males mounted a female, compared to 174 out of 281 (62%) *C. frigida* males. Notably, only 8 male *C. pilipes* mounted a female following 1 day of exposure to treatments. The influence of duration of exposure differed between the two species ($\chi^2_1 = 13.222$, $p < 0.001$). Harassment by *C. pilipes* males increased with longer exposure ($\chi^2_1 = 33.255$, $p < 0.001$), whilst the length of exposure had no significant effect on harassment by *C. frigida* males ($\chi^2_1 = 0.986$, $p = 0.321$).

Despite a non-significant interaction term, male harassment was influenced by treatment in *C. frigida* ($\chi^2_3 = 9.942$, $p = 0.019$) but not in *C. pilipes* males ($\chi^2_3 = 5.644$, $p = 0.130$). Differences between *F. serratus* & *F. vesiculosus* did not contribute to the altered harassment rates in *C. frigida* ($\chi^2_1 = 0.030$, $p = 0.862$) and so these treatments were combined in further analyses. There was no significant difference between treatment with *Fucus* seaweed and the control ($\chi^2_1 = 1.476$, $p = 0.224$), rather the effect of different treatment on male harassment in *C. frigida* was the result of a significant difference between *Laminaria* and the control ($\chi^2_1 = 9.549$, $p = 0.002$) and between *Laminaria* and *Fucus* ($\chi^2_1 = 5.432$, $p = 0.020$; Fig. 4.1).

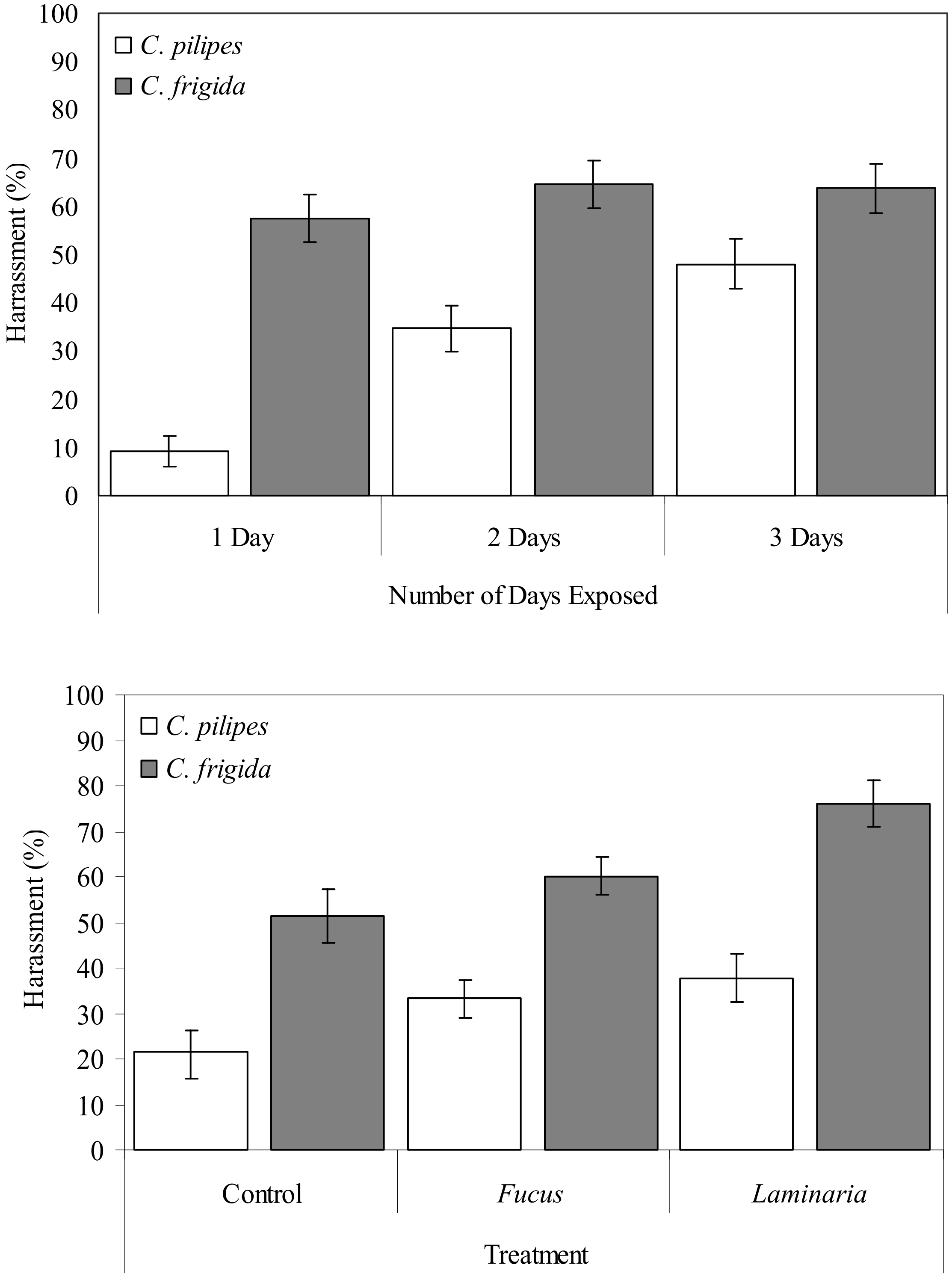


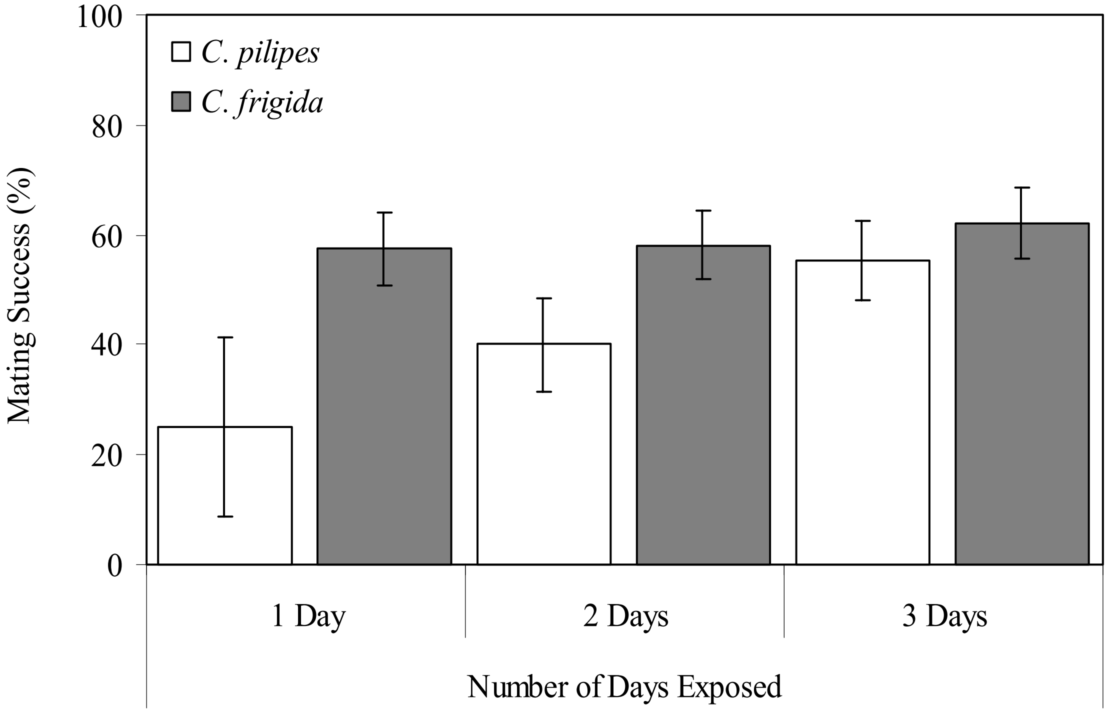
Figure 4.1 The effect of the duration of exposure to algae and exposure to different species of algae on harassment levels in *C. frigida* & *C. pilipes*. Harassment is shown as the percentage of males that mounted a female within 10 minutes.

4.3.2 Mating success

Mating success was determined primarily by an interaction between male size and female size ($\chi^2_1 = 6.981$, $p = 0.008$). This is the result of larger males being able to overcome the rejection response of smaller females more easily (Gilburn et al. 1992; Crean & Gilburn 1998). The influence of male size on mating success differed between the species ($\chi^2_1 = 4.890$, $p = 0.027$). Successful *C. frigida* males were larger than unsuccessful males whereas this difference was negligible in *C. pilipes*.

Analogous with harassment levels, mating success in *C. pilipes* was not affected by treatment ($\chi^2_3 = 0.021$, $p = 0.999$) compared to a significant influence on *C. frigida* ($\chi^2_3 = 11.549$, $p = 0.009$; Fig. 4.2). There was no difference in the effect of the different *Fucus* seaweeds on mating success ($\chi^2_1 = 2.438$, $p = 0.118$) and so these treatments were again combined in further analyses. Treatment with either *Fucus* or *Laminaria* algae led to a significant increase in mating success of *C. frigida* when compared to the control (*Fucus* $\chi^2_1 = 5.114$, $p = 0.024$; *Laminaria* $\chi^2_1 = 9.348$, $p = 0.002$), however there was no significant difference between the effects of *Laminaria* and *Fucus* ($\chi^2_1 = 1.689$, $p = 0.194$).

There was a difference in mating success following different lengths of exposure to algae, however this was not significant at the 5% level in a model including both species of coelopid ($\chi^2_1 = 3.465$, $p = 0.063$). In a model considering only *C. frigida*, length of exposure had no effect on mating success ($\chi^2_1 = 1.616$, $p = 0.204$). In contrast a model for *C. pilipes* did show a significant positive effect ($\chi^2_1 = 4.006$, $p = 0.045$).



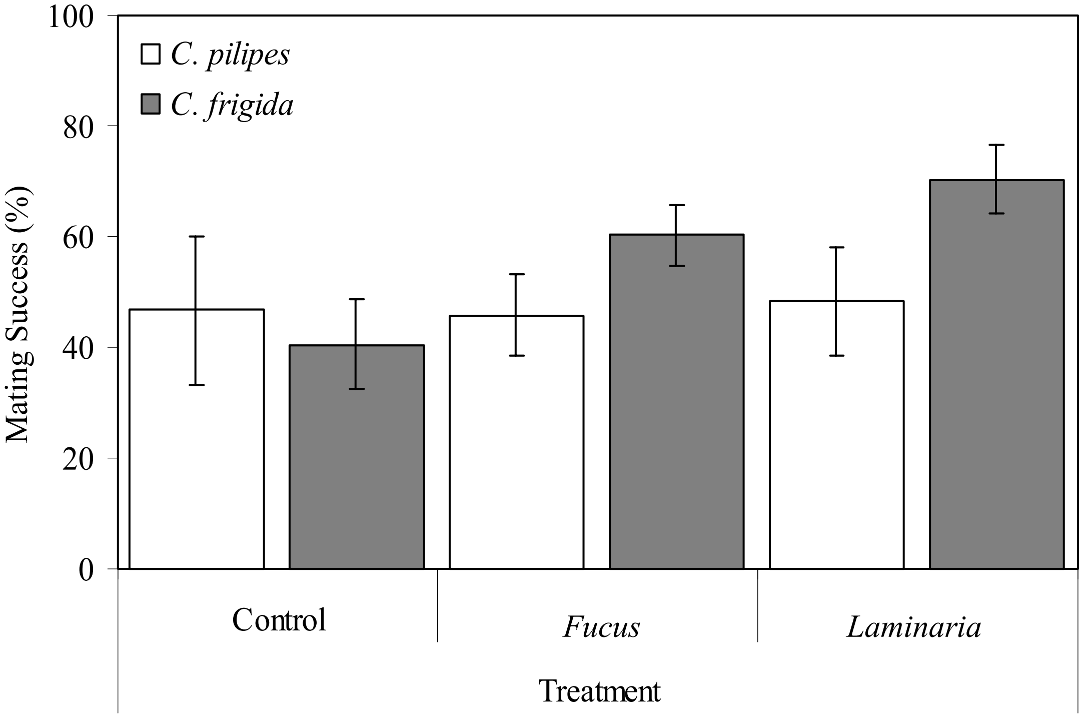


Figure 4.2 The effect of the duration of exposure to algae and exposure to different species of algal on the mating success of male *C. frigida* & *C. pilipes*. Mating success is shown as the percentage of males mounting a female that also copulated.

4.4 Discussion

4.4.1 Summary of results

Harassment of females by male *C. frigida* was stimulated by the presence of fucoid seaweeds. This finding is consistent with previous studies (Dunn et al. 2002). In this study males were exposed to a second genus of brown algae, *Laminaria*, also commonly found in Northern European wrack beds. This genus of seaweed was found to have a greater stimulatory effect on male harassment levels than fucoid seaweeds. The relative proportion of *Laminaria* and *Fucus* within wrack beds is likely to affect the level of male harassment by *C. frigida*, and therefore determine the level of sexual conflict within each population of this species.

Seaweed species composition of a wrack bed is unlikely to affect the level of sexual conflict within *C. pilipes* populations. Previous studies have found increased mating activity in *C. pilipes* when exposed to algae (Dunn et al. 2002), however we show that the duration of exposure to either algae or indeed sugar solution is the primary factor determining the level of harassment within this species. Given no significant interaction between duration of exposure and treatment, it is difficult to state whether *C. pilipes* require a period of maturation and/or exposure to seaweed in order to stimulate willingness to mate. Due to low levels of harassment in *C. pilipes* relative to *C. frigida*, particularly following short periods of exposure, a much greater sample size in combination with a more defined age range of flies would be required to determine the relative influence of age and exposure to algae.

Costs of multiple mating to female insects are well documented and include time costs, energy costs, increased predation, increased risk of injury and the risk of disease or parasite transmission (Arnqvist 1989). A number of female counter adaptations to reduce the costs of multiple mating have been documented (Stockley 1997). In contrast the cost of multiple mating to males has been overlooked as male fitness often increases with increasing number of successful matings. For *C. frigida* males the costs of mating are also expected to be significant, limiting mating capacity and leading to strategic allocation of resources (Chapter 1). A reduction in male mating effort when stimulated by *Fucus* is empirical evidence in support of strategic allocation of resources and further suggests a limit to male mating capacity in *C. frigida*.

The results of this study contribute to our understanding of coelopid life history and interspecific interactions. Preference of *C. frigida* males to reproduce when exposed to *Laminaria* in conjunction with increased female oviposition can explain the greater proportion of *C. frigida* found in wrack beds containing *Laminaria*. Delayed male mating behaviour in *C. pilipes* would contribute to the longer generation time observed for this species. Differential allocation of male mating effort can assist in maintaining temporal and spatial separation of these species, providing direct benefits for the maintenance of these behaviours.

4.4.2 Environmentally determined sexual conflict

Discovering that the intensity of male harassment is environmentally determined has important implications for attempts to measure the intensity of selection occurring as a result of sexual conflict. A single measurement of the intensity of conflict might not be a true reflection of the level within a population. Several measurements might be required at different time points and under different environmental conditions in order to gain a clear estimate of the average level of, and variability in, the intensity of conflict. As all previous studies of mating behaviour in *C. frigida* have used *Fucus* algae to stimulate mating behaviour the effects of sexual conflict in this species will likely have been underestimated.

Habitat variation can influence the outcome of sexual selection. For example turbidity inhibits mate choice in cichlid fish (Seehausen et al. 1997) and availability of breeding sites determines the strength of sexual selection in the European lobster and sand goby (Forsgren et al. 1996; Debuse et al. 2003). In water striders costs of mating are increased as a result of ecological conditions, such as hunger, and the female rejection response to multiple mating has been shown to increase in intensity (Ortigosa & Rowe 2002, Rowe 1992, 1994). Population density, which may vary in different environments, has been identified as fundamental to the evolutionary outcome of sexual selection and sexual conflict (Martin & Hosken 2003b; Kokko & Rankin 2006).

The extent of sexual dimorphism in *C. frigida* is known to vary between populations. This variation has previously been interpreted in terms of environmental factors such as tidal range that may influence the strength of female mating preferences (Gilburn & Day 1994a). Variation in sexual dimorphism may also be explained as the result of differences in male mating behaviour in different environments.

It is suggested that climate change may have both direct and indirect influences on coelopid distribution. Indirect effects include a change in seaweed distribution and the proportion of different seaweed species deposited in wrack beds (Chapter 3). As the coelopid mating system is influenced by different species of seaweed a change in climate may then also influence reproductive behaviour. The pattern and extent of sexual dimorphism is predicted to become altered with climate change through changes in wrack bed composition.

4.4.3 Manipulation of mating behaviour

Experimental manipulation of mating rates has been fundamental to the study and understanding of the processes underlying sexual conflict. This can be achieved with relative ease by altering either the operational sex ratio (Arnqvist 1992; Rowe 1992; Vepsalainen & Savolainen 1995; Rowe & Arnqvist 2002; Wigby & Chapman 2004) or population density (Arnqvist 1992; Martin & Hosken 2003b; Hardling & Kaitala 2005). In addition, other ecological factors such as food deprivation (Simmons & Bailey 1990; Rowe 1992; Sih & Krupa 1992; Ortigosa & Rowe 2002), predation (Sih & Krupa 1992; Sih 1994) and mating history (Shuker & Day 2001; Ortigosa & Rowe 2003) have also been found to influence the extent of sexual conflict. In the majority of studies there is a bias towards manipulation of female mating rates, with a relative inability to alter male mating behaviour (Sih & Krupa 1992; Ortigosa & Rowe 2002; Rowe & Arnqvist 2002). The greater reproductive investment made by females in a majority of taxa would likely explain the wider variation in susceptibility to the costs of mating when exposed to different environmental stresses. For example, in the water striders female hunger is found to influence mating frequency whilst male hunger does not (Rowe 1992; Ortigosa & Rowe 2002). Similarly, a male biased operational sex ratio does not affect the mating rate of male gerrids. It has been suggested that optimal male mating rates are relatively high and constant among gerrids in relation to females (Rowe & Arnqvist 2002, though see Lauer et al. 1996). Interspecific variation in male mating rate is not sufficient to explain behavioural covariation, which is instead most likely the result of variation in female mating rates (Rowe & Arnqvist 2002).

Following the results of this study, coelopids present an interesting system as male mating rates and mating success vary both inter- and intraspecifically. Male mating behaviour may be manipulated relatively easily by variation in the environment, either exposure to different species of algae or different durations of exposure. This presents an ideal model system for future comparative studies and population crosses to examine the impacts of sexual conflict and variable selection pressures.

Chapter 5 - A changing environment & male mating behaviour

5.1 Introduction

Once sexual maturity is reached finite resources must be allocated between essential activities in order to maximise individual reproductive success. As a consequence trade-offs may be required between elements of non-mating fitness such as survival, growth and condition in order to maximise current and future reproductive efforts (Stearns 1992; Stearns & Hoekstra 2005). In addition, trade-offs between current mating efforts and maintenance of sufficient resources for future matings are also predicted (Stearns 1992; Galvani & Johnstone 1998; Engqvist & Sauer 2002; Reinhold et al. 2002). Reducing mating effort when reproductive prospects are low in favour of more cost effective mating opportunities in the future could prove to be an advantageous strategy. One way for males to maximise reproductive success is to adjust mating effort in accordance with female fecundity or quality, dependent upon accurate assessment of females (Reinhold et al. 2002). During early reproductive maturity a male may do better to restrict mating effort in case a female of higher quality is encountered in the future (Galvani & Johnstone 1998).

A number of factors may determine the relative benefits of expending efforts in current versus future mating opportunities. As individuals age the number of future reproductive encounters will naturally decrease. It is observed that male sticklebacks (*Gasterosteus aculeatus*) increase signalling intensity during their lifetime (Candolin 2000) and ageing male scorpion flies (*Panorpa cognata*) allocate mating effort less discriminately between females of different quality (Engqvist & Sauer 2002). Parasite infection can similarly influence longevity and future reproductive success leading to increased mating effort in *Corophium volutator* amphipods (McCurdy et al. 2000), *Drosophila* fruit flies (Polak & Starmer 1998) and milkweed leaf beetles (Abbot & Dill 2001). Finally, the availability of resources may influence behavioural patterns. Male guppies (*Poecilia reticulata*) raised in environments with high food availability show higher levels of aggression towards other males whilst devoting less time to foraging (Kolluru & Grether 2005). In the absence of protein required for successful reproduction an alternative physiological mode is induced in the fruit fly (*D. melanogaster*) that effectively delays egg production and mortality (Carey et al. 1998). Through this process reproductive success and longevity can be optimised in the future despite sub-optimal conditions in the present.

Presence or absence of material suitable for offspring development has a direct effect on reproductive success and a corresponding association with oviposition preferences (Thompson 1988). Females of many invertebrate species, including coelopids, are found to express ovipositional preferences that may result in greater offspring survival or progeny of superior quality (Craig et al. 1989; Barker & Maczka 1996; Poore & Steinberg 1999; Dunn et al. 2002). Availability of oviposition material effectively limits the reproductive capacity of females and hence fecundity. Observations of increased male mating behaviour in *C. frigida* when exposed to seaweed are consistent with predictions of a trade-off between the success of reproduction at different life stages.

A previously unstudied aspect of *C. frigida* mating behaviour is the effect of an erratic and unpredictable environment. Generated at the ecotone between marine and terrestrial environments, wrack beds are a constantly fluctuating habitat, rapidly changing as tides and storm events wash away or deposit algal material. *C. frigida* are known to migrate, sometimes over long distances, so may encounter novel environments on a regular basis (Oldroyd 1954; Egglishaw 1961). To maximise reproductive success it is predicted that mating effort should track changing conditions, in response to the removal and deposition of algal material. The potential costs of remaining in a reproductively active state following removal of suitable oviposition material may be significant, especially if mating is costly. The true value of an adaptation may be measured not only in the response to a stimulus but the maintenance of that response following removal of the stimulus. In this study the effects of exposure and subsequent removal of algae on male mating behaviour are investigated to achieve a better understanding of allocation of mating effort in *C. frigida*.

5.2 Methods

5.2.1 Preparation

Virgin flies were collected from laboratory populations of *C. frigid*a established from wild larvae and adults collected at Crail, Fife (NO 6192 0782). Algae used in this study were collected from the same location.

5.2.2 Experimental procedure

Male flies were prepared for mating trials by exposure to *Laminaria*, *Fucus* or a sucrose only control. After 48 hours, all male flies were removed from containers and placed into new individual containers of the same size that contained only a sponge square soaked in 5% sucrose solution. Initial exposure of 48 hours to each treatment was chosen as it has previously been demonstrated that this duration of exposure results in a significant increase in male mating activity (Chapter 4). Subsets of males from each experimental group were entered into mating trials 2, 3, 4 or 5 days after initial preparation, equivalent to 0, 1, 2 or 3 days following removal from algae. Following mating trials the wing length of all flies was determined.

5.2.3 Statistical analysis

All data were checked for normality and transformed where necessary. Struggle duration required log transformation and the duration of copulation was cube root transformed. Binary logistic models were generated for analysis of male harassment and mating success. General linear models were generated for analysis of the duration of struggle and duration of copulation.

Separate models were created for each dependent variable; one for comparison of control treatments and another for comparison of the two algal treatments. Factors included in all models were time between initial preparation and mating trial (2, 3, 4 or 5 days), algal treatment (*Laminaria* or *Fucus*), male wing length and female wing length. Models were generated using SPSS v12.0.2 (SPSS Inc. Chicago IL, USA) and maximal models were selected based upon the Akaike Information Criterion calculated using R 2.5.1 (R Development Core Team 2007), non-significant terms being excluded from models. P-values for binary logistic models were determined using log-likelihood chi-squares.

5.3 Results

5.3.1 Sucrose treatments

The time that males were exposed to sucrose controls did not affect levels of harassment ($\chi^2_1 = 0.081$, $p = 0.776$), struggle duration ($F_{(3,159)} = 0.675$, $p = 0.569$), copulation ($\chi^2_1 = 0.067$, $p = 0.796$) nor copulation duration ($F_{(3,105)} = 1.456$, $p = 0.231$). Mating success was greater for larger males ($\chi^2_{(1)}=8.095$, $p=0.004$).

5.3.2 Algal treatments

Following exposure to algae, there was a significant effect of the time removed from this stimulus upon harassment level ($\chi^2_1 = 10.036$, $p = 0.002$), however the response to removal of seaweed was dependent upon the species of algae ($\chi^2_1 = 6.942$, $p = 0.008$). Though harassment levels remained consistent following removal of *Laminaria*, there was a significant reduction in harassment after removal of *Fucus* (Fig. 5.1). A similar difference in the effect of removal of *Laminaria* and *Fucus* is also evident in struggle duration ($F_{(4,288)} = 4.613$, $p = 0.001$; Fig. 5.2), longer struggle durations being observed following exposure to *Laminaria*.

Mating success was greater following exposure to *Laminaria* ($\chi^2_1 = 5.864$, $p = 0.015$) and was not affected by removal of algae ($\chi^2_1 = 0.468$, $p = 0.494$; Fig. 5.3). The duration of copulation was influenced by both the time removed from algae ($F_{(3,226)} = 3.288$, $p = 0.013$) and algal species ($F_{(1,226)} = 19.373$, $p < 0.001$). After removal of algae mating effort was maintained for longer following exposure to *Laminaria* when compared to *Fucus* (Fig. 5.4).

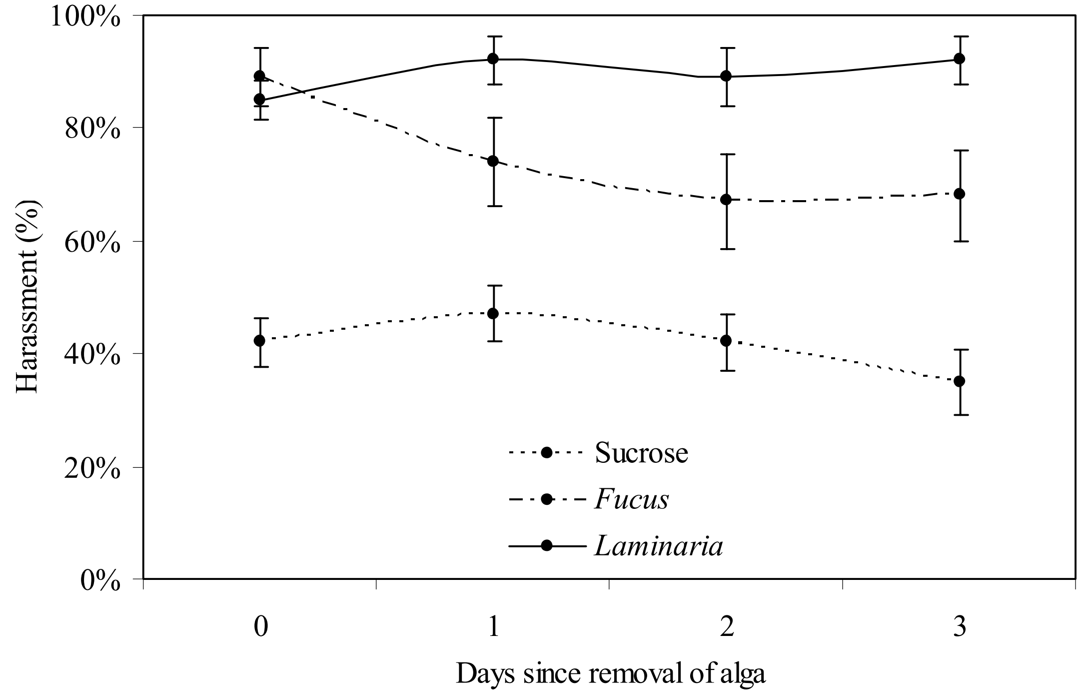


Figure 5.1 The level of harassment following removal of *Laminaria* and *Fucus* algae and during continued exposure to a sucrose control. Harassment is shown as the percentage of males that mounted a female. (Error bars show standard errors.)

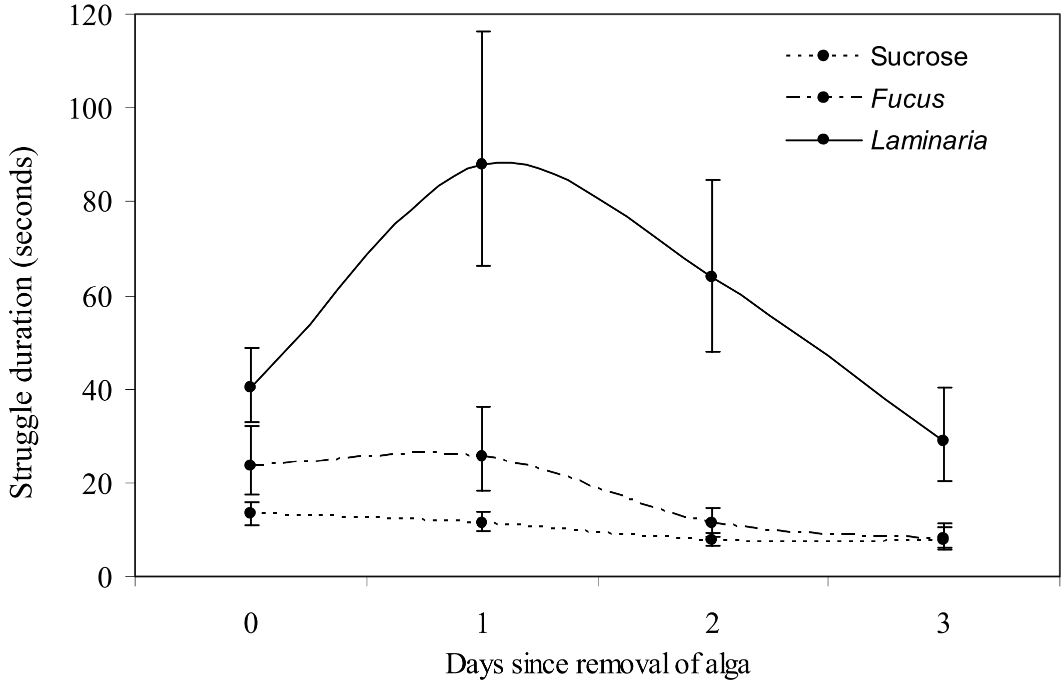


Figure 5.2 Struggle duration following removal of *Laminaria* and *Fucus* algae and during continued exposure to a sucrose control. (Error bars show standard errors.)

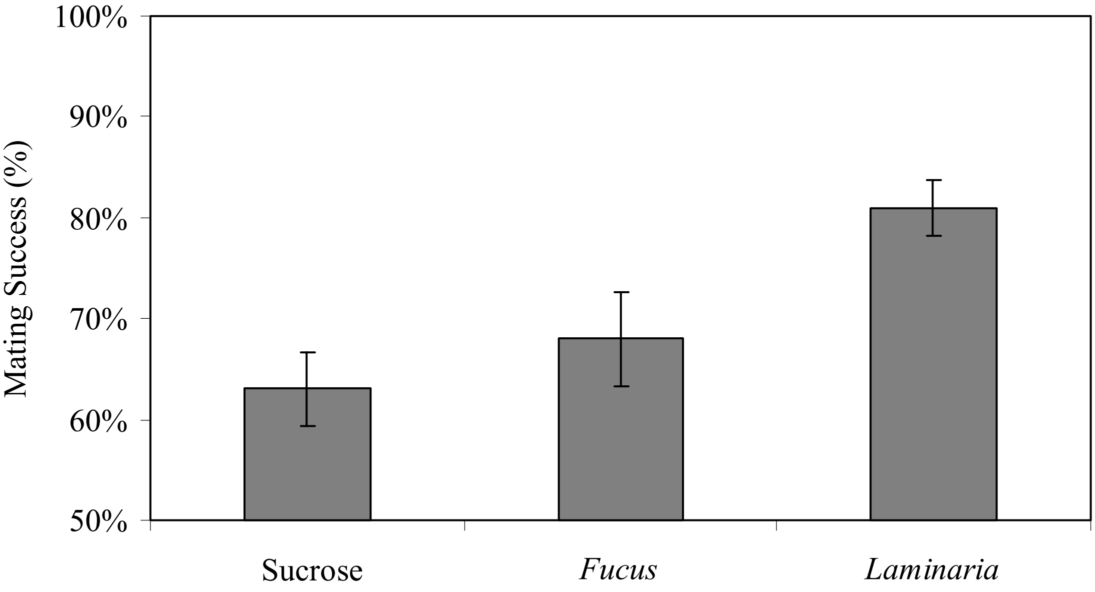


Figure 5.3 Mating success following exposure to *Laminaria*, *Fucus* and a sucrose control. Mating success is shown as the percentage of males that copulated. (Error bars show standard errors.)

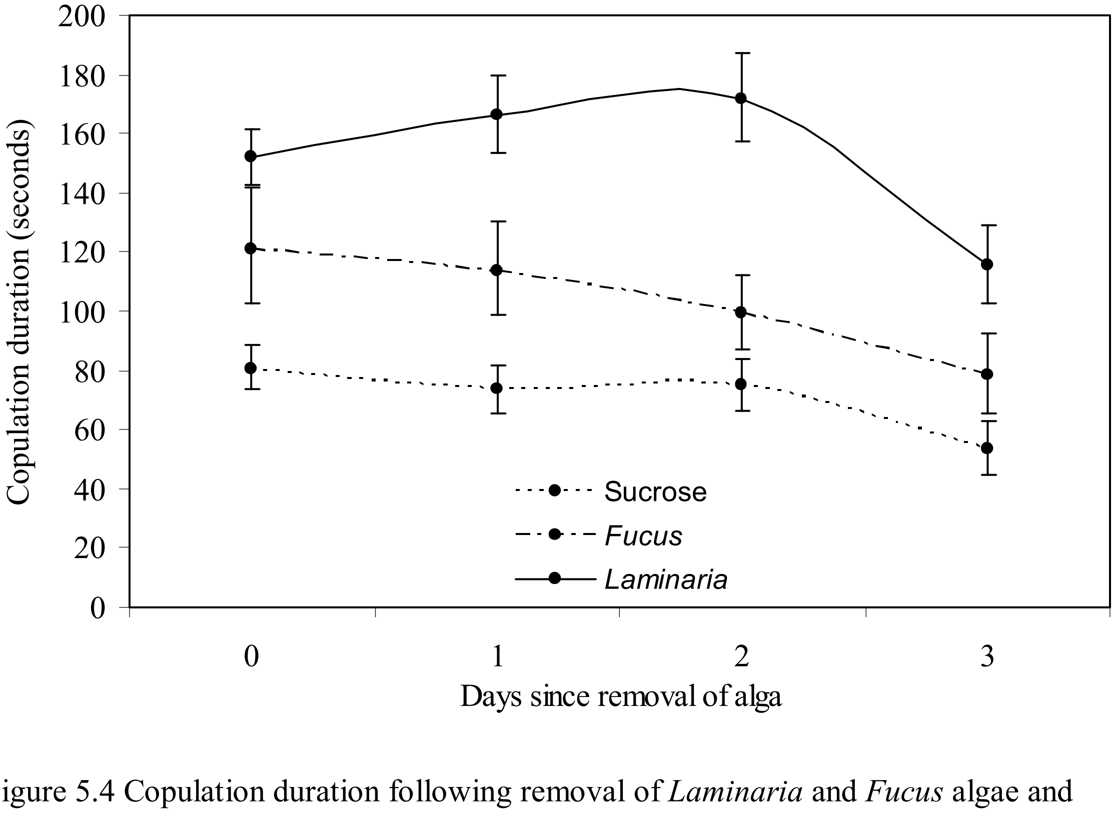


Figure 5.4 Copulation duration following removal of *Laminaria* and *Fucus* algae and during continued exposure to a sucrose control. (Error bars show standard errors).

5.4 Discussion

Results demonstrate that male *C. frigida* not only allocate mating effort following exposure to an algal stimulus but may reallocate effort after removal. Heightened male harassment following exposure to *Laminaria* confirms findings of previous work (Chapter 4). In addition it is shown that exposure to *Laminaria* can contribute to mating success and the effects of this alga are more sustained. The relative effects of *Laminaria* compared to *Fucus* on the *C. frigida* mating system may be even greater than previously estimated. When measuring the influence of a stimulus, the total effect may be considered a factor of both magnitude and duration. Two stimuli with effects of equal magnitude cannot be considered the same if this influence is not maintained for the same period. A stimulus with an effect of higher magnitude may be of limited impact if the influence is short lived. Adopting the correct experimental protocol is vital in drawing relevant conclusions. It is now possible to say with improved certainty that *Laminaria* has a greater effect than *Fucus* upon the mating system of *C. frigida*, not only in the magnitude but also the duration of effect.

In the absence of algae, and thus females ovipositing in material suitable for larval development, the potential reproductive benefits available to male *C. frigida* are greatly reduced. Following removal of algae, male mating success may depend upon the adoption of a successful trade-off strategy between mating effort under current versus future conditions. As female oviposition status can be determined by the species of algae present (Phillips et al. 1995b; Dunn 2001) this forms an indirect basis for male assessment of female fecundity. The observation of reduced harassment following removal of *Fucus* is consistent with expectations of a cost-benefit trade-off in response to reduced female oviposition (Galvani & Johnstone 1998; Reinhold et al. 2002). In comparison, relatively sustained mating activity following removal of *Laminaria* is paradoxical. If an adaptive explanation is assumed for this difference it is predicted that males exposed to *Laminaria* derive greater reproductive benefits following removal of this alga relative to *Fucus* exposed counterparts.

Behavioural preferences for *Laminaria* are likely to have evolved in response to this alga being more suited to development (Chapter 6). With the assistance of greater offspring viability and increased oviposition, matings when exposed to *Laminaria* may be more profitable. Improved allocation of future effort will be of greater necessity to individuals who have not prospered in previous encounters. Males exposed to *Fucus* may then have more incentive to preserve available reserves for the future. It is also possible that an ‘apparent’ absence of algae may lead to mistakes in the mating decision. The costs of such a mistake would be greater if *Laminaria* is perceived to be absent when it is not, as opposed to the perceived absence of *Fucus*. A delayed response to the removal of *Laminaria* may receive less penalty.

An alternative explanation lies in the physiological costs of exposure to seaweed as it has been found that exposure to algae incurs a cost to longevity in *C. frigida* (Dunn et al. 2002; Meader & Gilburn, unpublished). More recent results have further shown that costs to longevity are greater following exposure to *Laminaria* than *Fucus* (Chapter 8). Reduction in future reproductive prospects of *C. frigida* males following exposure to *Laminaria* would be expected to result in a greater investment in current opportunities.

Response to removal of a stimulus may be further confounded by physiological limitations. Very little is known of the basis to the algal stimulus, less so any difference in the mechanism of stimulus provided by *Laminaria* and *Fucus* (though see Kanmiya 1987). Assuming a chemical cue, this may be the effect of different compounds found in each species of algae or different concentrations of the same compound. Biochemical pathways leading to behavioural responses and the metabolism of these chemicals may ultimately dictate the ability to evolve an adaptive response.

This study has made a further significant contribution to our understanding of male mating behaviour in *C. frigida* and adaptation to local conditions. The implications of enhanced sexual conflict resulting from a sustained response to *Laminaria* echo those of the previous chapter. Trade-offs between current and future reproductive opportunities illustrate strategic male mating effort and a limited mating capacity of male *C. frigida*.

Chapter 6 - Condition dependent sexual conflict and the culture of *C. frigida* in different environments

6.1 Introduction

Female preference for male traits indicative of 'good genes' may provide indirect benefits and help to maintain costly female mating preferences (Chapter 1). Genetic variance in the male trait is required for female preference to be maintained through this mechanism. Strong selection directed by female preference is instead predicted to deplete genetic variance, creating the lek paradox (Borgia 1979; Falconer 1981; Taylor & Williams 1982; Kirkpatrick & Ryan 1991). Nevertheless mating preferences are widely observed and high additive genetic variance can be found in sexually selected traits (Pomiankowski & Møller 1995). Possible mechanisms sustaining variation in the face of directional selection have been the focus of a number of studies aimed at resolving the lek paradox (Taylor & Williams 1982; Pomiankowski & Møller 1995; Rowe & Houle 1996; Tomkins et al. 2004).

One resolution rests on the assumption that sexually selected traits are condition dependent and that there is high genetic variance associated with condition (Andersson 1982, 1986; Nur and Hasson 1984; Grafen 1990; Rowe and Houle 1996). By definition, sexually selected traits benefit an individual indirectly and do not contribute to the acquisition or efficient use of resources that would provide a net direct benefit to the bearer. Only individuals in better condition with the resources available can afford to exaggerate costly traits that contribute to reproductive fitness (Rowe & Houle 1996). A complex array of biological processes contribute to condition representing far greater potential for accumulating genetic variance. A process of female preference increasing covariance between a sexually selected trait, male condition and variance in genetic determinants of condition has been termed ‘genic capture’ (Rowe & Houle 1996). An important prediction of genic capture is that all sexually selected traits will develop condition dependence, including those under Fisherian selection (Rowe & Houle 1996; Lorch et al. 2003). A number of studies have confirmed condition dependence of sexually selected traits (Wilkinson and Taper 1999; Kotiaho et al. 2001; Rantala et al. 2003; Bonduriansky and Rowe 2005; Hedrick 2005).

Female preferences may not always make a direct contribution to fitness and their expression can be costly (Pomiankowski, 1987; Reynolds and Gross, 1990). Females in enhanced condition may be better able to express strong mating preferences so that the principles of genic capture may similarly apply to female preference. Awareness of condition dependence of female preferences and implications for the evolution of mating systems is attracting increased attention (Tomlinson and O’Donald 1996; Cotton et al. 2006). Local conditions affecting hunger in female water striders, *Gerris buenoi*, can affect mating rates and selection for large male size (Ortigosa and Rowe, 2002). Female black field crickets, *Teleogryllus commodus*, in better condition are more sexually responsive and exhibit greater preference for male call rate and dominance (Hunt et al. 2005). Linkage disequilibrium between genetic determinants of female condition and preference may present additional indirect ‘good genes’ benefits of assortative mating (Tomlinson and O’Donald, 1996).

Sexual selection for good genes requires that sexually selected traits are heritable (Chapter 1). In *C. frigida* this has been demonstrated as the *αβ* chromosomal inversion system significantly contributes to the size of adults (Butlin et al. 1982a;

Wilcockson et al. 1995). Up to 25% of variance in size may be attributed to the inversion system in natural populations (Wilcockson et al. 1995). Due to heterokaryotypic advantage selection for large males may not be advantageous to all females dependent upon local conditions. Alternatively it is proposed that female mating preferences are maintained through convenience polyandry and Fisherian selection (Chapter 1).

Adult size is determined by the contribution of both genetic and environmental variance. Furthermore, the level of expression of a genotype and relative success of genotypes can be environmentally determined. The relative contribution of genetic variance to size (the heritability of size) will then depend on the local environment. Genotype by environment interactions may be particularly important in the evolution of mating systems through determining condition and the relative benefit of different genotypes. The influence of such processes has previously been demonstrated in *C. frigida* through variation in tidal range (Gilburn & Day 1994c).

Coelopa frigida are entirely dependent upon marine algae in wrack beds for development and reproduction and so are highly adapted to this resource. It is perhaps not surprising then, that variation in this habitat should influence reproductive behaviour (Phillips et al. 1995b; Dunn 2001; Dunn et al. 2002; Chapters 4 & 5). If algal specific behaviours are the result of selection to reproduce on more nutritious substrates it is expected that *C. frigida* development will differ between algae. Differences in development may involve the condition of individuals, success of different genotypes and heritability of sexually selected traits; factors that may have significant influence on different mechanisms maintaining a mating bias. All work considering mating behaviour in *C. frigida* prior to this thesis has adopted *Fucus* algae as the culture medium. No comparison of mating behaviour following development on other algae has been undertaken; indeed no comparison of development on different algae has been completed.

6.2 Objectives

The purpose of this study is to further quantify the role of the environment in shaping the mating system of *C. frigida*. This is achieved by examining the relationship between larval development in different algae and subsequent mating patterns. Adult size in *C. frigida* depends upon larval nutrition and can be positively correlated with survival (Butlin and Day, 1985), fecundity (Butlin and Day, 1985; Pitafi et al., 1995) and mating success (Gilburn et al., 1992). Being a sexually selected trait in males, size is predicted to be condition dependent and hence a reliable measure of condition in this species. If different species of algae are of different nutritional quality this provides the opportunity to rear flies of different condition. The condition dependence of sexually selected traits and sexual preferences can then be compared in different populations. In addition, if culture alga contributes to condition this should also be evident in female fecundity. As behaviour in *C. frigida* is oriented towards *Laminaria* it is hypothesised that this alga is more suited to larval development, producing adults of higher condition with superior female preferences and male sexually selected traits.

Genotype by environment interactions and different contributions of environmental variance are predicted to influence the development of flies with different inversion karyotypes. Development on different algae is predicted to affect aspects of life history such as heterokaryotype advantage, development time, egg to adult viability and the relative size of flies. If the success of different genotypes varies between algal diets then the relative contribution of good genes selection, Fisherian selection and convenience polyandry in the maintenance of sexual preferences may also differ between populations.

Male size has previously been identified as a male persistence trait in coelopids (Gilburn et al. 1992; Crean et al. 2000; Weall and Gilburn 2000); however, it is probable that males possess multiple persistence traits (Rowe et al. 2005; Moore and Pizzari 2005). Consequently, two further potential persistence traits in addition to male size are analysed; struggle duration and the time preceding a mount. Struggle duration may be a direct measure of the ability of a male to withstand the female rejection response and thus a positively selected trait. Time preceding a mount is equivalent to the time taken to locate and mount a female and so is an indirect measure of the intensity with which a male searches for and harasses females.

To address these objectives, this study has been completed in two parts. In the first part of the study *C. frigida* are cultured on different algae and subjected to mating trials. Techniques were subsequently improved so that in the second part of this study the density of larvae in different cultures is controlled. This allowed for more direct comparison of development and success of inversion karyotypes between cultures. A repeat of mating trials in conjunction with analysis of oviposition preferences were conducted on this second set of flies.

6.3 Methods – Part I

6.3.1 Preparation

C. frigida larvae were collected from 4 locations around the Firth of Forth in Autumn 2005 and Spring 2006; Cellardyke (NO578039), Crail (NO620079), St Monans (NO521013) and Whitesands (NT709774). Algae, *Fucus* spp. and *Laminaria* spp., were collected from Crail in Autumn 2005. *Fucus* and *Laminaria* were carefully sorted and rinsed to remove other algal species and epiphytes. Cultures of *C. frigida* were initiated using either *Fucus* or *Laminaria* algae.

6.3.2 Mating trials

Virgin adult flies from both culture media were used in mating trials. Prior to mating trials male flies were exposed for 48 hours to either *Laminaria* or *Fucus* algae. A full factorial experimental design was adopted as females cultured on *Fucus* and *Laminaria* were paired with males cultured on and exposed to *Fucus* and *Laminaria*. Following mating trials the wing length of all flies was determined.

6.3.3 Statistical analyses

All data were checked for normality prior to analysis. The time preceding each mount and struggle duration were both positively skewed requiring logarithmic and reciprocal cube root transformations respectively. Binary logistic models for male harassment and mating success and General Linear Models of time preceding mount and struggle duration were created separately using SPSS v12.0.2 (SPSS Inc. Chicago IL, USA). In order to investigate different male persistence traits contributing to mating success additional general linear models were created that also included struggle duration and time preceding the mount. Male culture alga, female culture alga, male algal stimulus and the wing length of each sex were initially included in all models. Maximal models were selected based upon the Akaike Information Criterion calculated using R 2.5.1 (R Development Core Team 2007); non-significant terms being excluded from models. Preference functions were illustrated in cubic splines with 10,000 bootstrap replicates to estimate 95% confidence intervals using GLMS version 4.0 (Schluter, 1988).

6.4 Results – Part I

6.4.1 Summary of results

A total of 347 mating trials were completed comprising a minimum of 40 mating trials within each experimental group. Flies cultured using *Laminaria* were significantly larger than those raised on *Fucus* (Males: $t_{345} = 3.203$, $p = 0.001$, Females: $t_{345} = 5.748$, $p < 0.001$; Fig. 6.1). Males were larger than females when bred on either media. The relative size of male and female flies did not differ significantly between treatments ($t_{345} = 0.789$, $p = 0.431$), females being approximately 97% the size of their average male counterparts when raised on either alga.

6.4.2 Male harassment

Small males were more likely to harass females than large males ($\chi^2_1=13.350$, $p <0 .001$). A modest, yet significant, difference in harassment rate between males bred on the different algae was observed ($\chi^2_1 = 4.975$, $p = 0.026$). 82% of males raised on *Laminaria* attempted to mate with their given female compared to 77% of males raised on *Fucus*. The difference in harassment rates between large and small males was more evident when reared on *Fucus* than *Laminaria* ($\chi^2_1 = 3.843$, $p = 0.050$; Fig. 6.2).

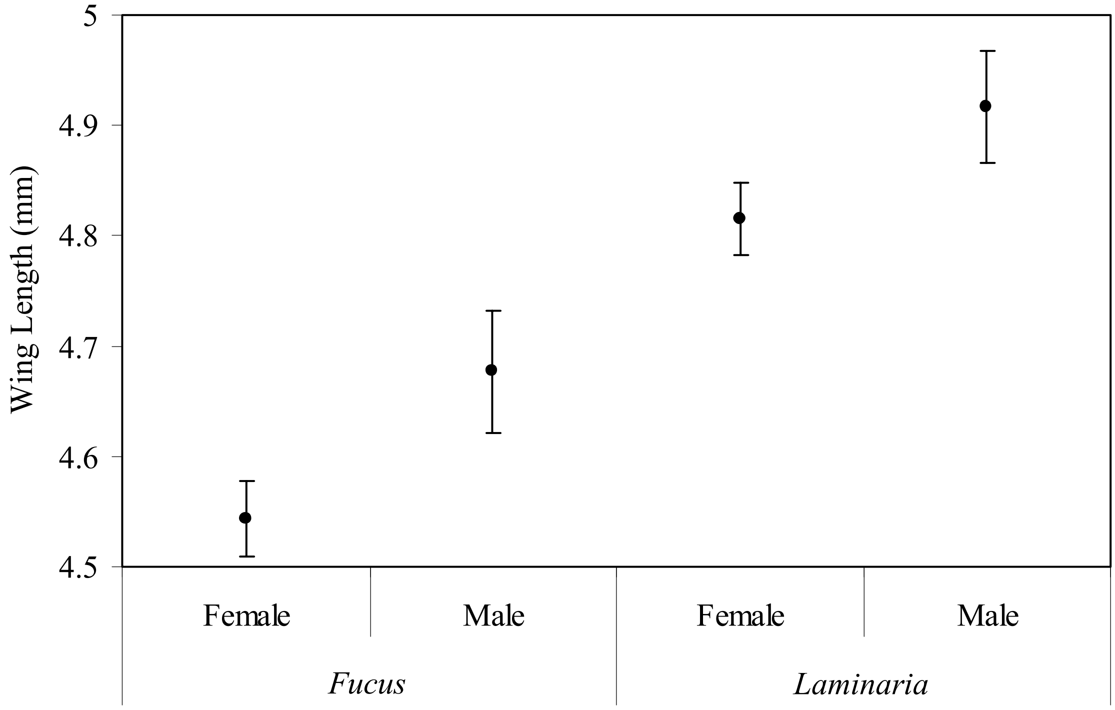


Figure 6.1 The mean size of male and female *C. frigida* when bred on *Laminaria* and *Fucus*. (Error bars show standard errors.)

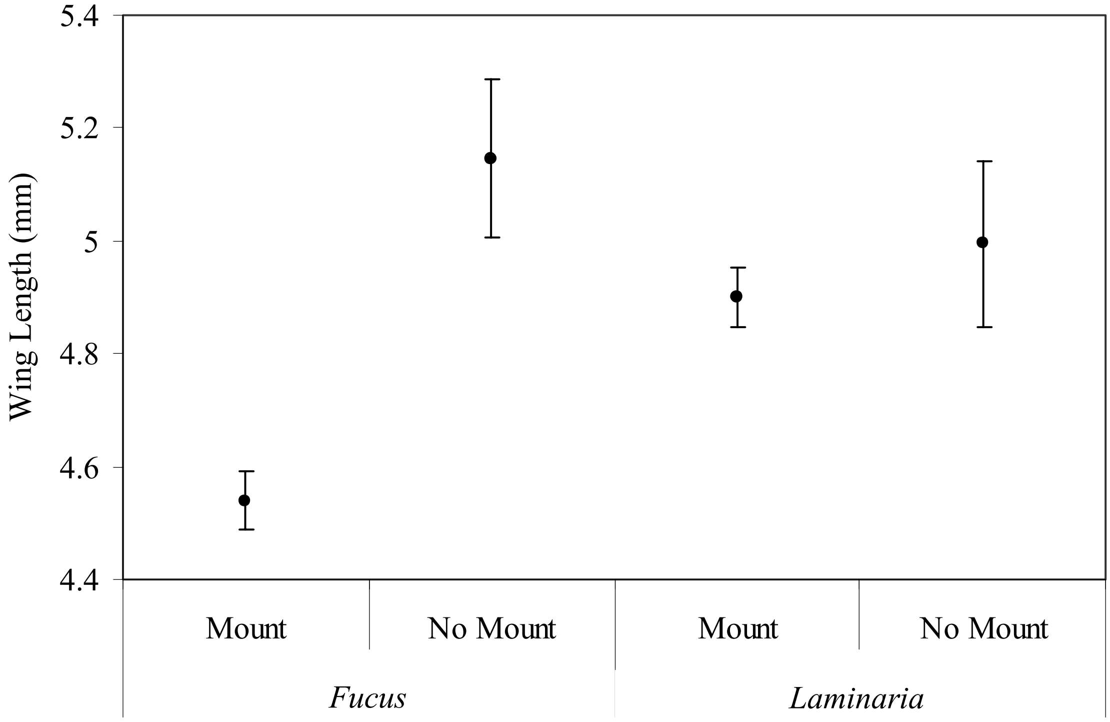


Figure 6.2 The mean size of males bred on *Fucus* and *Laminaria* that did and did not mount a female. (Error bars show standard errors.)

6.4.3 Time preceding a mount

The time taken for a male to mount a female was also determined by culture alga, but that of the female rather than the male ($F_{(1,273)} = 4.760$, $p = 0.030$). Males mounted females reared on *Laminaria* sooner than those reared on *Fucus* (Fig. 6.3). Despite the larger size of females raised on *Laminaria*, there was no correlation between female size and the time before a male mounted ($F_{(1,274)} = 0.219$, $p = 0.640$).

6.4.4 Struggle duration and copulation success

Struggle duration was positively influenced by both male ($F_{(1,271)} = 12.523$, $p < 0.001$) and female ($F_{(1,271)} = 13.332$, $p < 0.001$) size. This effect is reflected in the struggle durations of pairs of flies from different culture combinations following the correlation between size and culture alga (Fig. 6.4). The success of male mating attempts was determined by an interaction between male breeding and male stimulus ($\chi^2_1 = 5.368$, $p = 0.021$; Fig. 6.5). Both culture with and exposure to *Laminaria* algae resulted in increased mating success.

6.4.5 Male persistence traits

The most significant male persistence trait influencing copulation success was the time preceding a mount ($\chi^2_1 = 9.387$, $p = 0.002$), however there was a significant interaction between this term and male culture alga ($\chi^2_1 = 4.000$, $p = 0.045$). There was a much greater difference in the time preceding a mount between successful and unsuccessful males raised on *Fucus* than *Laminaria* (Fig. 6.6). Overall there was no significant difference in the time preceding a mount between *Fucus* and *Laminaria* reared males ($t_{274} = 0.801$, $p = 0.424$).

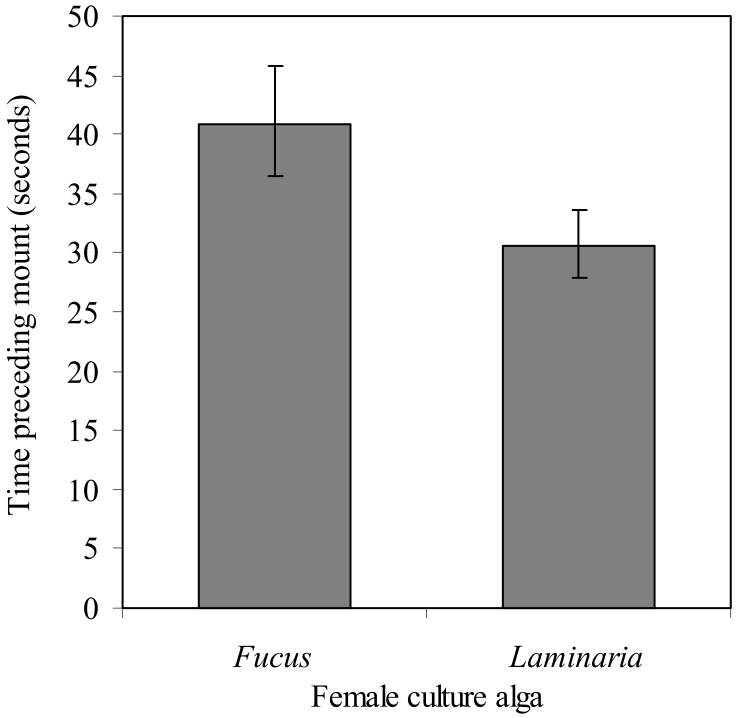


Figure 6.3 The mean time preceding a mount for females bred on *Laminaria* and *Fucus*. (Error bars show standard errors.)

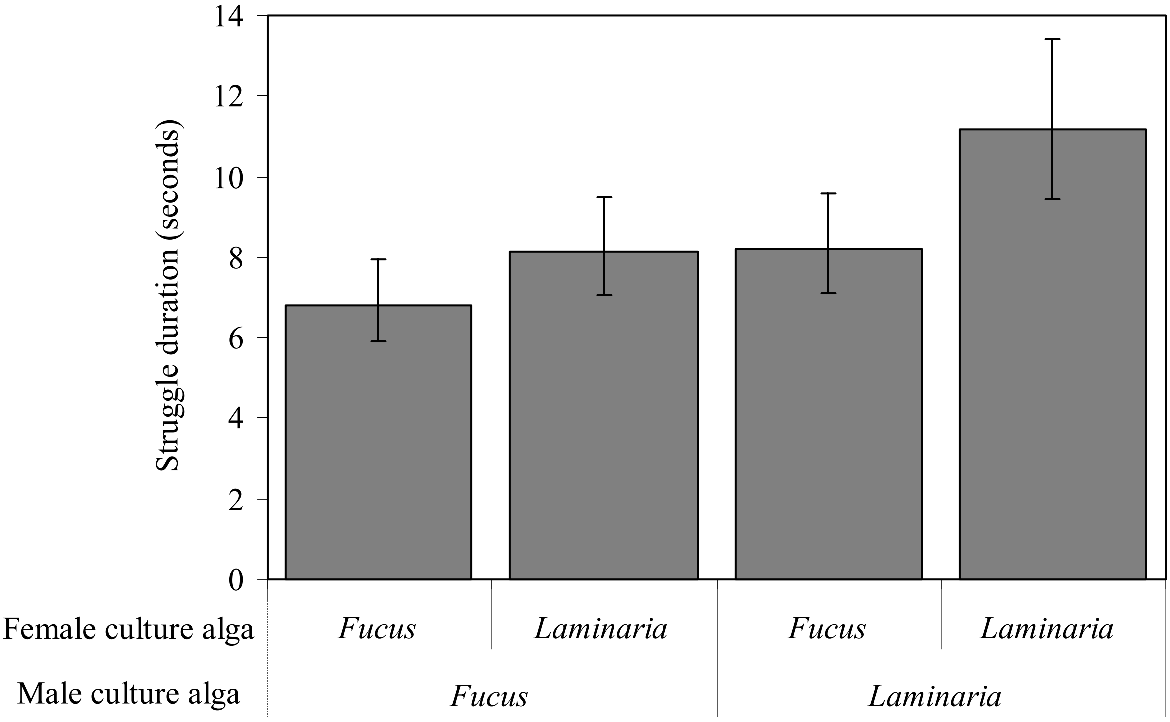


Figure 6.4 The mean struggle duration for pairs of male and female flies cultured on either *Laminaria* or *Fucus*. (Error bars show standard errors.)

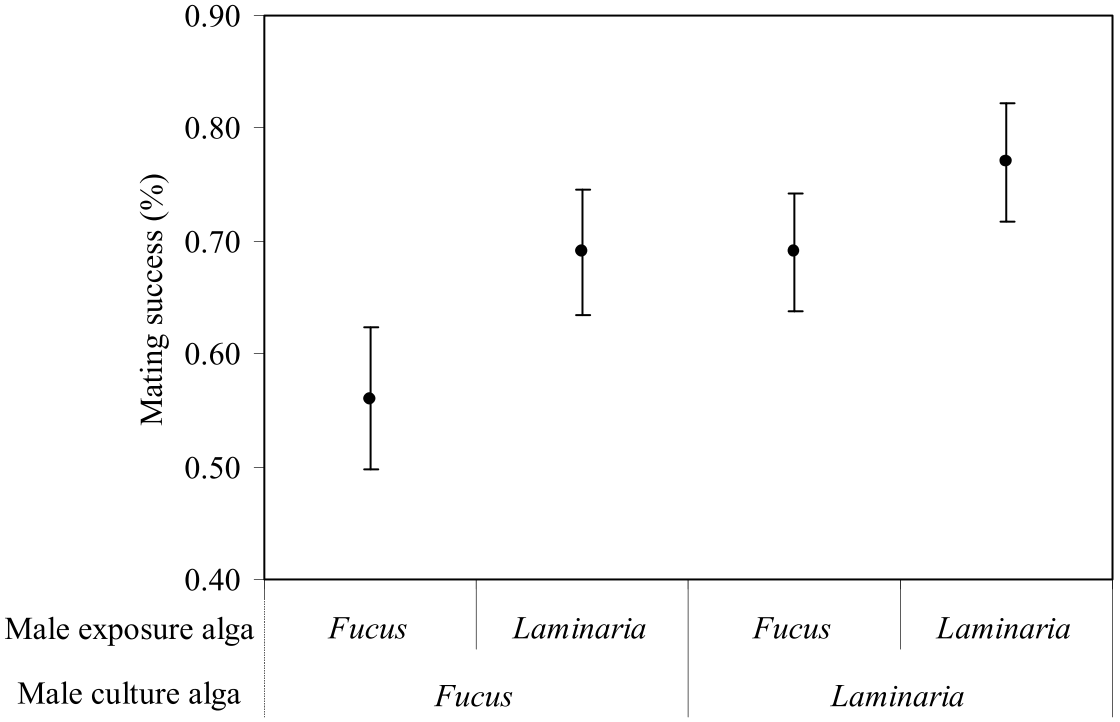


Figure 6.5 Mating success of males cultured on and exposed to either *Laminaria* or *Fucus*. Mating success is shown as the percentage of males that copulated. (Error bars show standard errors.)

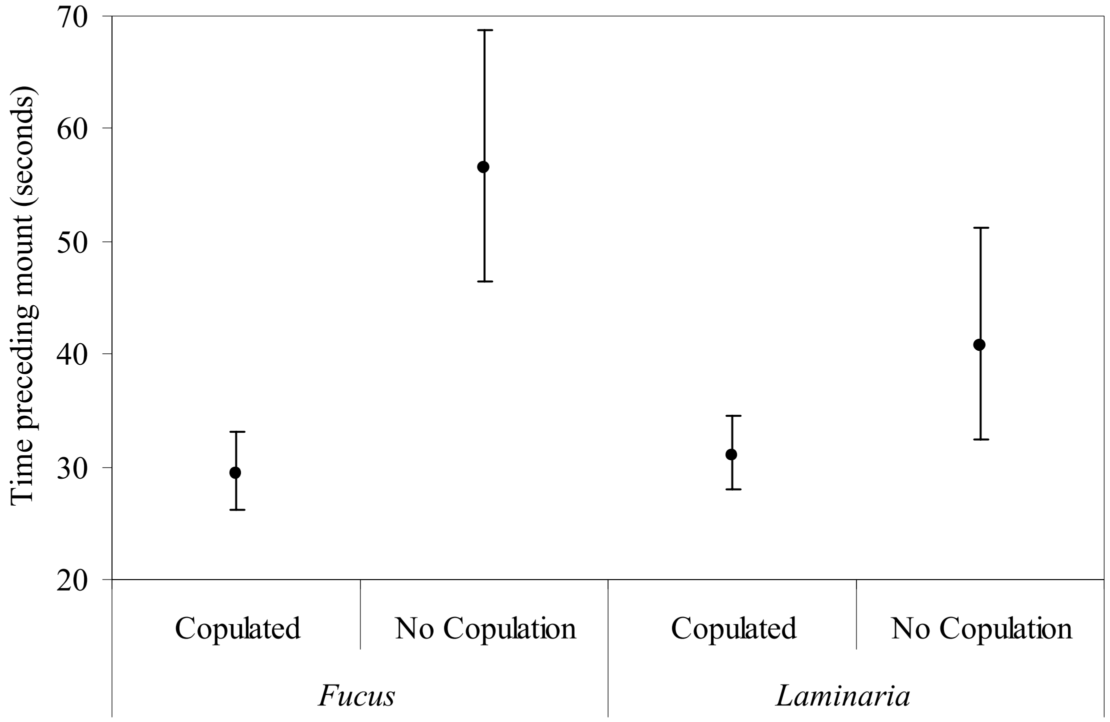


Figure 6.6 The time preceding a mount for successful and unsuccessful males cultured on either *Laminaria* or *Fucus* algae. (Error bars show standard errors.)

Separate models were used to identify male persistence traits in *Fucus* and *Laminaria* reared males. These models confirmed that neither male size ($\chi^2_1 = 0.197$, p = 0.657), struggle duration ($\chi^2_1 = 0.023$, p = 0.879) nor time preceding a mount ($\chi^2_1 = 1.439$, p = 0.230) were sensitive to female preference in *Laminaria* reared males. In *Fucus* reared males there was a significant female preference for shorter times preceding a mount ($\chi^2_1 = 8.938$, p = 0.003; Fig. 6.6) and for male size ($\chi^2_1 = 4.834$, p = 0.028; Fig. 6.7). Clear differences in female preference function are apparent for the time preceding a mount and male size when presented with a male raised on either *Laminaria* or *Fucus* (Fig. 6.8).

A difference can also be seen in preference function when females are reared on either *Fucus* or *Laminaria* algae (Fig. 6.9). When raised on *Laminaria* female preference was not sensitive to male size ($\chi^2_1 = 2.793$, p = 0.095), struggle duration ($\chi^2_1 = 0.836$, p = 0.360) or time preceding a mount ($\chi^2_1 = 0.785$, p = 0.376). However, when reared on *Fucus* there was a significant female preference for males that mounted sooner ($\chi^2_1 = 10.949$, p = 0.001). The difference in preference when reared under different conditions can again be seen in the sexual selection functions (Fig. 6.10).

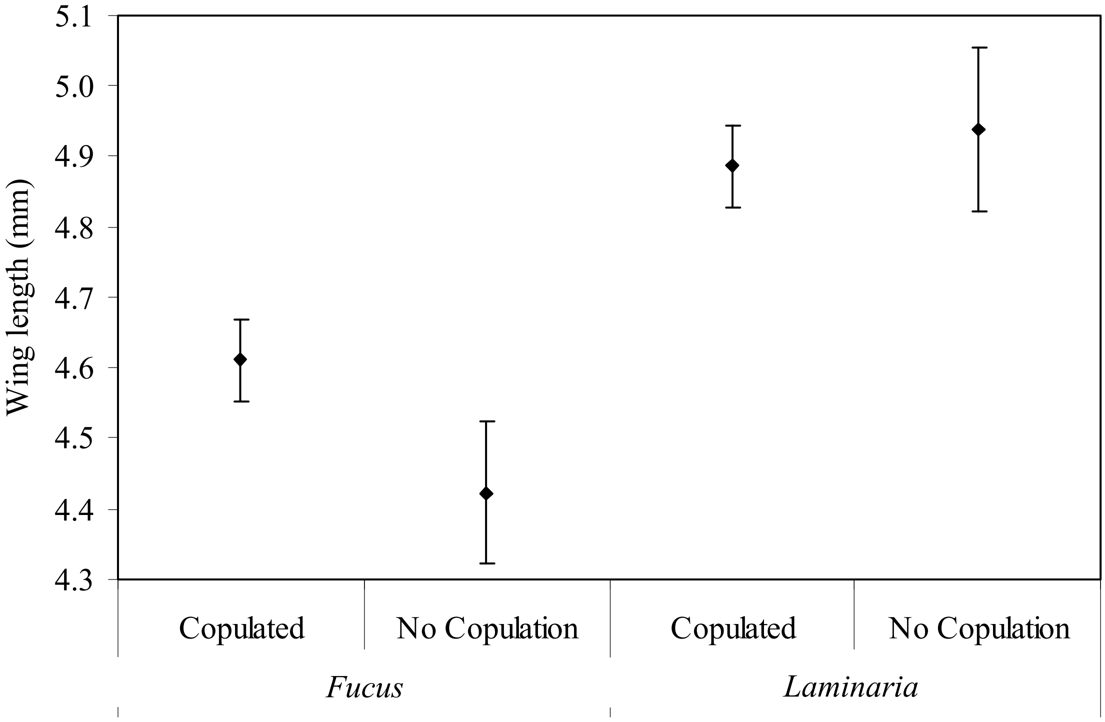


Figure 6.7 The size of successful and unsuccessful males cultured on either *Laminaria* or *Fucus* algae. (Error bars show standard errors.)

Figure 6.8 (a-d) Female preference for time preceding a mount and male size when males are raised on *Fucus* & *Laminaria* algae. a) Female preference for time preceding a mount when males are raised on *Fucus.* b) Female preference for time preceding a mount when males are raised on *Laminaria.* c) Female preference for male size when males are raised on *Fucus.* d) Female preference for male size when males are raised on *Laminaria*. Dashed lines show 95% confidence intervals.

Figure 6.8a

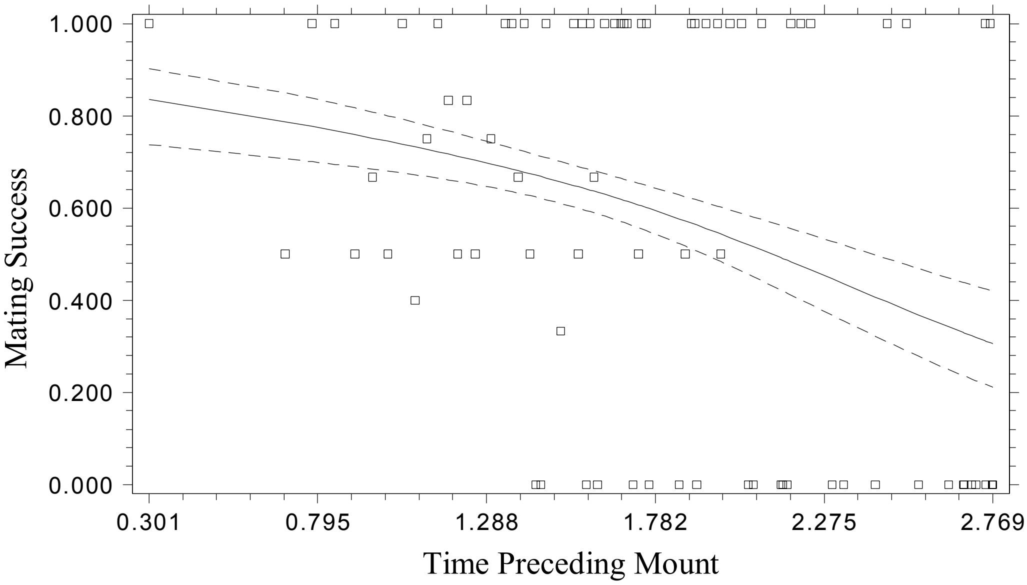


Figure 6.8b

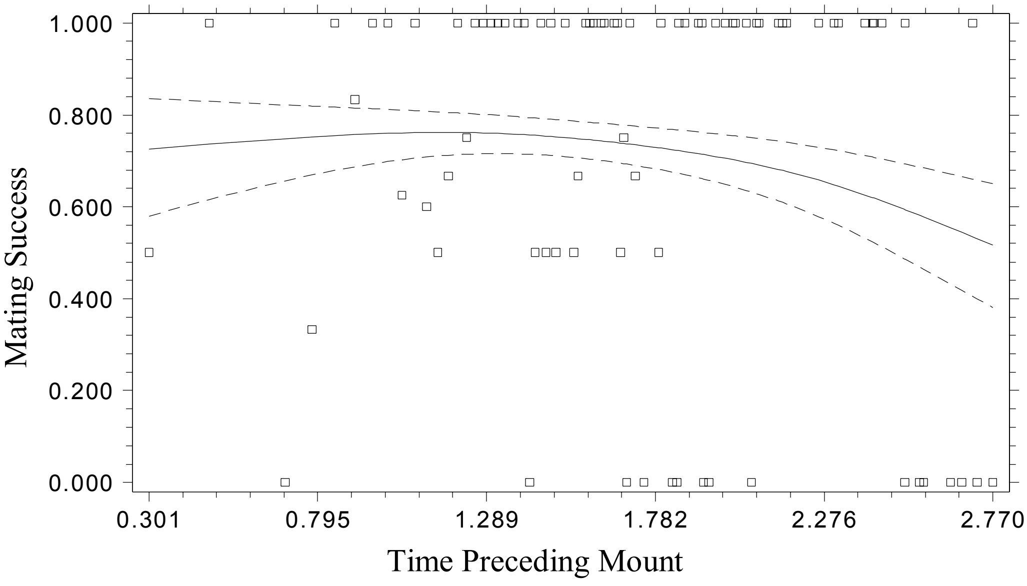


Figure 6.8c

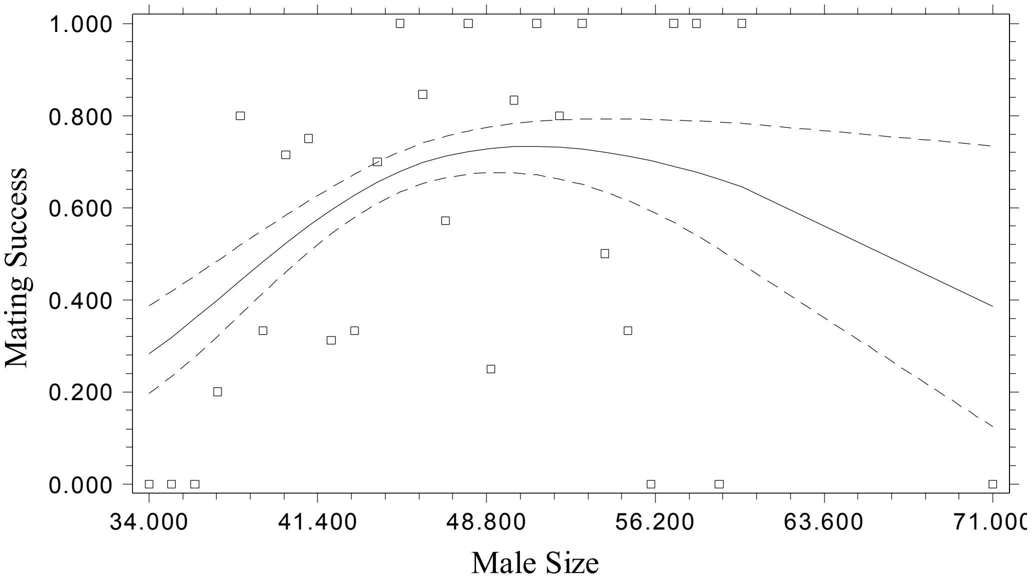
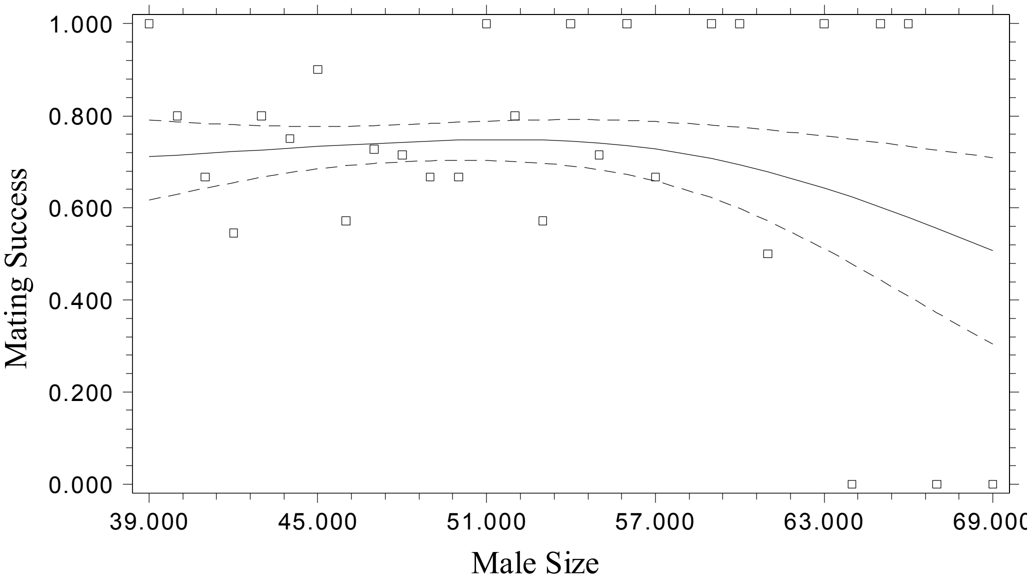


Figure 6.8d



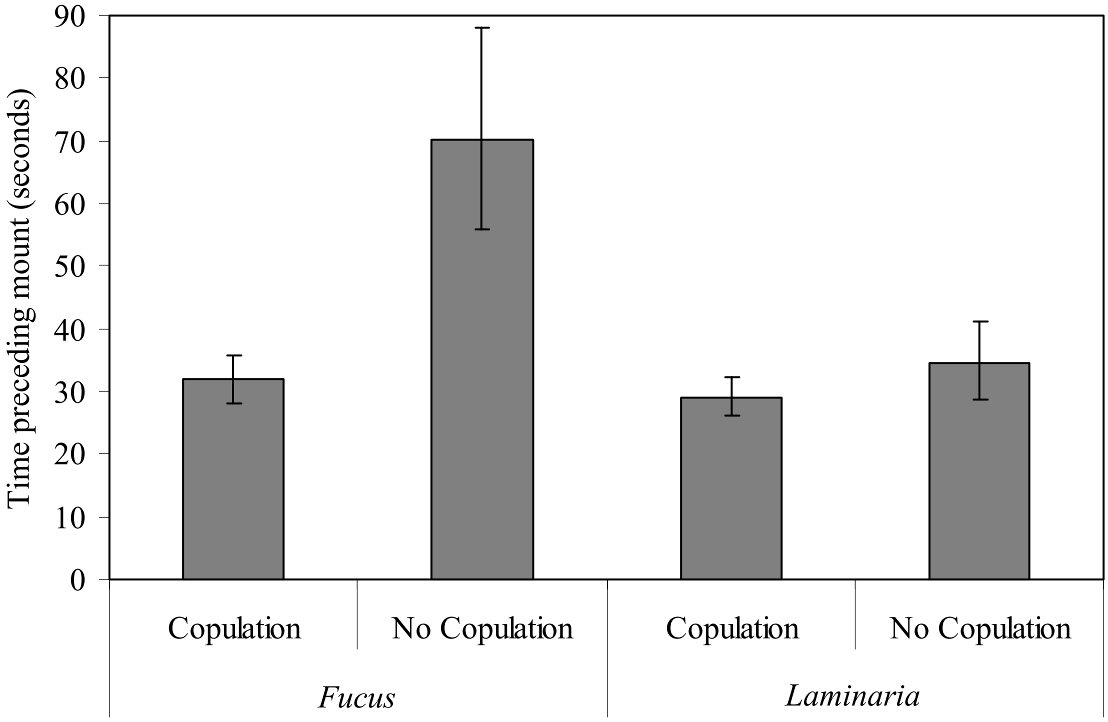


Figure 6.9 The time preceding a mount for successful and unsuccessful females cultured on either *Laminaria* or *Fucus* algae. (Error bars show standard errors.)

Figures 6.10a-b Female preference for time preceding a mount when females are raised on (a) *Fucus* & (b) *Laminaria* algae. Dashed lines show 95% confidence intervals.

Figure 6.10a.

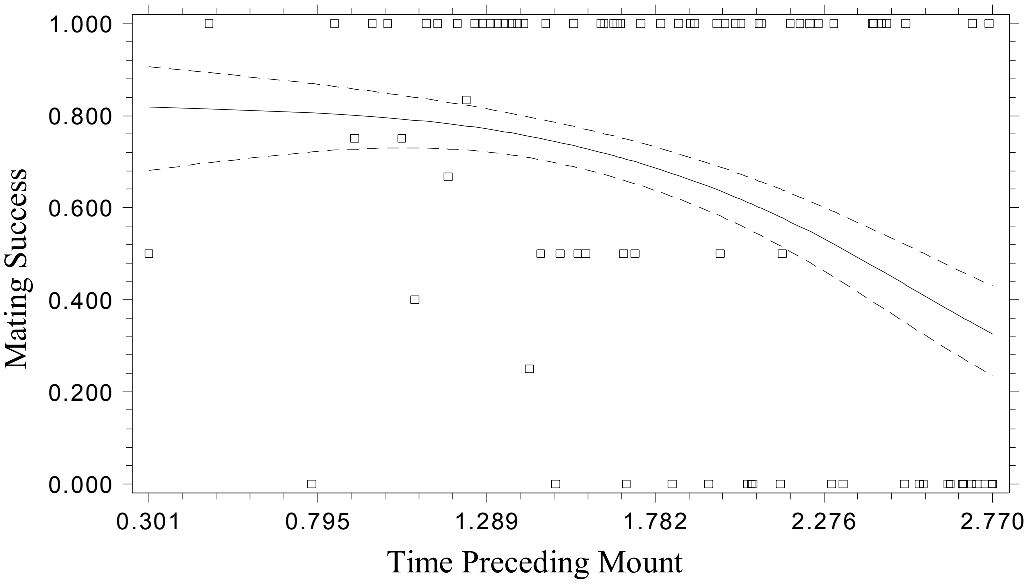
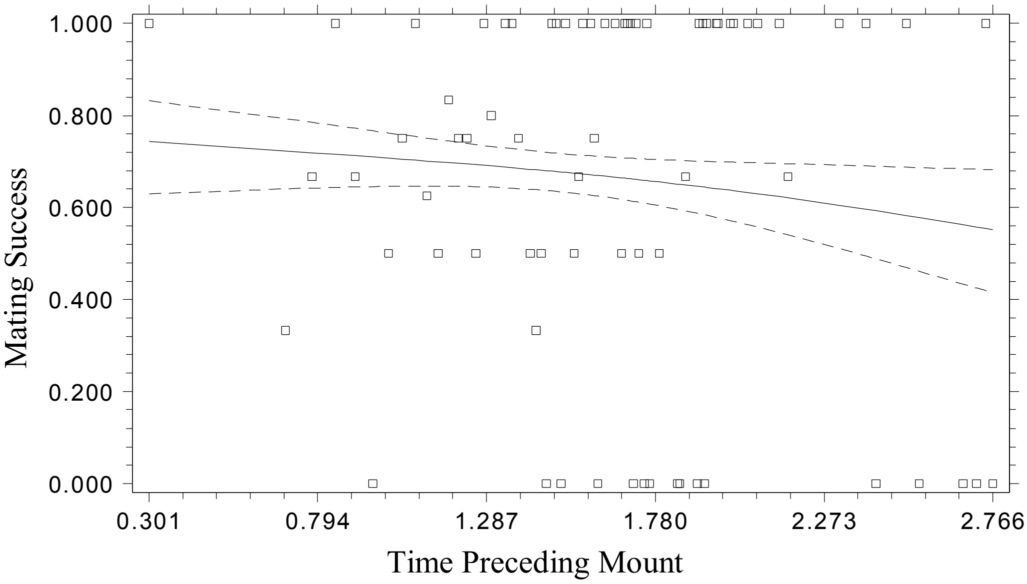


Figure 6.10b.



6.5 Discussion – Part I

6.5.1 Harassment by small males

Small males were found to be more likely to harass females than large males. Increased willingness of small males to mount a female has previously been recorded in *C. frigida* (Dunn et al. 1999). This behaviour may increase the lifetime mating success of small males when compared to larger males. Smaller males, with a corresponding shorter development time, have a greater mating advantage soon after eclosion as they will get first access to females. Expending greater effort during this period may be more profitable than preserving resources.

The reduced difference in size dependent harassment when reared on *Laminaria* may be explained in a number of ways. Firstly, as males reared on *Laminaria* are more likely to harass a female the number of males not harassing females is lower. The ability to detect a size difference between these two groups of males would then be made more difficult from a statistical perspective because of sample sizes. Though there was a difference in harassment rates between males cultured on *Fucus* and *Laminaria* this was small relative to the number of mating trials completed so this is not an entirely sufficient explanation.

Alternatively, when reared on *Fucus* large males may be attempting to mate at a lower frequency, rather than small males attempting to mate at a higher frequency. Due to high promiscuity the costs of mating to males may be considerable and of a similar nature to the costs of mating normally associated with females; increased risk of predation, reduced foraging time, energetic costs, time costs etc. (Stockley 1997). Males must balance these costs against the benefits of increased reproductive success resulting from multiple mating. When raised on *Fucus* the costs of mating may be increased for both large and small males if it is assumed this algae yields poorer condition. Large males may offset these costs through a reduced frequency of mating attempts whilst maintaining an element of reproductive success as large size is an advantage on the few occasions that an attempt is made. The large male mating advantage dictates that there is less cost to large males that show any form of mate choice or decrease in their willingness to mate. Small males may not be able to afford to lower the frequency of mating attempts if the chances of success are less likely. The improved condition of males cultured on *Laminaria* could provide the additional reserves required to withstand the costs of a high frequency of multiple matings regardless of size.

The Ghiselin-Reiss hypothesis predicts that small males require less time to forage, so can afford to expend more time on reproductive activities (Ghiselin 1974; Reiss 1989; Dunn et al. 1999). It is not clear to what extent adult *C. frigida* require foraging though a source of moisture is necessary for survival. If *Laminaria* provides more resources to adult *C. frigida* then large males may have to expend less time foraging. Finally, the increase in male size as a result of female rejection may have drawn males away from their optimum size to the extent that they have suffered a reduction in fitness that is demonstrated in a decreased willingness to mate. This reduction in fitness may be more evident when reared on *Fucus*.

6.5.2 Females cultured on *Laminaria* mounted sooner

The size of *C. frigida* females is positively associated with fecundity and it is suggested that male *C. frigida* demonstrate mate choice on the basis of gravidity (Pitafi et al. 1995). Males may also be able to assess the future reproductive prospects of individual females and adjust mating behaviour accordingly (Dunn et al. 2001). The larger size of females reared on *Laminaria* and shorter time to mount these females is consistent with predictions of male mate choice. However, previous predictions of male mate assessment have considered choice on the basis of post-mounting behaviour, after direct contact between two flies has been made (Pitafi et al 1995; Dunn et al. 2001). If males are choosing to mount more fecund females sooner this would require the ability to assess female condition before contact is made. The lack of a significant relationship between female size and the time preceding a mount suggests that this assessment is carried out irrespective of female size.

Two possible mechanisms to explain this result are suggested. Firstly, there may be chemical mediation of attraction between the sexes. This could be a pheromone produced by the female for the purpose of attracting a mate; though given the female rejection response such behaviour would be contradictory. The metabolism of *Laminaria* may produce either a distinct chemical compound or a greater amount of a chemical compound that is attractive to male*s*. This compound may attract males directly or merely stimulate males to search more intensively for a mate. Secondly, female *C. frigida* raised on *Laminaria* may be more active as a result of improved condition. A more active female, whether searching for a mate, oviposition site or merely foraging, will be more likely to encounter a male. The time preceding a mount in *C. frigida* may be determined as much by the relative levels of activity of the sexes as any form of mate choice.

Irrespective of the mechanism, the decreased time preceding a mount for females raised on *Laminaria* can be interpreted as a greater level of sexual conflict. The average time preceding a mount for a female raised on *Laminaria* is just 30.6 seconds, compared to 40.9 seconds for females raised on *Fucus*. Thus, a *Laminaria* raised female may be mounted 1.3 times more often than a *Fucus* raised female.

6.5.3 Struggle duration

The duration of mating struggles was positively associated with the size of both male and female flies. As culture conditions are related to size this relationship is evident in the struggle durations of different *Laminaria* and *Fucus* raised pairs. The longest struggles are observed when both male and female flies are raised on *Laminaria* (Fig. 6.4). In the three other pair combinations struggle durations are much reduced. This may be due to reduced combined fitness, in the case of *Fucus-Fucus* pairs, or an inequality of strengths if flies have different culture histories, so that one fly may overcome the other sooner. As individual resources are required to maintain struggle duration these results are consistent with the prediction of improved condition of larger flies and flies reared on *Laminaria*.

6.5.4 Sexual conflict in different environments

Males raised on *Laminaria* are larger than those raised on *Fucus*. As smaller males are more likely to harass females it is then perhaps more significant that males cultured on *Laminaria* harass females more often. Greater harassment by males cultured on *Laminaria* is further indication of improved condition.

Individuals cultured on and exposed to *Laminaria* showed the highest mating success. The relative importance of exposure and rearing on *Laminaria* for mating success appears to be equal. Males cultured on *Laminaria* and exposed to *Fucus* had a mating rate similar to that of males raised on *Fucus* and exposed to *Laminaria* (Fig.

6.5). It is interesting to note the magnitude of difference between the success of males raised and exposed to *Laminaria* (77%) compared to those males raised on and exposed to *Fucus* (56%). Considering that culture conditions can also influence the time preceding a mount (Fig. 6.3) and struggle duration (Fig. 6.4) this highlights the importance of considering not only current conditions to which an individual is exposed, but the life history of that individual in order to place behavioural observations in the correct context. A combination of both larval and adult experience contributes to the mating success of male *C. frigida*.

6.5.5 Condition dependent sexual conflict

Individual condition can be defined in terms of the amount of resources available for allocation to fitness enhancing traits (Lorch et al. 2003). A number of results from this study suggest that flies reared on *Laminaria* are in better condition. In addition to greater size of both males and females, higher harassment rates and mating success of males reared on *Laminaria*, shorter time to mount for females raised on *Laminaria* and longer struggle durations of *Laminaria* raised pairs are all consistent with improved condition. Analysis of multiple male persistence traits demonstrates interesting results previously unrecorded in this species (Fig 6.8). None of the male persistence traits appear sensitive to female preference when males are cultured on *Laminaria*; relatively consistent success occurring irrespectively. Conversely, males reared on *Fucus* were preferred on the basis of both larger size and shorter time to mount. This result concurs with the large male advantage reported in previous studies but further highlights that other male persistence traits may also be undergoing selection. The consistent success of males raised on *Laminaria* may be explained through improved condition conferred by this alga.

If females raised on *Laminaria* are in better condition, the condition-dependent female preference hypothesis predicts these flies will express stronger preferences (Cotton et al. 2006). Results of this study do not support this prediction, and in fact suggest that the strength of female preference is greater when in poorer condition (Fig. 6.10). Variation in the strength of female preference is apparently dictated by the condition of males (Fig. 6.8). This contradiction may have arisen as a result of female preference being the passive side effect of convenience polyandry rather than adaptive mate choice.

The condition-dependent female preference hypothesis predicts that females in improved condition are better able to express strong preferences due to improved ability to pay the costs of choice (Cotton et al. 2006). Though the principle of this hypothesis is well founded, it must also consider that the costs of expressing a preference can be dependent upon male condition. The influence of male condition upon the costs of resistance may be predicted to vary widely between species. If male and female condition covaries in a population and male condition contributes to an increased cost of mate choice then female preferences may not necessarily develop condition dependence. In the *C. frigida* mating system it is evident that males in improved condition will increase the costs of the rejection response for females. If the corresponding increase in female condition is not sufficient to counteract this then convenience polyandry is predicted. This has important implications for the prediction that strong female preferences will become associated with genetic factors that contribute to condition (Cotton et al. 2006). The fact that lower condition female *C. frigida* exhibit stronger mating preferences adds further credence to female mate choice occurring as a non-adaptive side effect of sexual conflict in this species.

Sexually antagonistic coevolution has been proposed to explain observations of apparently non-adaptive reproductive behaviour resulting from sexual conflict. Female adaptations to counter male persistence traits in turn select for greater persistence (Parker 1979; Holland & Rice 1998, Gavrilets et al. 2001, Arnqvist and Rowe 2005). In *C. frigida,* increased sexual conflict when males are exposed to and cultured on *Laminaria* may then result in greater selection for male persistence traits through increased female resistance. Contrary to this prediction, the results of this study show that the strength of female preference is reduced when males are raised on *Laminaria*, effectively limiting an evolutionary arms race. Our results accord with the model of Rowe and co-workers (2005) in which both the shape and threshold of female preference can evolve. Under these circumstances, inter-locus sexual conflicts may be limited by a relative insensitivity of female resistance to the male persistence trait. Consequently, future diversion of male persistence and female resistance traits between populations according to the local algal community is less likely to occur than previously expected (Chapter 4).

The results of this study complement earlier work and further characterise the close association between mating behaviour in *C. frigida* and the algal community. Rather than demonstrating condition dependence of female preferences these results illustrate that alternative outcomes can occur in species with mating preferences occurring as a side effect of sexual conflict. It is shown that a plastic female preference function, dependent upon male condition, may act as a resolution to sexually antagonistic coevolution in *C. frigida*.

6.6 Methods – Part II

6.6.1 Controlled density cultures

To create cultures of equal density the following protocol was adapted from Leggett et al. (1996). This procedure meant it was possible to determine the approximate density of larvae in cultures and ensure that all cultures had the same starting density.

500 male and 500 female wild *C. frigida* were collected from Crail, Fife. Male flies were introduced to a culture box containing a thin layer, approx 1cm, of minced *Laminaria* algae. At the same time, female flies were stored in a 500ml powder round containing a ball of cotton wool soaked in 5% sucrose solution. The culture box containing algae and male flies and the powder round containing female flies were stored in a controlled temperature room set at 25°C on a 12h:12h light dark cycle for 24 hours. This period of time was used to increase the willingness of male flies to mate. *Laminaria* was used as this alga is known to have a greater effect upon both male mating behaviour and female oviposition rate.

Female flies were then introduced to the culture box containing male flies and stored at 25°C to allow mating and oviposition. After 16 hours all flies were removed from the box. This length of time was chosen in order to maximise yield of fertilised eggs whilst minimising the number of eggs hatching prior to the start of cultures. Eggs were collected from the algae by shaking vigorously in 3% saline solution. This process also separated clutches of eggs. Algae was then removed from the solution by straining the liquid through a mesh. Eggs were diluted into a final volume of 1500ml.

150ml of egg solution was filtered onto a piece of black cotton cloth so that eggs were widely spaced and could be easily identified. This cloth was placed inside a lidded culture box upon damped tissue paper and left at 25°C to allow viable eggs to hatch. At the same time 75ml aliquots of egg solution were filtered onto smaller squares of cloth that were placed upside down into small culture boxes (13 x 13 x 14cm; Stewart Plastics, Croydon, UK) containing 200g of either *Fucus* or *Laminaria* algae. Small culture boxes were then also stored at 25°C.

After 48 hours the number of hatched eggs on the larger piece of black cloth was counted and smaller pieces of cloth were removed from each of the culture boxes. Culture boxes were maintained at 25°C to allow coelopids to develop. During development cultures were checked regularly to see that larvae had sufficient algae but that there was not an abundance of undigested algae. If larvae were seen to be moving up the sides of the culture boxes in large numbers it was assumed resources were becoming limited. Additional algae of the same genera was added to all culture boxes in equal measure as required.

As development progressed, newly eclosed adult flies were collected twice daily at intervals of no more than 16 hours in order to prevent development of a further generation. Each culture box was checked for newly emerged flies until none had eclosed for 48 hours. The wing length of all flies collected was recorded in addition to a subset being selected for weighing and determination of *αβ* chromosomal inversion karyotype by starch gel electrophoresis. This subset consisted of five male and five female flies selected at random from each culture box on each day that flies were collected. If there were less than five flies of either sex taken from a box then all flies of that sex were selected. Flies were stored at -20°C prior to weighing and electrophoresis.

6.6.2 Statistical analysis of controlled density cultures

For the purpose of analysis day zero was defined as the day that female flies were first introduced to males. Development times therefore include the entire period of development from conception to eclosion. Condition of flies was calculated from the mass and size of each fly and defined as weight (mg) / (wing length (mm))^3. All data was checked for normality prior to analysis.

It is often found that smaller flies produce a less visible banding pattern following electrophoresis so that alcohol dehydrogenase allozymes may not be determined. As size is influenced by the inversion system this may have biased results against β-homokaryotypes. A t-test and χ^2 test were used to ensure no significant bias in the size of flies whose allozymes could not be identified and also that these flies were not biased to cultures of either alga. The frequency of occurrence of α and β forms of the inversion system were compared within each sex between algal cultures using a χ^2 square test.

Separate General Linear Models were used to determine the influence of development time, algae and karyotype upon the size and condition of males and females. Non-linear effects of development time were incorporated into all models by inclusion of the quadratic term. As only partial data was available for the karyotype of flies models were first constructed excluding this factor and subsequently rebuilt using a subset of the results. All analyses were carried out using SPSS v12.0.2 (SPSS Inc.

Chicago IL, USA). Maximal models were chosen based upon the Akaike Information Criterion calculated using R 2.5.1 (R Development Core Team 2007).

6.6.3 Mating and oviposition trials

Flies used in this part of the study were a random selection of flies taken from the controlled density cultures. Males and females were exposed, in individual containers, to *Laminaria*, *Fucus* or a sucrose control for 48 hours before mating trials. As females were stored with seaweed in this study, female pots were the same size as male containers (55mm diameter by 75mm) for the 48 hour exposure period. This increased handling efficiency and the ability to identify if eggs had been laid. Before mating trials containers were checked for the presence of eggs and females transferred to smaller containers (30mm diameter by 55mm) in which mating trials took place. Following mating trials flies were weighed and wing length measured.

6.6.4 Statistical analysis of mating trials

All data were checked for normality prior to analysis. Time preceding a mount and duration of copulation were both log transformed, struggle duration was reciprocal root transformed. The wing lengths of males and females from each algal culture were compared using a t-test.

General linear models were used to identify factors affecting the time preceding a mount, struggle duration and copulation duration. Binary logistic regression was used to identify factors affecting harassment rate and mating success. All models were constructed in SPSS 12.0.2 (SPSS Inc. Chicago IL, USA). Independent variables included in all models were male culture algae, female culture algae, male algal exposure, female algal exposure, oviposition status of the female, male wing length and female wing length. To account for non-linear effects quadratic terms for male size and female size were also considered in all models. To account for additional male persistence traits the binary logistic model for mating success also included the time preceding a mount, struggle duration and respective quadratic terms. In the general linear model for struggle duration, mating success was also included as this is predicted to have an effect. All possible two-way interactions were considered in the models. Maximal models were chosen based upon the Akaike Information Criterion, calculated using R 2.5.1 (R Development Core Team 2007). Preference functions were illustrated in cubic splines with 10,000 bootstrap replicates to estimate 95% confidence intervals using GLMS version 4.0 (Schluter, 1988).

6.6.5 Statistical analysis of oviposition trials

Female wing length was found to be normally distributed. Binary logistic regression was carried out in SPSS 12.0.2 (SPSS Inc. Chicago IL, USA) to examine the relative influence of culture algae, treatment group, female size and all two way interactions on the probability of oviposition. Maximal models were chosen based upon the Akaike Information Criterion, calculated using R 2.5.1 (R Development Core Team 2007).

6.7 Results – Part II

6.7.1 Controlled density cultures

6.7.1.1 Summary of results

1138 hatched eggs were counted on the larger piece of black cloth. This estimated the starting density of each culture to be approximately 208 larvae per gram. Four cultures of *Laminaria* and four cultures of *Fucus* were prepared. A further 150g of algae was added to each box during development to give a final estimated density of 1.65 larvae per gram.

A total of 2326 flies were collected from all the cultures. 1904 of these flies were measured and the remaining 422 were measured, weighed and gelled. Of these 422 flies, 52 possessed Adh alleles not in linkage disequilibrium with the inversion system and the banding patterns of a further 34 flies could not be accurately identified. Consistent with expectations, these latter flies were smaller than flies whose allozymes could be determined, though not significantly so ($t_{408} = 0.396$, $p = 0.692$). These flies were also not significantly biased towards cultures of either alga ($\chi^2_1 = 0.058$, $p = 0.810$).

6.7.1.2 Total number of flies

Flies started to eclose from both *Laminaria* and *Fucus* cultures following 9 days of development. A clear difference can be observed in the pattern of emergence of flies following culture on *Laminaria* and *Fucus* (Fig. 6.11). Though the number of flies that eclosed per day was initially greater on *Fucus*, this was soon surpassed so that by day 11 more flies were eclosing per day from *Laminaria* cultures. The number of flies eclosing per day rapidly decreased for *Fucus* cultures compared to a more moderate decline in the numbers of adults retrieved from *Laminaria* cultures following a peak at 10 days. Males had a longer development time than females so that sex ratios were initially female biased. After all flies had eclosed there was no sex bias or difference in sex ratio between algal cultures. The sex ratio took longer to reach equivalence on *Laminaria* due to the different patterns of development of males and females on the two algae. More flies eclosed in total from *Laminaria* cultures than *Fucus* cultures, none of the *Fucus* cultures exceeding the yield of any *Laminaria* culture (Fig. 6.12).

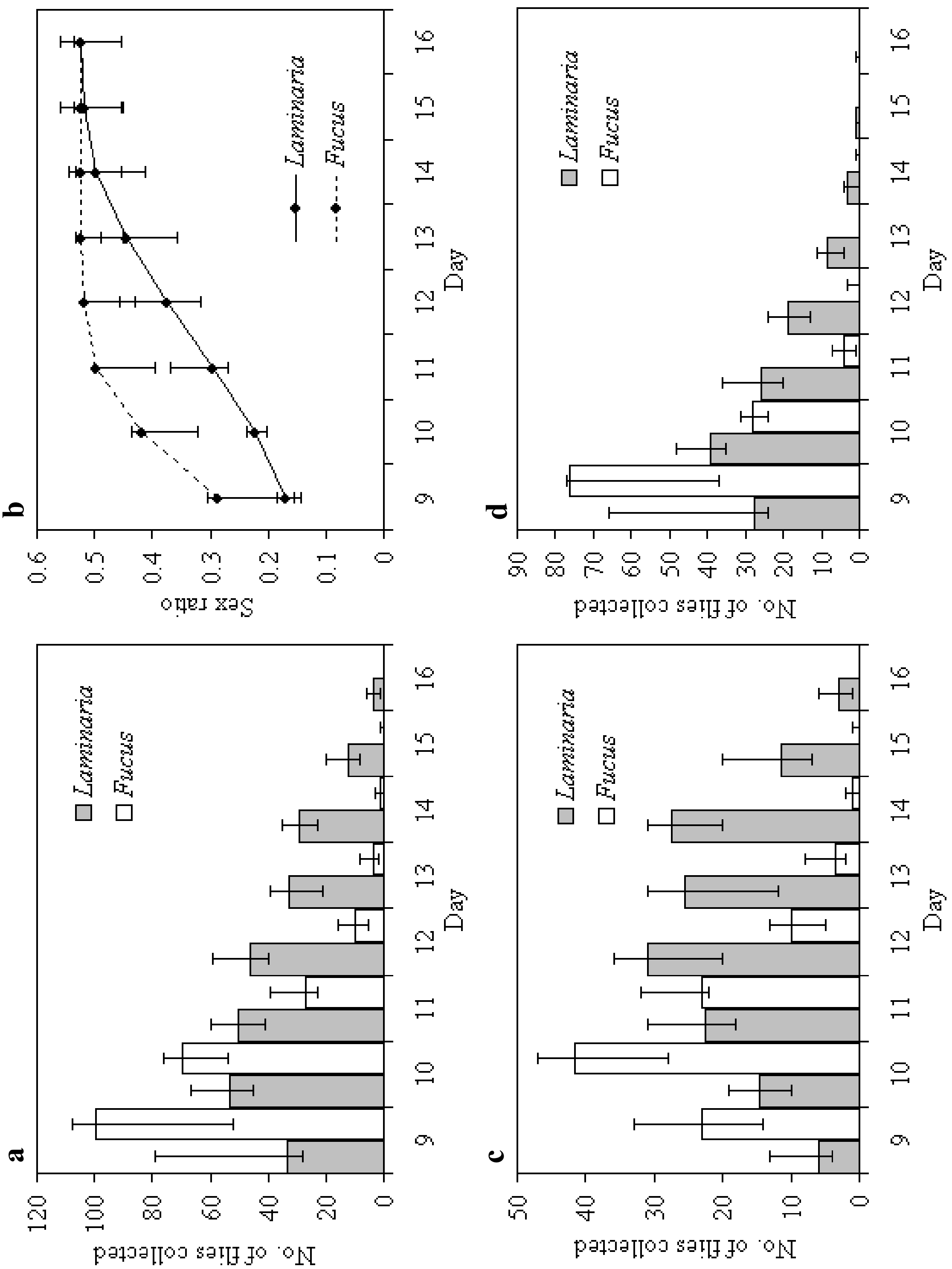


Figure 6.11 The total number of flies eclosing and sex ratio of each algal culture. (a) shows the total number of flies eclosing each day (b) the cumulative proportion of males having eclosed (sex ratio) (c) the total number of males eclosing each day and (d) the total number of females eclosing each day. Data points represent medians and error bars show the full range of values.

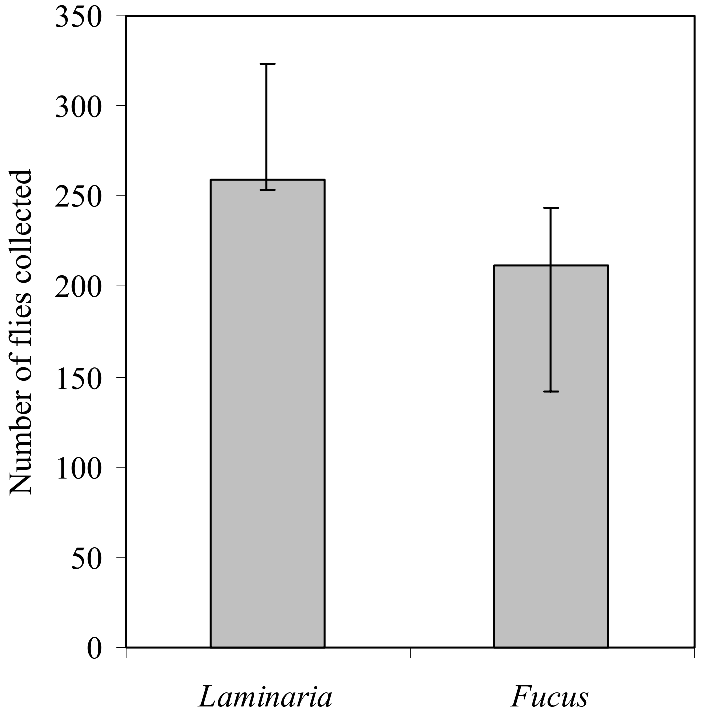


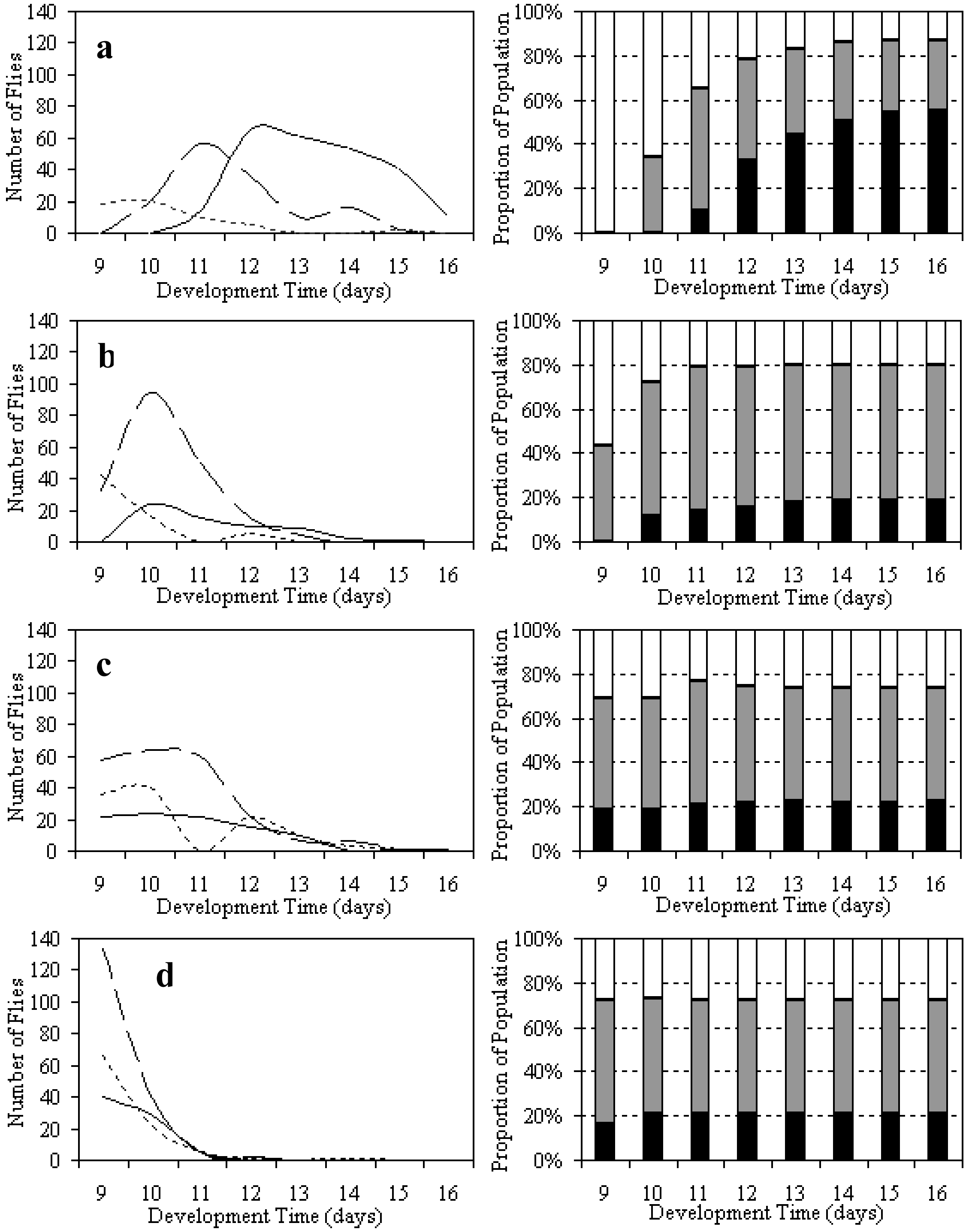
Figure 6.12 The total number of flies collected from each culture. Columns show median values and error bars the range of values.

6.7.1.3 Inversion karyotypes

The pattern of emergence of male and female karyotypes and the karyotype composition of populations reared on each alga are shown in Figure 6.13. On both algae the emergence of each female karyotype is relatively constant over time when compared to males. This yields little variation in the ratio of each female karyotype in populations during the eclosion period. Once all females had eclosed there was no significant difference in the frequency of α and β forms of the inversion in *Fucus* and *Laminaria* populations (*Laminaria* $P_{(\alpha)}=0.48$, *Fucus* $P_{(\alpha)}=0.47$; $\chi^2_1 < 0.001$, $p = 0.982$). In neither population did the frequency of karyotypes differ from predictions of Hardy-Weinberg equilibrium (*Laminaria*: $\chi^2_2 = 0.088$, $p = 0.957$; *Fucus*: $\chi^2_2 = 0.010$, $p = 0.995$).

In contrast, the male population is initially dominated by β-homokaryotypes, followed by the emergence of heterokaryotypes and finally α-homokaryotypes. This succession is most pronounced when culturing with *Laminaria*. After all males had eclosed there was a significant difference in the frequency of α and β forms of the inversion in *Fucus* and *Laminaria* populations (*Laminaria* $P_{(\alpha)}=0.71$, *Fucus* $P_{(\alpha)}=0.49$; $\chi^2_1 = 9.973$, $p = 0.002$). Though karyotype frequencies differed between algal cultures, within cultures these frequencies did not differ significantly from Hardy-Weinberg equilibrium (*Laminaria*: $\chi^2_2 = 2.619$, $p = 0.270$; *Fucus*: $\chi^2_2 = 2.418$, $p = 0.298$).

Figure 6.13 The number of each karyotype emerging each day (left panel) and cumulative proportion of each karyotype in the population (right panel) for (a) males eclosing from *Laminaria* cultures (b) males eclosing from *Fucus* cultures (c) females eclosing from *Laminaria* cultures and (d) females eclosing from *Fucus* cultures. Solid lines (left panel) and filled bars (right panel) show $\alpha\alpha$-homokaryotypes, long-dashed lines and shaded bars show heterokaryotypes and short-dashed lines and open bars show $\beta\beta$-homokaryotypes.



6.7.1.4 Size and condition of male flies

Male size was greater following development on *Laminaria* than *Fucus* ($F_{(1,934)} = 46.770$, $p < 0.001$). There was a non-linear relationship between development time and male size ($F_{(1,934)} = 73.710$, $p < 0.001$), an effect which was greater using *Fucus* as male size decreased following 11 days of development (Fig 6.14). There was a distinct size hierarchy from large *αα*-homokaryotypes, through heterokaryotypes to small *ββ*-homokaryotypes ($F_{(2,178)} = 29.061$, $p<0.001$). The magnitude of this hierarchy was influenced by the algal environment as *αα*-homokaryotypes and heterokaryotypes were larger when reared on *Laminaria* ($F_{(2,178)} = 4.896$, $p = 0.009$; Fig 6.15).

Male condition was positively correlated with development time ($F_{(1,241)} = 37.296$, $p < 0.001$). Though there was no independent effect on condition of either culture alga ($F_{(1,240)} = 1.391$, $p < 0.239$) or karyotype ($F_{(2,184)} = 0.307$, $p = 0.736$) these factors can be indirectly associated with condition (Fig. 6.16). In a model that excludes development time, condition varies between karyotypes ($F_{(2,182)} = 3.858$, $p = 0.023$) though not between culture algae ($F_{(1,182)} = 1.521$, $p = 0.219$). In a final model that excludes development time and karyotype, males raised on *Laminaria* are in better condition than those raised on *Fucus* ($F_{(1,241)} = 8.235$, $p = 0.004$; Fig. 6.16).

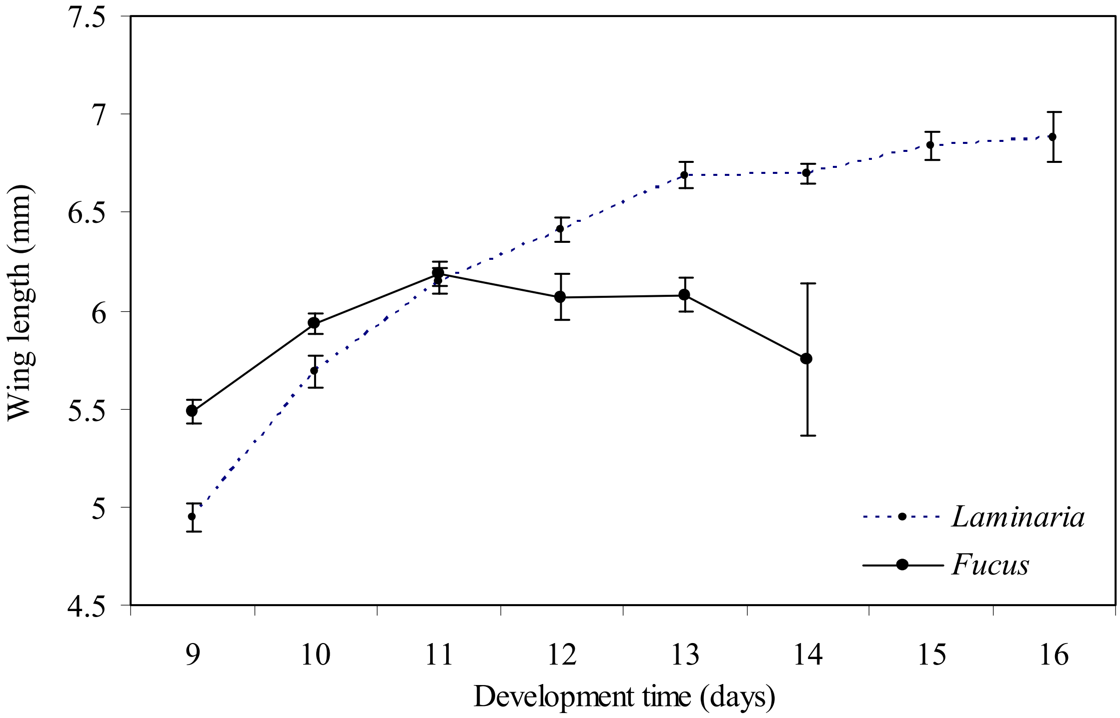


Figure 6.14 The size of male flies following different durations of development on *Laminaria* and *Fucus* algae. (Error bars show standard errors.)

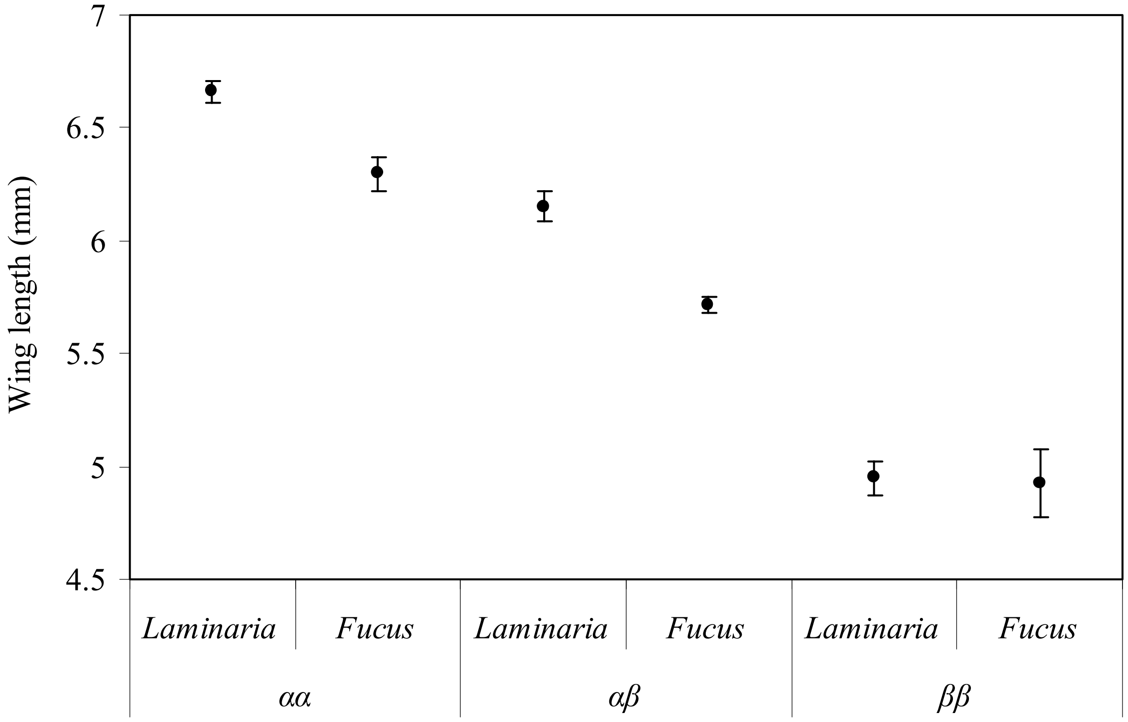


Figure 6.15 The size of male flies of different karyotype when raised on either *Laminaria* or *Fucus* algae. (Error bars show standard errors.)

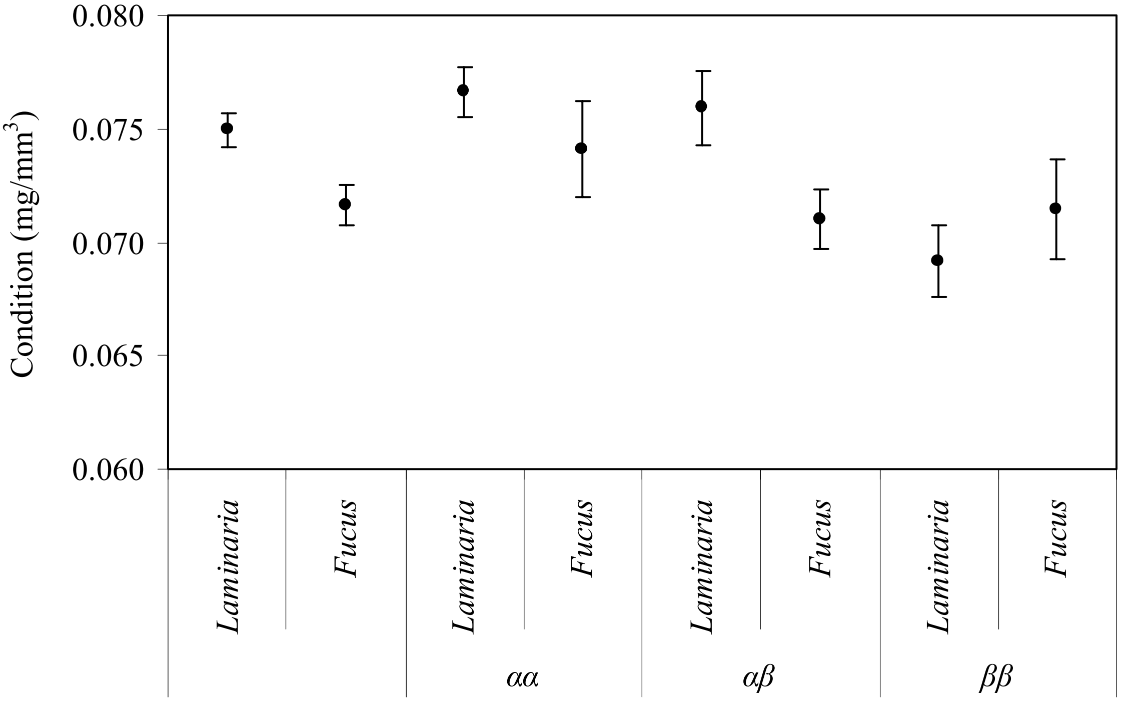


Figure 6.16 The condition of male flies of different karyotype and when raised on either *Laminaria* or *Fucus* algae. (Error bars show standard errors.)

6.7.1.5 Size and condition of female flies

Female size was greater following development on *Laminaria* than *Fucus* ($F_{(1,896)} = 5.996$, $p = 0.015$), though this is dependent upon development time ($F_{(1,896)} = 4.342$, $p = 0.037$; Fig. 6.17). In contrast to males, female size was negatively correlated with development time; a reduction in size which happened sooner when raised on *Fucus*. Female size did vary with karyotype as *ββ*-homokaryotypes were smaller ($F_{(2,132)} = 3.808$, $p = 0.025$), though the size of karyotypes did not vary significantly between algal cultures ($F_{(2,130)} = 0.753$, $p = 0.886$; Fig. 6.18).

Female condition was positively correlated with development time ($F_{(1,165)} = 13.317$, $p < 0.001$) with no independent effect of either culture alga ($F_{(1,164)} = 0.697$, $p = 0.405$) or karyotype ($F_{(2,135)} = 0.031$, $p = 0.970$). Differences in condition of females from different cultures and of different karyotype reflect differences in development time (Fig. 6.19). In a model that excludes development time, females raised on *Laminaria* are in better condition than those raised on *Fucus* ($F_{(1,165)} = 5.199$, $p = 0.024$), however there is no difference in condition between female karyotypes ($F_{(2,135)} = 0.104$, $p = 0.901$).

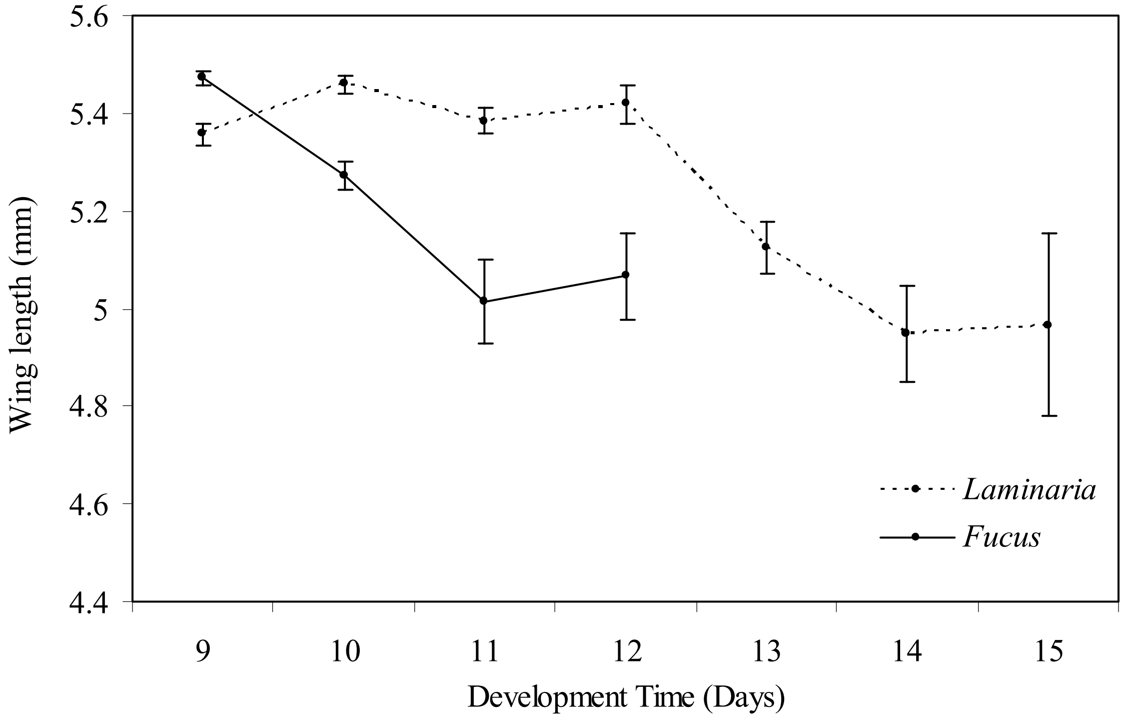


Figure 6.17 The size of female flies following different durations of development on *Laminaria* and *Fucus* algae. (Error bars show standard errors.)

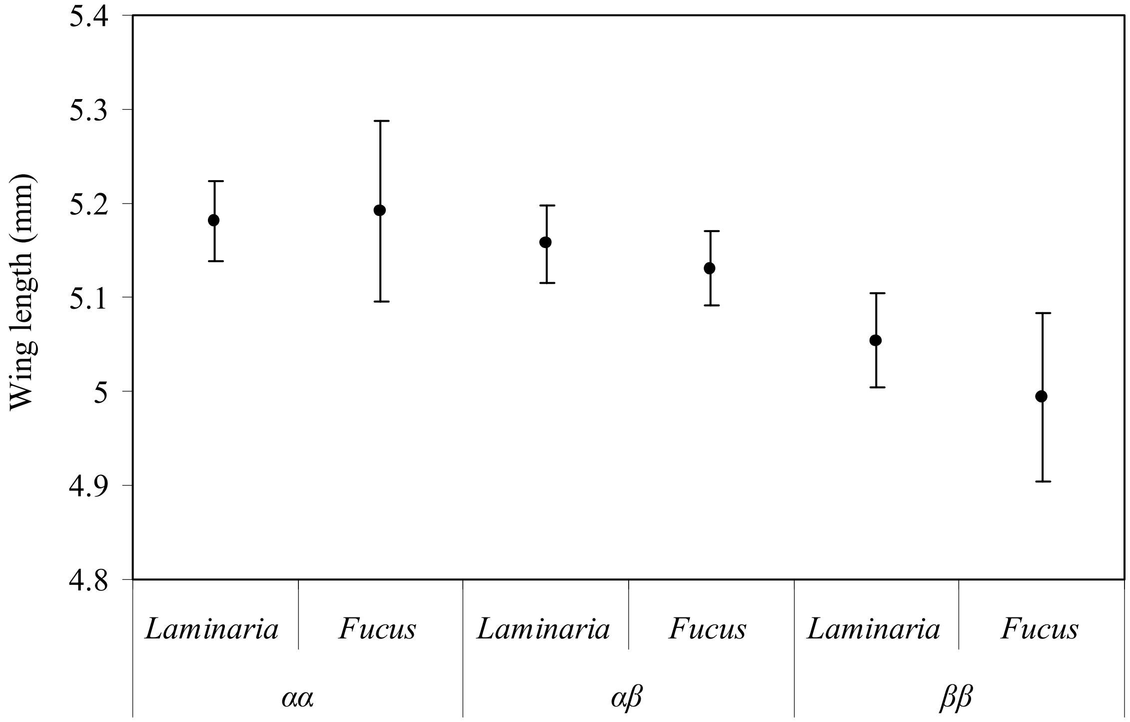


Figure 6.18 The size of female flies of different karyotype when raised on either *Laminaria* or *Fucus* algae. (Error bars show standard errors.)

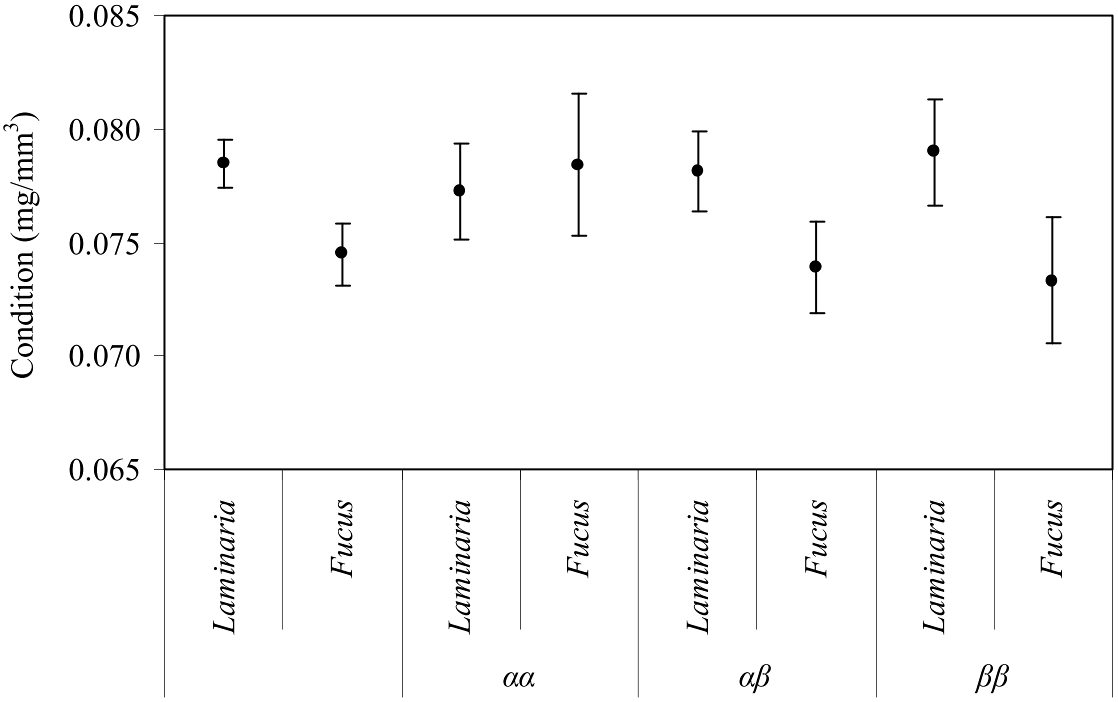


Figure 6.19 The condition of female flies of different karyotype and when raised on either *Laminaria* or *Fucus* algae. (Error bars show standard errors.)

6.7.2 Mating trials

A total of 288 mating trials were successfully completed. There was no difference in female wing lengths ($t_{283} = 0.581$, $p = 0.562$), but males reared on *Laminaria* were larger than males reared on *Fucus* ($t_{281} = 7.779$, $p < 0.001$).

6.7.2.1 Harassment rates

Smaller males were more likely to harass females ($\chi^2_1 = 16.849$, $p < 0.001$). Exposure of males to algae or a sucrose control had a significant effect on levels of harassment ($\chi^2_2 = 11.198$, $p = 0.004$), though this was the result of lower harassment following exposure to the control rather than a difference between the two algal treatments ($\chi^2_1 = 0.584$, $p = 0.445$; Fig. 6.20). Males were more likely to mount a female that had previously oviposited ($\chi^2_1 = 10.718$, $p = 0.001$). There was no effect of the algae that males had been cultured on ($\chi^2_1 = 1.530$, $p = 0.216$) nor a preference to mount females that had been raised on either algae ($\chi^2_1 < 0.001$, $p = 0.984$).

6.7.2.2 Time preceding mount

Consistent with smaller males being more likely to harass a female, small males also harassed a female sooner ($F_{1,207} = 5.184$, $p = 0.024$). There was no difference in the time preceding a mount following exposure to either alga ($F_{2,207} = 0.773$, $p = 0.568$). The time preceding a mount was not influenced by the algae that males had been reared on ($F_{1,206} = 0.038$, $p = 0.847$), though there was a marginally significant effect of female algal culture as females raised on *Laminaria* were mounted sooner ($F_{1,207} = 3.779$, $p = 0.053$; Fig. 6.21).

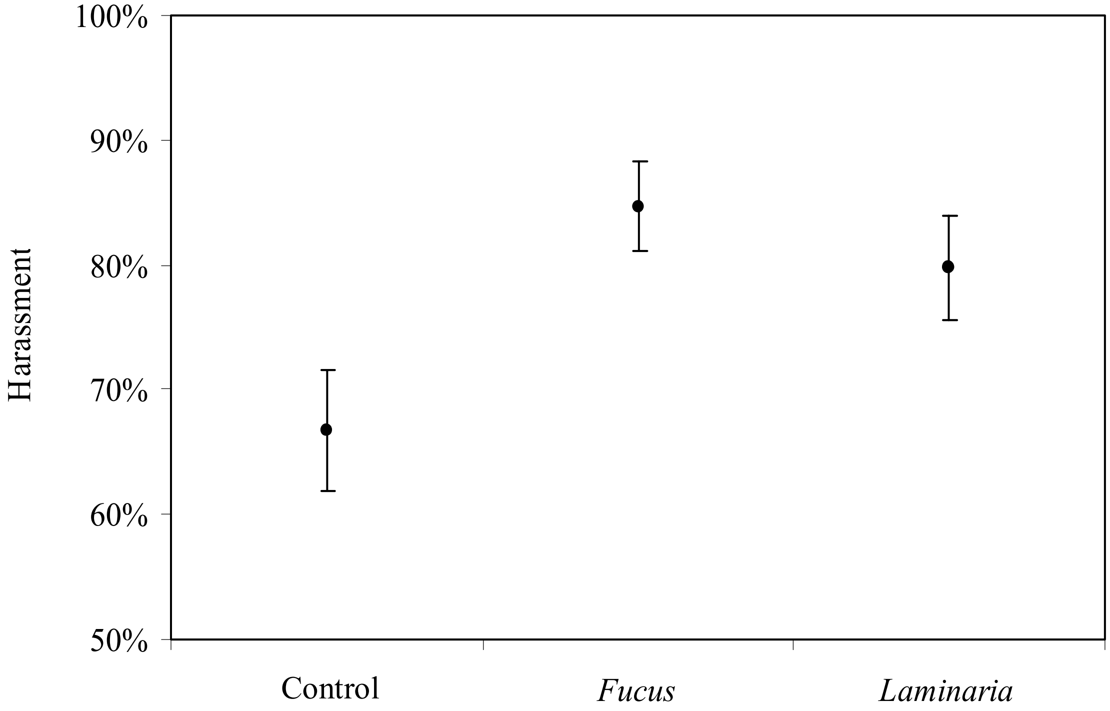


Figure 6.20 Levels of harassment when males are exposed to *Fucus*, *Laminaria* or a sucrose only control. (Error bars show standard errors.)

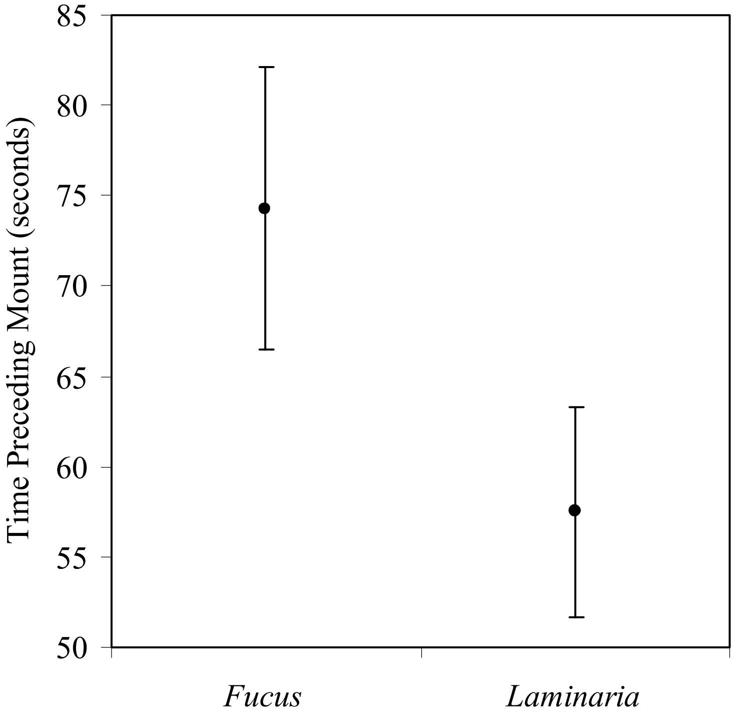


Figure 6.21 The time preceding a mount when females are cultured on *Fucus* and *Laminaria* algae. (Error bars show standard errors.)

6.7.2.3 Mating success

Mating success resulted from non-linear associations with struggle duration ($\chi^2_1 = 12.590$, $p < 0.001$; Fig. 6.22) and male size ($\chi^2_1 = 4.802$, $p = 0.028$; Fig. 6.23). There was also a significant interaction between male treatment and female oviposition status as males exposed to *Laminaria* were more likely to mate with females that had oviposited ($\chi^2_2 = 12.329$, $p = 0.002$; Fig. 6.24). There was no difference in the mating success of males reared on different algae ($\chi^2_1 = 0.121$, $p = 0.728$) or of females reared on different algae ($\chi^2_1 = 0.827$, $p = 0.363$).

6.7.2.4 Struggle duration

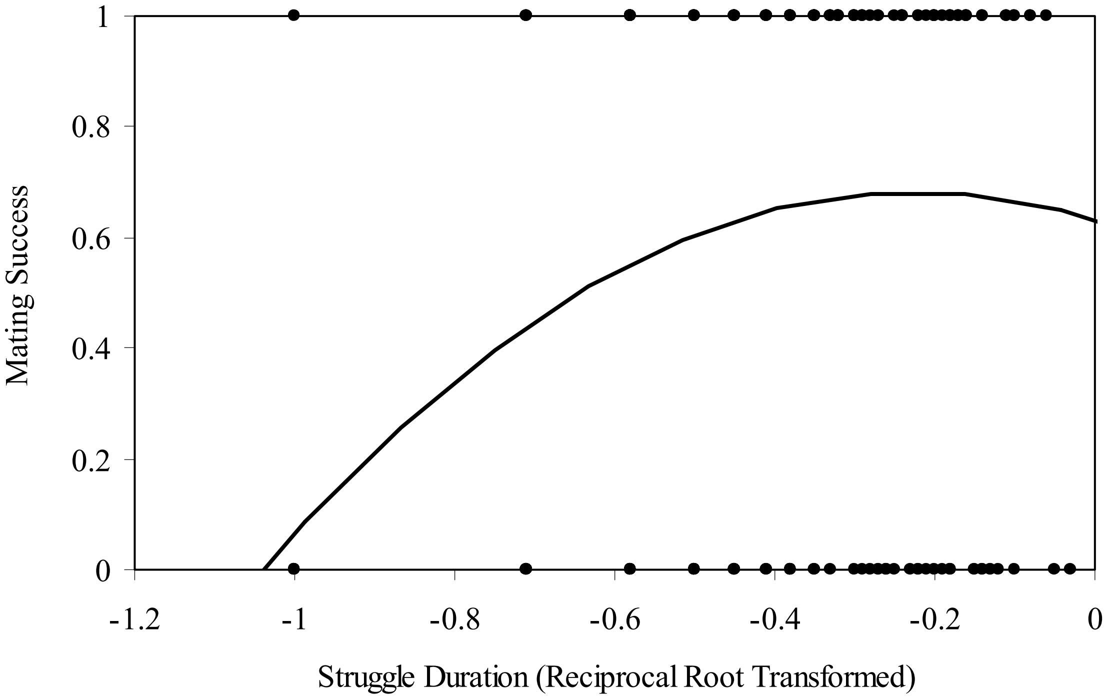
Struggles were longer if ended by copulation ($F_{(1,211)} = 6.405$, $p = 0.012$), if males had been exposed to algae ($F_{(2,211)} = 3.465$, $p = 0.033$) and if females were larger ($F_{(1,211)} = 4.390$, $p = 0.037$).

6.7.2.5 Copulation duration

The duration of copulation was dependent upon an interaction between male culture alga and female oviposition status ($F_{(1,122)} = 4.456$, $p = 0.037$). Males reared on *Laminaria* copulated for a significantly shorter period with females that had recently oviposited (Fig 6.25). The algae that females had been cultured with also affected copulation duration, but again was dependent upon whether the female had recently oviposited ($F_{(1,122)} = 8.496$, $p = 0.004$). Females that had oviposited had shorter copulation durations, but the difference was far greater when reared on *Fucus* (Fig. 6.26). Finally, struggle durations decreased with increasing male size, but only when males were reared on *Laminaria* ($F_{(1,122)} = 5.593$, $p = 0.020$; Fig. 6.27).

6.7.2.6 Preference functions

Cubic splines showing female preference function for male size when males are cultured on *Laminaria* and *Fucus* show very different selection pressures (Fig. 6.28a-b). The non-linear component of selection is only applicable to males reared on *Laminaria*, with greatest success achieved by males with a wing length of approximately 5.5mm. When raised on *Fucus* the strength of selection is weaker and shows a positive preference for larger males. Female preferences for male size are also non-linear. The strength of this preference and optimum male size differed depending on the culture algae; females cultured on *Fucus* successfully rejecting a greater number of large males (Fig. 6.28c-d).



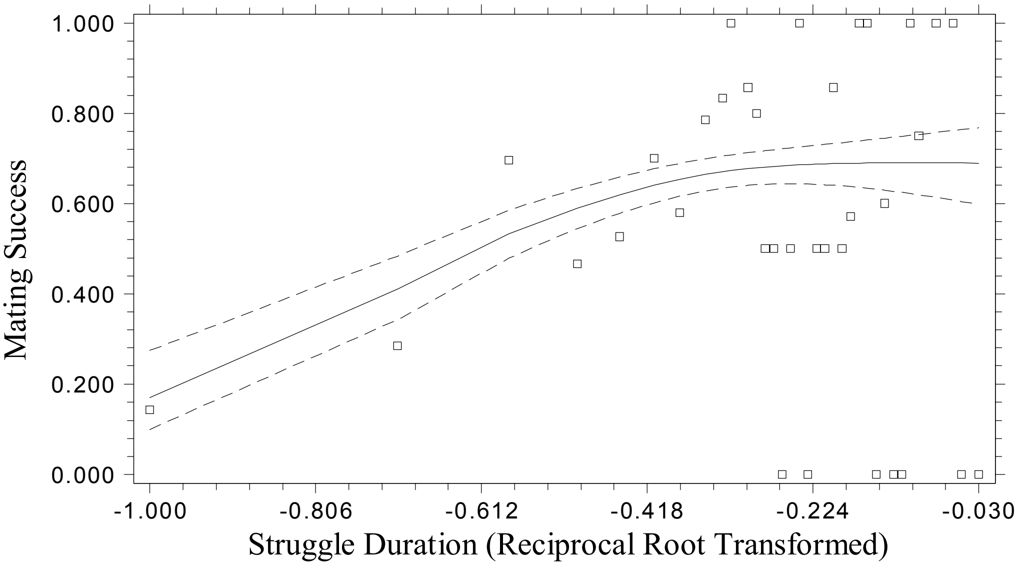
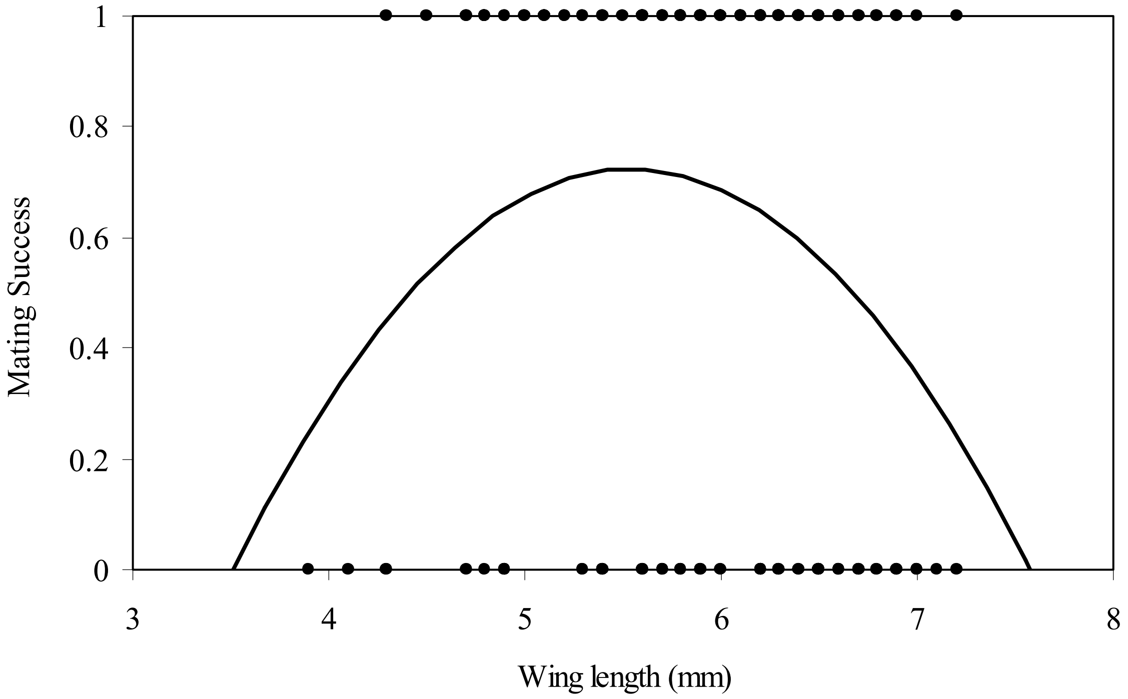


Figure 6.22 Non-linear association between struggle duration and mating success. The top figure shows a line of best fit following second order polynomial regression. The bottom figure shows the same data following cubic spline analysis.



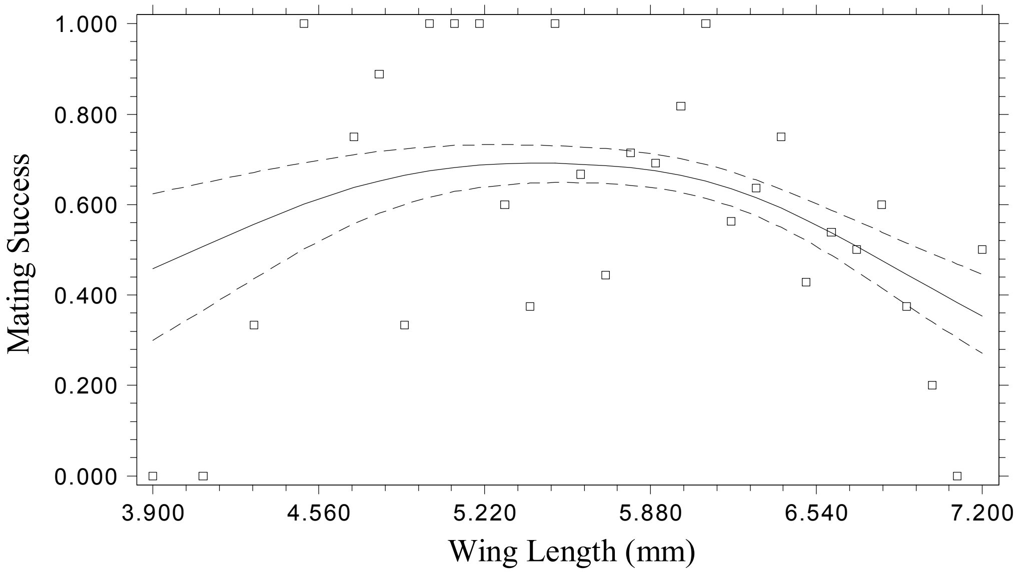


Figure 6.23 Non-linear association between male size and mating success. The top figure shows a line of best fit following second order polynomial regression. The bottom figure shows the same data following cubic spline analysis.

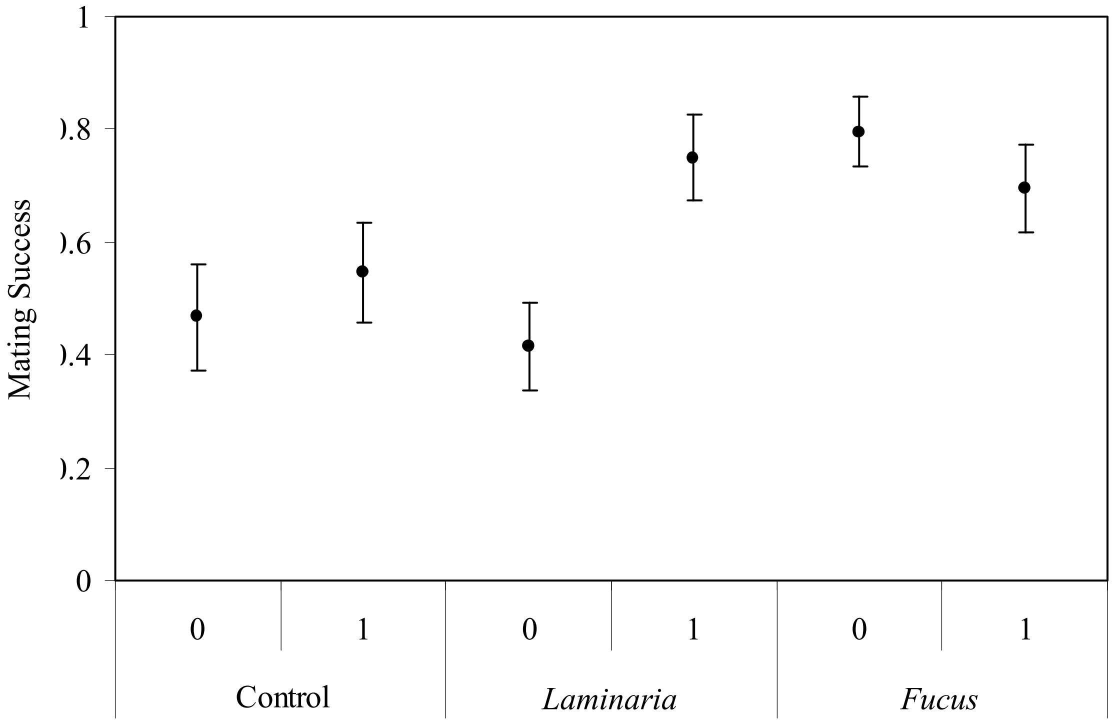


Figure 6.24 Mating success of males exposed to *Laminaria*, *Fucus* and a sucrose only control when paired with females that had (1) and had not (0) oviposited. (Error bars show standard errors.)

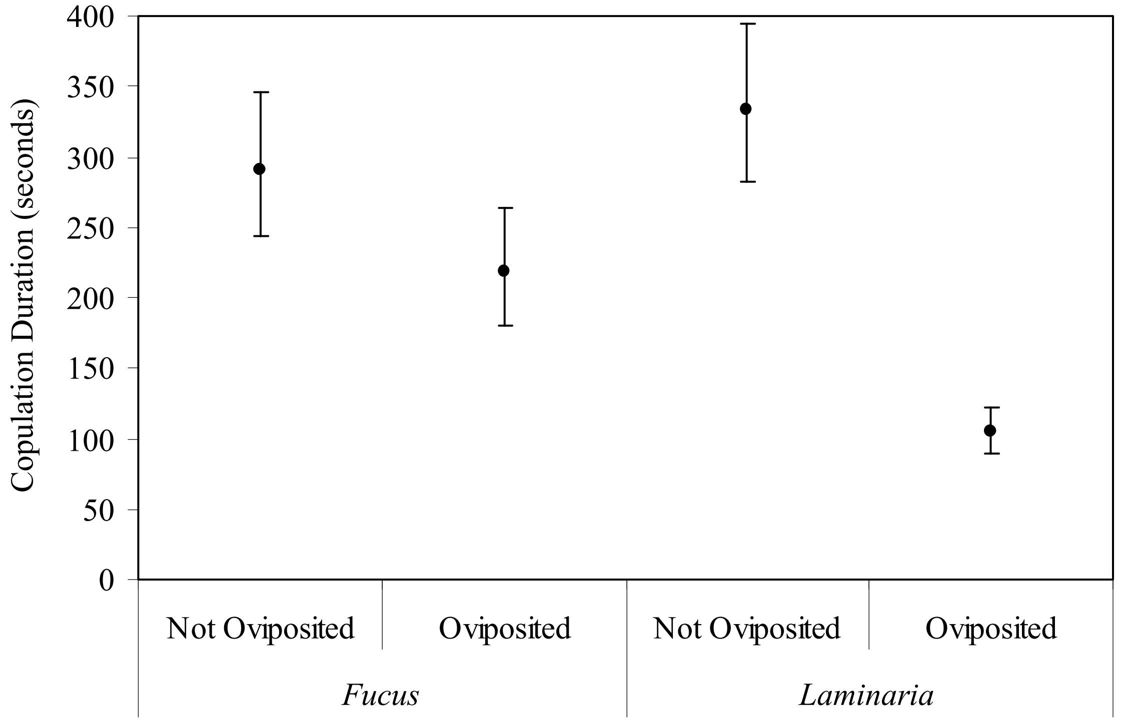


Figure 6.25 Copulation duration of males cultured on *Fucus* and *Laminaria* when paired with females that had and had not oviposited. (Error bars show standard errors.)

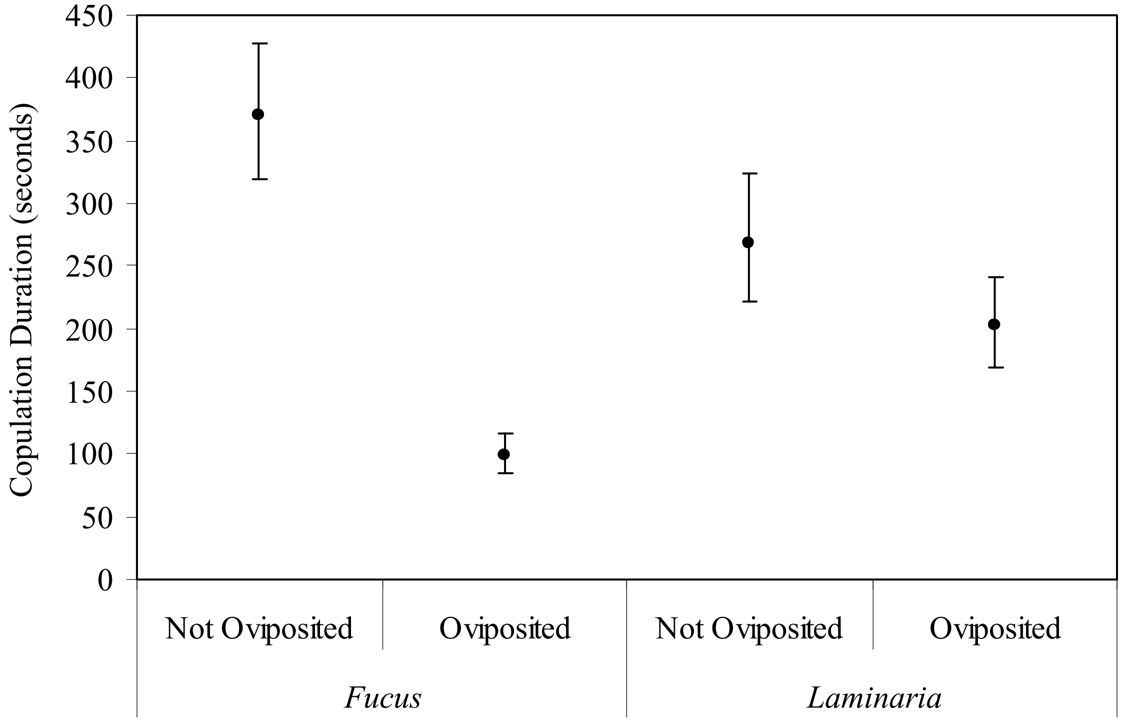


Figure 6.26 Copulation duration of females cultured on *Fucus* and *Laminaria* that had and had not oviposited. (Error bars show standard errors.)

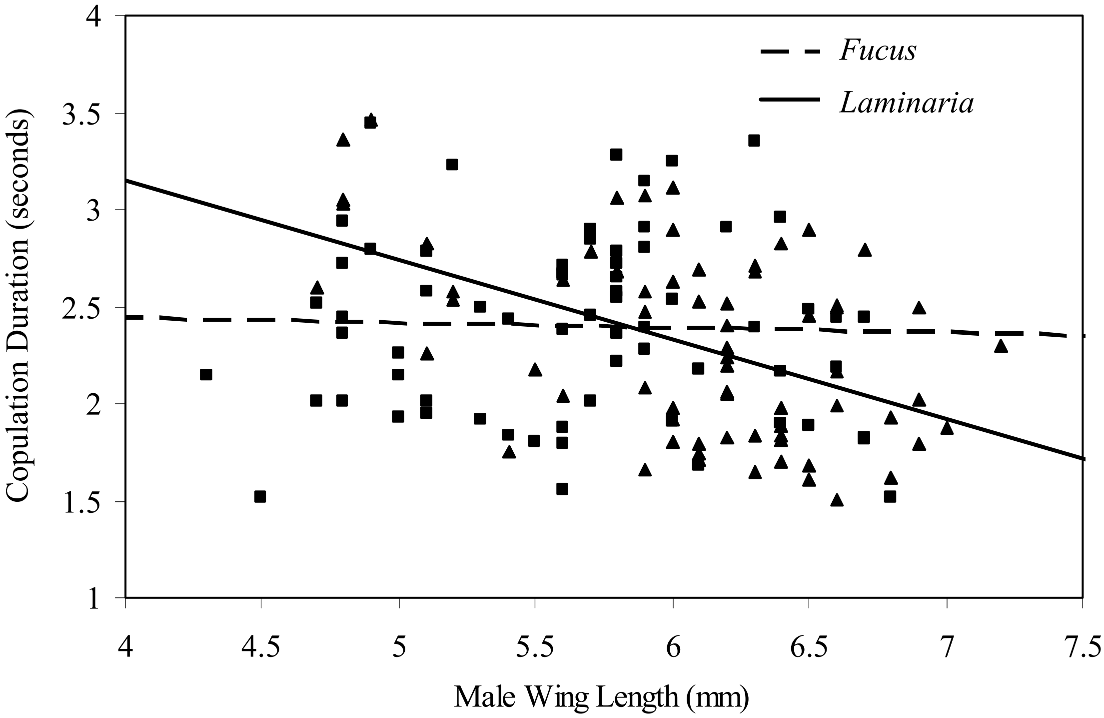


Figure 6.27 – Copulation duration vs. size for males cultured on *Laminaria* (▲) and *Fucus* (■).

Figure 6.28a-d Female preference functions for male size when (a) males are cultured on *Fucus*, (b) males are cultured on *Laminaria*, (c) females are cultured on *Fucus* and (d) females are cultured on *Laminaria*. Dashed lines show 95% confidence intervals.

Figure 6.28a

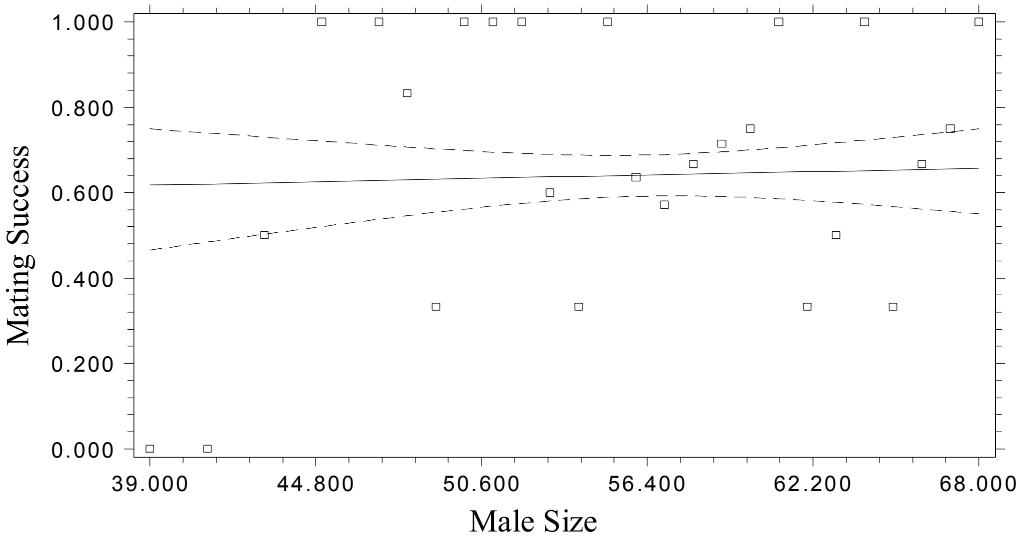


Figure 6.28b

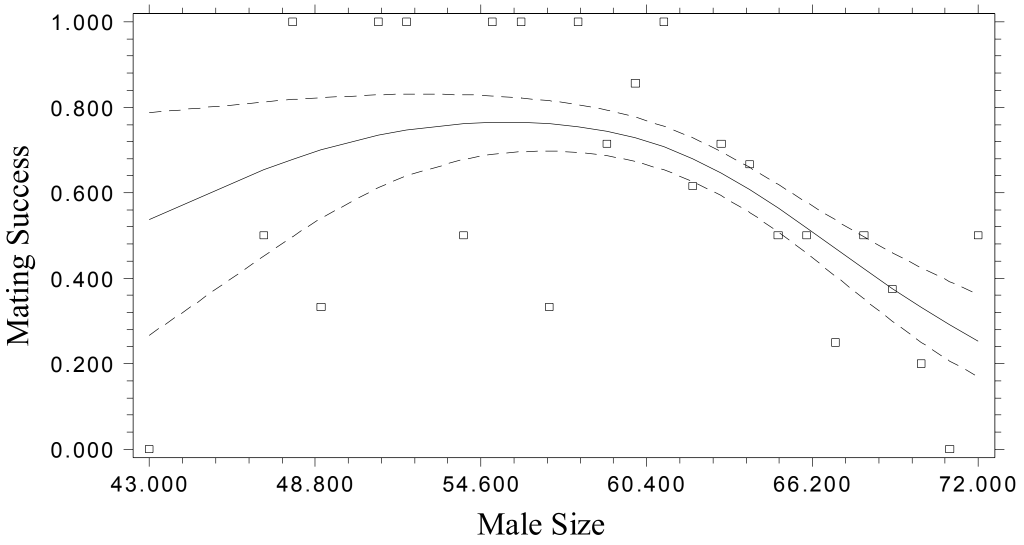


Figure 6.28c

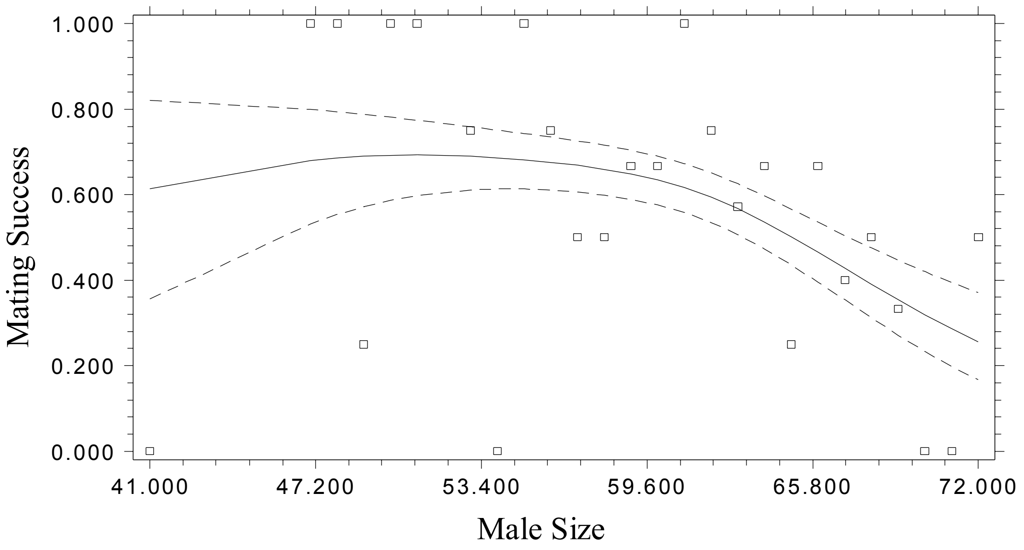
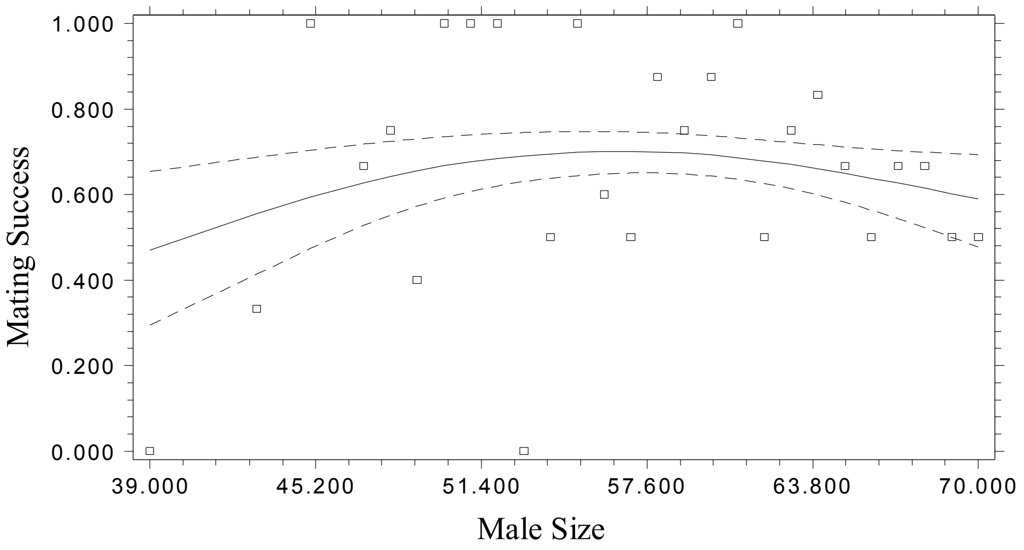


Figure 6.28d



6.7.3 Oviposition trials

A total of 292 females were successfully trialled. There was no difference in wing lengths of females from the two algal cultures ($t_{287} = 0.531$, $p = 0.596$). Treatment group had a highly significant effect on the probability of oviposition as only one out of 109 females not exposed to algae laid eggs ($\chi^2_2 = 157.281$, $p < 0.001$; Fig. 6.29). There was no difference in the number of females that oviposited following exposure to *Fucus* or *Laminaria* ($\chi^2_1 = 0.021$, $p = 0.886$; Fig. 6.29). Females cultured on *Laminaria* were more likely to oviposit than those cultured on *Fucus* ($\chi^2_1 = 4.467$, $p = 0.035$; Fig. 6.30).

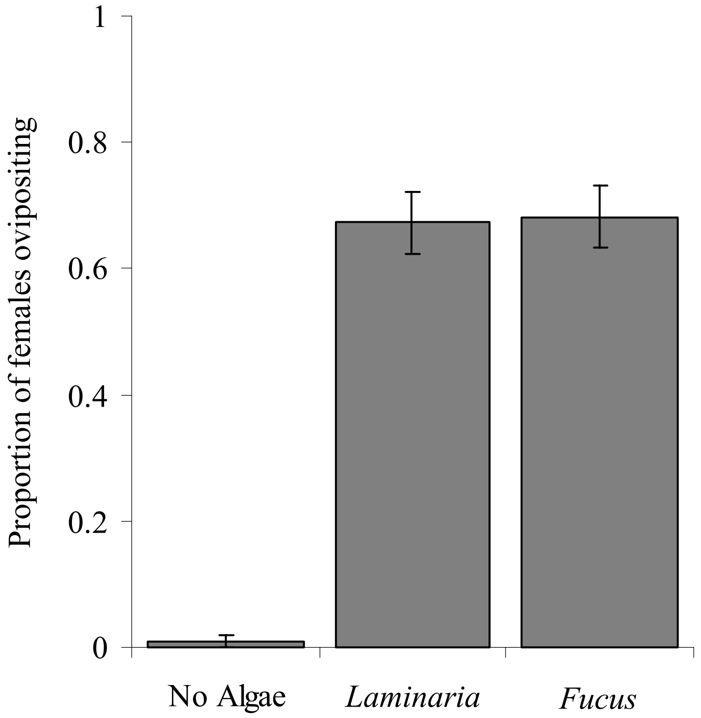


Figure 6.29 The proportion of females ovipositing when exposed to *Laminaria*, *Fucus* or a sucrose only control. (Mean ± SE).

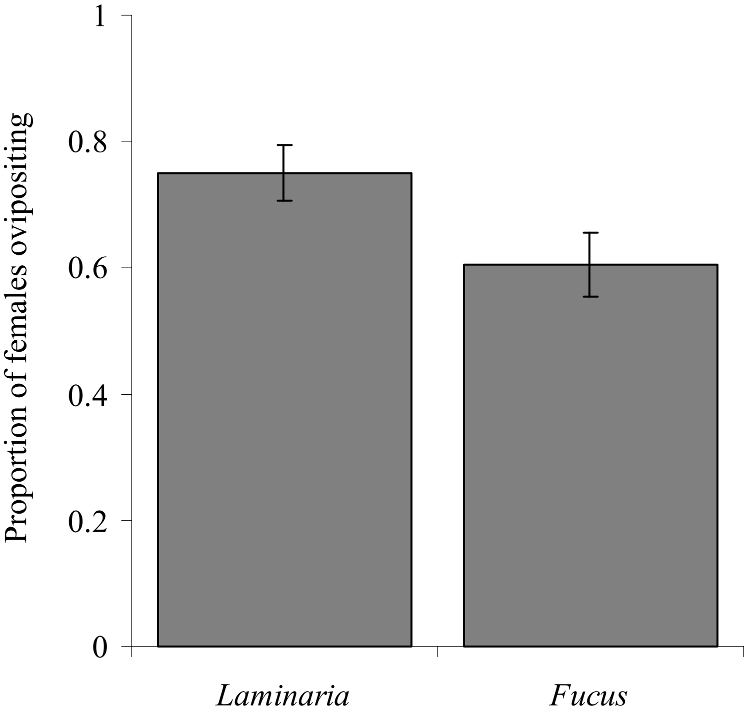


Figure 6.30 The proportion of females ovipositing when cultured on either *Laminaria* or *Fucus*. (Mean ± SE).

6.8 Discussion – Part II

6.8.1 Oviposition trials

The results of oviposition trials fail to support the findings of previous studies as exposure to *Laminaria* did not increase oviposition rate (Phillips et al. 1995b; Dunn 2001). It is possible that the different developmental conditions used in this study have not accurately replicated previous work, though this explanation is merely speculative. It is not believed that the results of this study provide the basis to disregard previous results without further investigation. This inconsistency is all the more puzzling as the benefit of larval development in *Laminaria* has been demonstrated; reinforcing predictions that females will prefer this alga for oviposition. Indeed, females that had been raised on *Laminaria* were more likely to oviposit; further confirming the benefits to condition of development on *Laminaria*.

The mating behaviour of males towards females that have recently oviposited has rarely been studied in this mating system (though see Pitafi et al. 1995). As females are known to regularly oviposit in the wild irrespective of mating history (Dunn et al. 2002) and recent oviposition will affect male reproductive success due to last male sperm precedence (Thompson 1951; Burnet 1961; Blyth & Gilburn 2005) male mate choice with regard to oviposition status is predicted to be of benefit. The finding that males are more likely to mount a female that has oviposited is inconsistent with predictions. Pitafi et al. (1995) also found no preference of males to mount gravid females. A number of explanations have been proposed for the shorter time to mount of females raised on *Laminaria* (see 6.5.2). These explanations may similarly apply to females that have oviposited. Chemicals produced by the female that attract males may be associated with more fecund individuals irrespective of oviposition status. More fecund females in better condition may also be more active irrespective of oviposition status. Male confusion over female status appears to continue even after direct contact is made as mating success is not associated with oviposition status; indeed males cultured on *Laminaria* were more likely to copulate with females that had already oviposited. It is only in copulation duration that males expend less effort on females that had oviposited.

6.8.2 Controlled density cultures

The contribution of diet to survivorship, fecundity and offspring fitness in invertebrates has been comprehensively studied (Delisle & Hardy 1997; Jann & Ward 1999; Wacker & von Elert 2002; Kaspi et al. 2002; Shelly et al. 2002, 2005; Engqvist & Sauer 2003; Shelly & McInnis 2003). There can be wide variation in the quality of marine algae available as a food source for invertebrates and this is reflected in selective feeding patterns in a variety of algivorous species (Neighbors and Horn 1991; Kennish & Williams 1997; Wakefield & Murray 1998; Poore and Steinberg 1999). The results of controlled density cultures show that algal diet also has an important influence on the development of *C. frigida*. The basis of the difference in nutritional value between *Laminaria* and *Fucus* is unknown but may be related to nitrogen content which is known to be a limiting factor in the growth of detritivores (Tenore 1981; Findlay 1982). *Fucus* species are often found to grow in the intertidal zone, so are regularly left exposed at low tide, whereas *Laminaria* species are primarily subtidal. Adaptations of *Fucus* species to resist fluctuating conditions and desiccation will likely influence nutritional value. Variation in patterns of successful development are likely to form the basis of selection for behaviours biased towards reproduction in different environments.

The difference in size and mating behaviour found between flies raised on different species of algae in the first part of this study served as an indication of their relative merits. In the second part of this study the greater size and improved condition following development on *Laminaria* has been confirmed. The improved condition of flies reared on *Laminaria* suggests greater resources may be available for allocation to fitness enhancing traits such as egg production. The relative benefits of each alga are further described as *Laminaria* also confers higher egg to adult viability.

Benefits of development on *Laminaria* come at a cost of longer development times, particularly for males. Individuals with longer development time are at a disadvantage in transitory wrack beds and males will have later access to earlier eclosing females. Different patterns of changing sex ratio following development on *Fucus* and *Laminaria* also present a dilemma for female development. Females that eclose earlier are in poorer condition, though may benefit from reduced harassment as sex ratios are female biased. When developing in a *Laminaria* dominated environment the advantage of a female biased sex ratio is prolonged. The relative benefits of development strategies in each alga are evidently not straightforward.

Central to the evolutionary process is the genetic variability and heritability of traits under selection. The relative contribution of genetic and environmental components to phenotypic variation can change with environmental conditions; the result of differential gene expression, genotype-environment interactions and environmental variance (Hoffmann & Merila 1999; Sgro & Hoffmann 2004; Charmantier & Garant 2005). Taking into account this variation it may be questioned whether laboratory based studies can accurately estimate the additive genetic variance of a particular trait. The relative contributions of genetic and environmental factors in determining male size have previously been determined for *C. frigida* (Wilcockson et al. 1995). Results reported here show that the contribution of the chromosomal inversion system to male size may have been underestimated in earlier work in which only *Fucus* was adopted as the culture medium. There is greater variation in the size and development time of flies following culture on *Laminaria* and this can be directly attributed to the *αβ* inversion system (Fig 6.15).

Heterokaryotype advantage has previously been described in *C. frigida* (Butlin et al. 1982b; Day et al. 1983; Butlin & Day 1989; Gilburn & Day 1996), though again work only employed *Fucus* as the culture medium. When cultured on *Laminaria* it is now shown that there can be an *αα*-homokaryotype advantage. This finding has important implications for the maintenance of female preference as both *αα* and *ββ*-homokaryotype females would benefit from mating with large αα-homokaryotype males in this environment. In this instance there is no predicted conflict between the processes of good genes selection, Fisherian selection and convenience polyandry, all of which may act in concert to maintain female preference for large males.

Heterokaryotype advantage is suggested to be the primary factor maintaining both forms of the inversion system in populations of *C. frigida*. It follows that the *β* form of the inversion would be lost if heterokaryotype advantage is replaced by *αα*-homokaryotype advantage in populations reproducing in *Laminaria* algae. This presents an interesting opportunity to examine maintenance of the inversion system in future studies. Predicting loss of the *β* form of the inversion following repeated development on *Laminaria* would be mistaken however. The relatively short development time of small $\beta\beta$-homokaryotypes is enhanced on *Laminaria*, so the relative benefits are also increased. This may be of particular advantage where wrack beds are transient. A shorter development time on *Laminaria* also provides earlier access to females. Though the increased size of $\alpha\alpha$-homokaryotype and heterokaryotype males demonstrates the nutritional benefits of *Laminaria*, $\beta\beta$-homokaryotype males do not appear to be taking full advantage of this alga (Fig 6.15). This may be evidence of a strategy that instead profits from the increased inequality of development times; something that has not been considered fully in relation to this species. Furthermore, results of mating trials (discussed below) show that selection for male size, and hence karyotype, can be stabilising when males are reared on *Laminaria*. Thus, processes maintaining female preferences, homokaryotype advantage and maintenance of the inversion system require fundamental questioning in light of these results.

6.8.3 Mating trials

There are a number of inconsistencies in the results of mating trials conducted in the first and second parts of this study. Smaller males were still found to be more likely to harass females and also mounted females sooner. Females reared on *Laminaria* were mounted sooner, consistent with earlier results. However, there was no effect of male culture algae or the algae to which males were exposed on either harassment rates or mating success. The influence of male size on struggle duration was also reduced. Perhaps the most significant difference between results is that mating success is determined by non-linear relationships with male size and struggle duration in the second part of this study (Fig 6.22 & 6.23). In particular, large males reared on *Laminaria* are at a significant disadvantage (Fig 6.28b). The strength of female preferences differs between culture populations in both parts of this study. On both occasions the strength of preference exhibited when females are cultured on *Fucus* is greater.

There are a number of differences in both the methods and analysis of the two parts of this study that may have led to differences in the results. The use of alternative statistical techniques and exposure of females to algae may appear to be the most significant changes in protocol. However, both of these changes represent improvements of the previous experimental design that are expected to have contributed additional information to the original findings rather than contradict them. This approach to the repetition of the study is then justified. Alternatively, evidence suggests that the culture conditions of the two studies were sufficiently different to influence results. The size of flies eclosing from cultures in the first part of this study were considerably smaller than in the second part of the study. In the first study flies ranged in size from between 4.5 and 5.0 mm. In the second study the average size of flies was greater than 5.0mm. Furthermore, flies cultured on *Fucus* in the second part of this study were larger than flies reared on *Laminaria* in the first part. The reason for the dissimilarity in the size of flies is almost certainly owing to differences in the density of larvae in cultures, which is known to influence size.

Differences in larval density may be expected to influence condition. Indeed, this is the reason for developing cultures on different algae that are of comparable density. As it has been shown that the condition of flies can have a dramatic effect on behaviour it would not be surprising to find that rearing flies at different densities also has an effect on behaviour. The results of neither part of this study are necessarily flawed but are likely to represent differences in behaviour resulting from the effect of larval density on condition. This study is the first to employ controlled density cultures for the comparison of mating behaviour in coelopids. In the same way that mating behaviour can be compared between algal cultures, comparison may also be made between populations reared at different densities. This presents an interesting dimension to future work and a massive undertaking if a full investigation is to be completed. At the very least the use of techniques to control larval density need to be employed more widely in the future so that results are comparable.

Sexual selection causes male traits to diverge from naturally selected optima. Though large males may initially have a mating advantage, it is to be expected that this should be reduced at extreme sizes. The largest males used in this study were found to be increasingly less mobile than smaller males, particularly in flight. Large males also appeared ungainly when handling females that were increasingly smaller than themselves (pers. obs.). Wrack beds composed solely of *Laminaria*, or indeed *Fucus* algae, may be rare in the wild as the distribution of these algae often overlaps. Though males may have attained what appears to be an excessive size in this study, this may occur only rarely in natural populations. Wider analysis of a more complex array of mixed cultures, in addition to different densities, would be required in this respect.

The large male mating advantage in *C. frigida* has been a focus of numerous studies (Chapter 1). The directional nature of this advantage has rarely been questioned, though it has been found that mating success plateaus at a large size (Gilburn et al. 1992). The results of this study show that there can be stabilising selection for male size when cultured using *Laminaria*; an algae that has been overlooked as a culture medium in previous work. This has certainly biased our current understanding of the coelopid mating system and provides a novel focus for future work.

Chapter 7 - Analysing multivariate selection in *C. frigida*

7.1 Introduction

Natural and sexual selection are processes affecting more that just the mean value of a trait. Selection may be directional, non-linear, either stabilising or disruptive, and can influence covariance between different traits (Phillips & Arnold 1989; Brodie et al. 1995). In addition, traits do not evolve in isolation but within a context of simultaneously evolving traits. The strength of selection for any one trait can be considered the sum of the direct attributes of that trait leading to its selection, but also the sum of effects of other correlated traits that may be indirectly advantageous (Phillips & Arnold 1989). This can lead to an incomplete interpretation of empirical data. A more accurate interpretation of selection is to consider a multidimensional surface described by selection and co-selection acting on many traits simultaneously (Lande & Arnold 1983; Phillips & Arnold 1989). By failing to interpret multivariate selection correctly it is suggested that the strength of non-linear selection will be underestimated (Blows & Brooks 2003).

Non-linear selection can be examined by the extension of multiple regression to include quadratic terms of the original traits (Lande & Arnold 1983; Brodie et al. 1995; Blows & Brooks 2003). With the inclusion of all cross-product terms such models also consider trait interactions. The quadratic and cross product selection gradients can be interpreted as an n-dimensional surface described in a symmetrical matrix, γ (Blows & Brooks 2003; Blows 2007). Unfortunately, the ability to statistically detect and interpret this multidimensional space in an informative way can be limited by the number of terms (Blows 2007) and spatial perception (Phillips & Arnold 1989).

Canonical analysis utilises the symmetrical nature of the γ matrix to identify an alternative matrix (M) that defines an alternative set of orthogonal axes (eigenvectors) and selection gradients (eigenvalues) that describe the relationship between traits and fitness using fewer components (Box & Draper 1987; Phillips & Arnold 1989). The original trait axes are rotated in multidimensional space to eliminate all cross product terms (diagonalisation). Importantly, the eigenvectors are a linear combination of the original axes that describe exactly the same information as the original axes (Phillips & Arnold 1989; Blows 2007). This process therefore reduces the number of terms that one must subject to statistical tests in order to identify significant aspects of multivariate selection. The ability to visualise selection and identify non-linear selection are thus much improved (Phillips & Arnold 1989; Blows & Brooks 2003; Blows et al. 2003).

Non-linear selection has been largely ignored in previous studies of seaweed fly behaviour, in spite of early results showing non-linear selection for large males (Gilburn et al. 1992). To improve our understanding of selection resulting from sexual conflict in *C. frigida*, I apply a canonical analysis to a matrix of selection gradients resulting from laboratory mating trials. First, this technique is applied to a relatively small dataset (Dataset A; N = 219) in which males and females have been reared on two different algal genera, *Fucus* and *Laminaria* (Chapter 6). In addition to investigating multivariate selection in this dataset, it is hypothesised that female preferences may be directly influenced by environmental variables that influence condition. To examine this prospect we compare the shape of selection when raised on different algae.

The shape and strength of selection may be miscalculated through a lack of either empirical data or phenotypic variation over which the full range of selective pressures may act. To address these possibilities we investigate multivariate selection in *C. frigida* using a second larger dataset (Dataset B; N = 1331) comprising mating trials conducted over the past three years in the preparation of this thesis. These mating trials are varied in a number of respects including food source, larval density, exposure of flies to different algae, duration of exposure to algae and oviposition status of females. Dataset B represents greater phenotypic variation and statistical power. This provides the opportunity to measure and compare the shape and strength of selection under a variety of conditions.

7.2 Methods

The measure of fitness used was mating success. Following an attempt by a male to mate, each mating trial was scored as either 0 if the female successfully dislodged the male before genital contact or 1 if genital contact was achieved. Three male traits were included in the analysis; male size, struggle duration and the time taken for the male to mount a female. Male size and struggle duration can be considered measures of the ability of the male to confront female resistance whilst the time preceding a mount is a measure of the willingness of the male to mate.

Canonical analysis was carried out following the methods of Phillips & Arnold (1989) and Blows & Brooks (2003). All traits were transformed to a mean of 0 and standard deviation of 1 prior to multiple regression that included all linear, quadratic and cross-product terms to determine non-linear selection gradients. Non-linear selection gradients were entered into a symmetrical matrix, γ, describing the shape of selection. Eigenanalysis of this matrix was conducted in Microsoft Excel (Microsoft Corp., Redmond, WA) using the PopTools add-in (Hood 2006). This analysis produced a matrix (M) containing the eigenvalues in a diagonal, with all off-diagonal values being zero. The relationship between the eigenvalues and the original traits is described by a series of eigenvectors that relate the rotated axes to original trait space. Thus for each individual we could perform a transformation of the raw phenotypic scores by each eigenvector to obtain the phenotype expressed in M space. These phenotypic scores and their squared terms were re-entered into a full second order polynomial regression on mating success to determine how phenotype related to fitness. Significant quadratic terms in this regression indicate significant stabilizing (negative beta values) or disruptive (positive beta values) selection. To visualize non-linear selection we fitted and plotted a thin plate spline in R 2.5.1 (R Development Core Team 2007) using the Tps function in the fields package (Fields Development Team 2006).

To compare selection between populations (Dataset A) and studies (Dataset B) interactions of these factors and both linear and quadratic components of the M matrix were entered into logistic regression models. All significant interactions were included in models ($p < 0.05$) in addition to all linear components of the M matrix. If the interaction was with a quadratic term then all quadratic components of the M matrix were also included.

7.3 Results

7.3.1 Dataset A

Selection gradients prior to and following canonical analysis are shown in Table 7.1. There was significant non-linear (stabilising) selection for both male size and struggle duration, shown by the negative values for γ, in addition to linear (directional) selection in favour of larger males. None of the cross product terms were significant, suggesting little interaction between traits. Following canonical analysis the eigenvectors are found to closely resemble the original traits (Table 7.1). This is consistent with the lack of correlation found between traits. Axes m_2 and m_3 both exhibit significant stabilising selection. These results are consistent with analysis prior to rotation though the significance of relationships is marginally more significant. Stabilising selection can be easily visualised in three dimensional plots of the results (Fig. 7.1).

When comparing the preferences of females reared on either *Fucus* or *Laminaria*, both populations show positive directional selection for m_2 (approximating to struggle duration). However, selection for m_3 (approximating to male size) is in opposing directions (Fig. 7.1c-d). An interaction term between culture algae and m_3 is found to be significant ($\chi^2_1 = 4.453$, $p = 0.035$). The interaction between culture algae and male size is not found to be significant in original, pre-rotated, trait space ($\chi^2_1 = 2.928$, df = 1, $p=0.087$).

Table 7.1 a) Linear selection gradients (β) and the γ matrix of quadratic and cross-product selection gradients. b) The matrix of eigenvectors (M) following canonical analysis that describes the loading of each original trait to each eigenvector (m). The linear (θ) and quadratic (λ) selection gradients of each eigenvector, λ being equivalent to the eigenvalue. (* $p < 0.05$, ** $p < 0.01$)

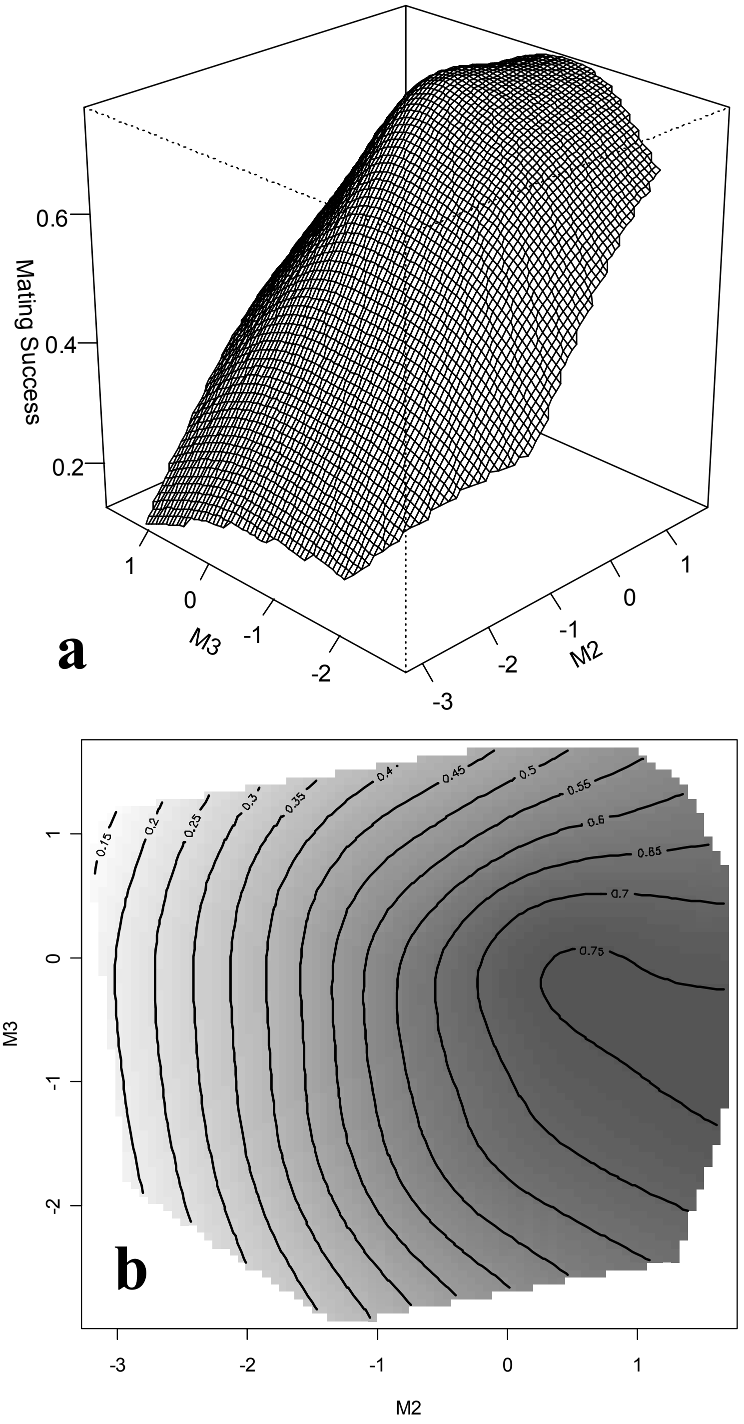
a

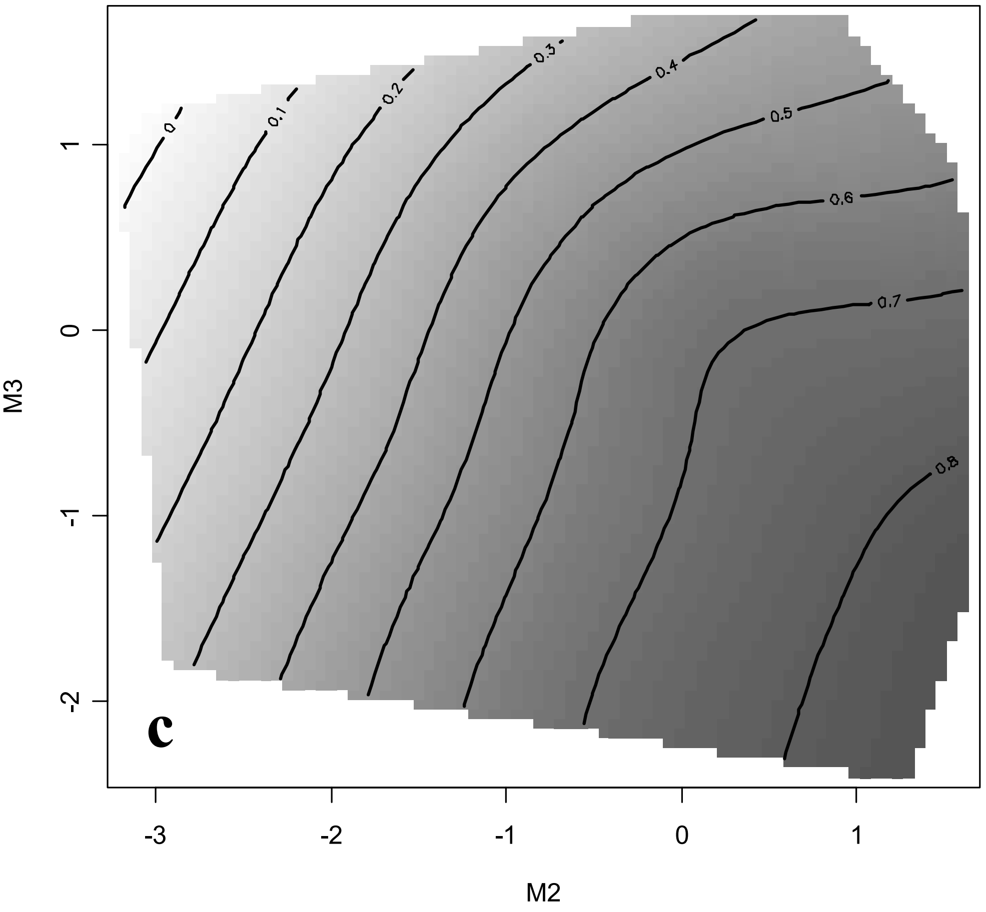
	β	**γ**		
		Time Preceding Mount	**Struggle Duration**	**Male Wing Length**
Time Preceding Mount	-0.014	-0.036		
Struggle Duration	0.059	0.003	-0.044*	
Male Wing Length	-0.088*	0.026	-0.009	-0.085**

b

	M			**θ**	**λ**
	Time Preceding Mount	**Struggle Duration**	**Male Wing Length**		
m_1	0.971	0.020	0.240	-0.034	-0.032
m_2	0.004	0.995	-0.101	0.067	-0.043*
m_3	-0.240	0.099	0.966	0.076*	-0.088**

Figure 7.1 Three dimensional plots of thin plate splines show selection along axes m_2 and m_3 following canonical analysis of Dataset A. Three dimensional perspective plot (a) and contour plot (b) of the entire dataset. Mating preferences of females when cultured using *Fucus* (c) and *Laminaria* (d).




c
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
M3
1
0
-1
-2
M2
-3
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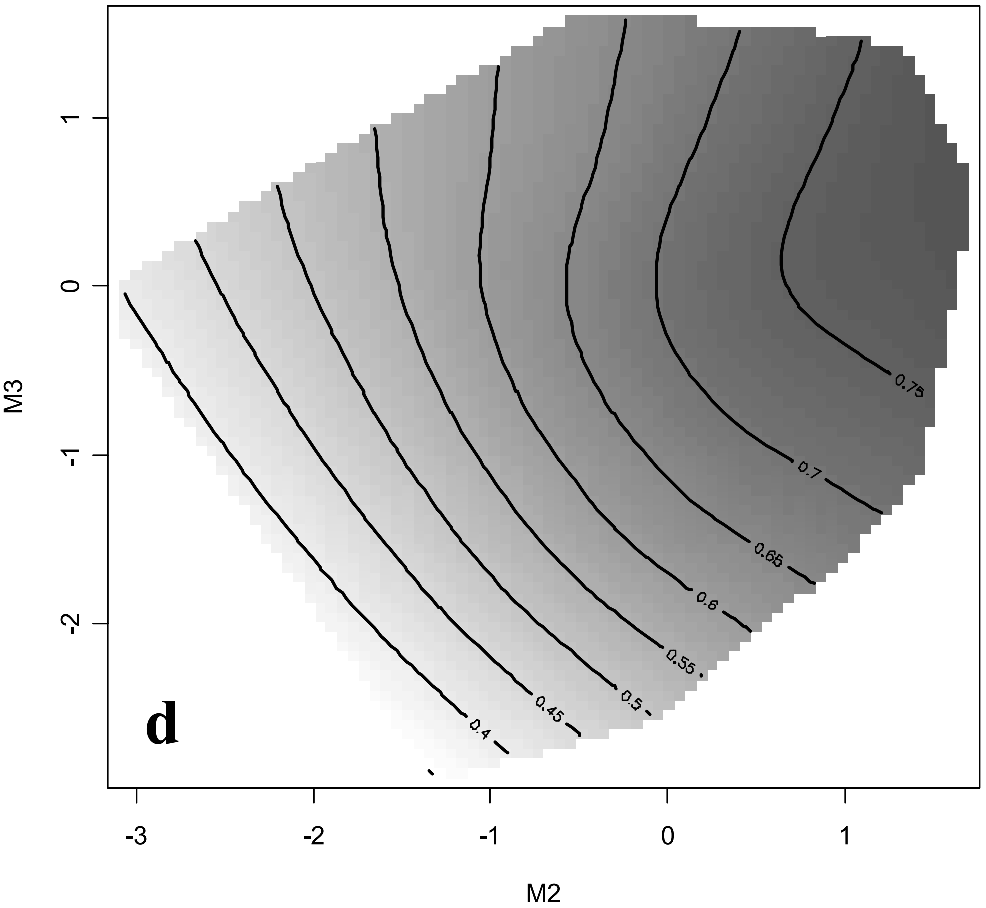
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7.3.2 Dataset B

Selection gradients prior to and following canonical analysis are shown in Table 7.2. Significant stabilising selection was found for both struggle duration and male size, consistent with Dataset A. There was also highly significant directional selection for shorter times before the male mounted a female and a significant interaction between this term and male size. Following canonical analysis, the loading of each original trait to the new vectors is more evenly weighted than for Dataset A. Thus it is not possible to approximate any of the new vectors to the original axes. All of the eigenvectors show highly significant selection, directional along m_1 and stabilising along m_2 and m_3. There is evidence for stabilising selection around a peak when the latter variables are visualised (Fig. 7.2). Variation in selection between different studies is evident through interactions between this factor and quadratic components of both m_2 ($\chi^2_6 = 16.518$, $p = 0.011$) and m_3 ($\chi^2_6 = 14.467$, $p=0.025$; Fig. 7.3). There was no significant variation in selection for m_1 between studies ($\chi^2_6 = 4.073$, $p = 0.667$).

Table 7.2 (a) Linear selection gradients (β) and the γ matrix of quadratic and cross-product selection gradients. (b) The matrix of eigenvectors (M) following canonical analysis that describes the loading of each original trait to each eigenvector (m). The linear (θ) and quadratic (λ) selection gradients of each eigenvector, λ being equivalent to the eigenvalue. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

a

		γ		
	β	**Time Preceding Mount**	**Struggle Duration**	**Male Wing Length**
Time Preceding Mount	.024***	-.017		
Struggle Duration	-.068	.011	-.049***	
Male Wing Length	-.003	.023*	.012	-.043***

b

	M				
	Time Preceding Mount	**Struggle Duration**	**Male Wing Length**	**θ**	**λ**
m_1	0.908	0.199	0.369	-0.053***	-0.012
m_2	-0.416	0.540	0.732	0.044***	-0.045***
m_3	0.054	0.818	-0.573	-0.019	-0.053***

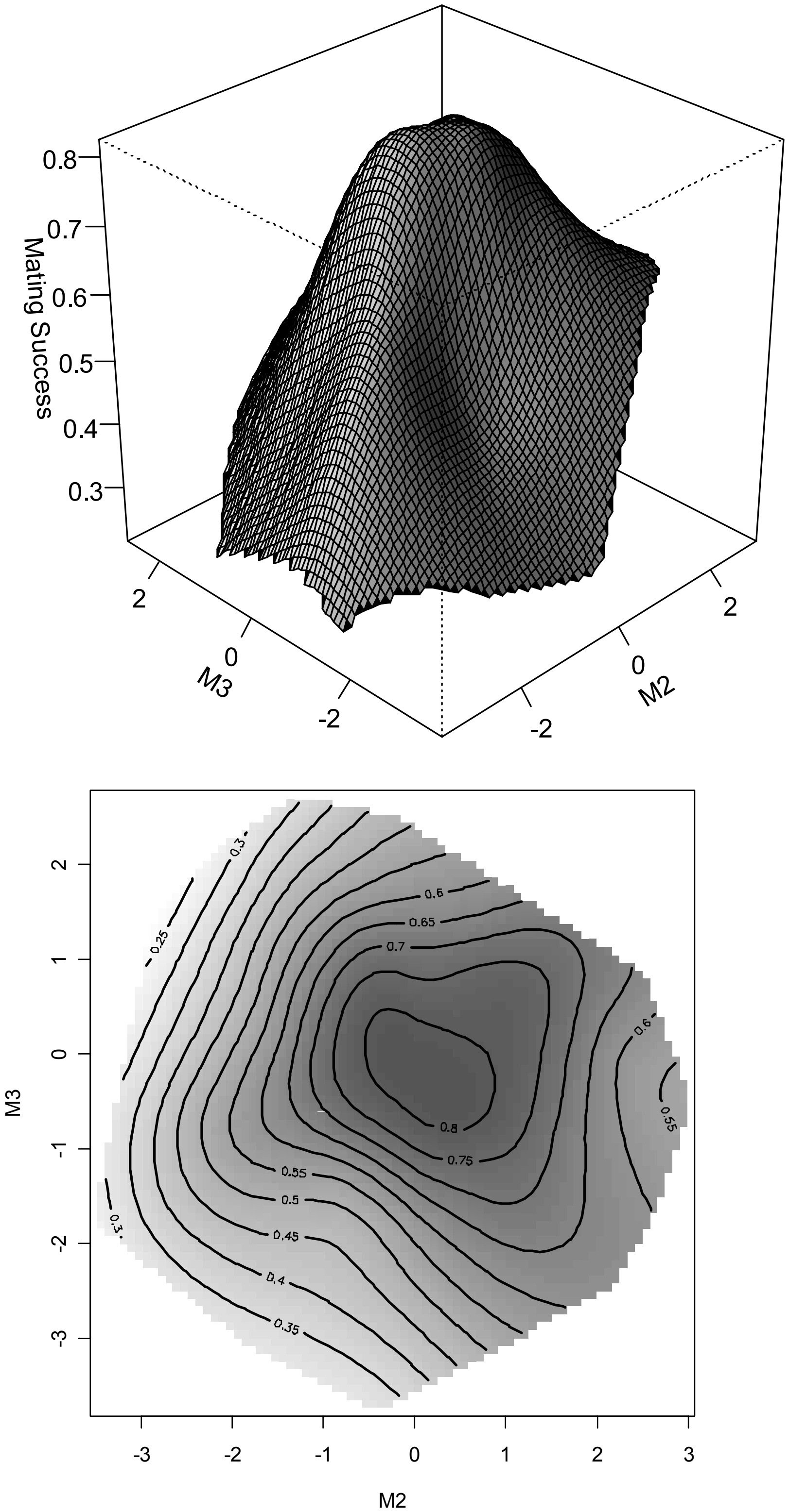
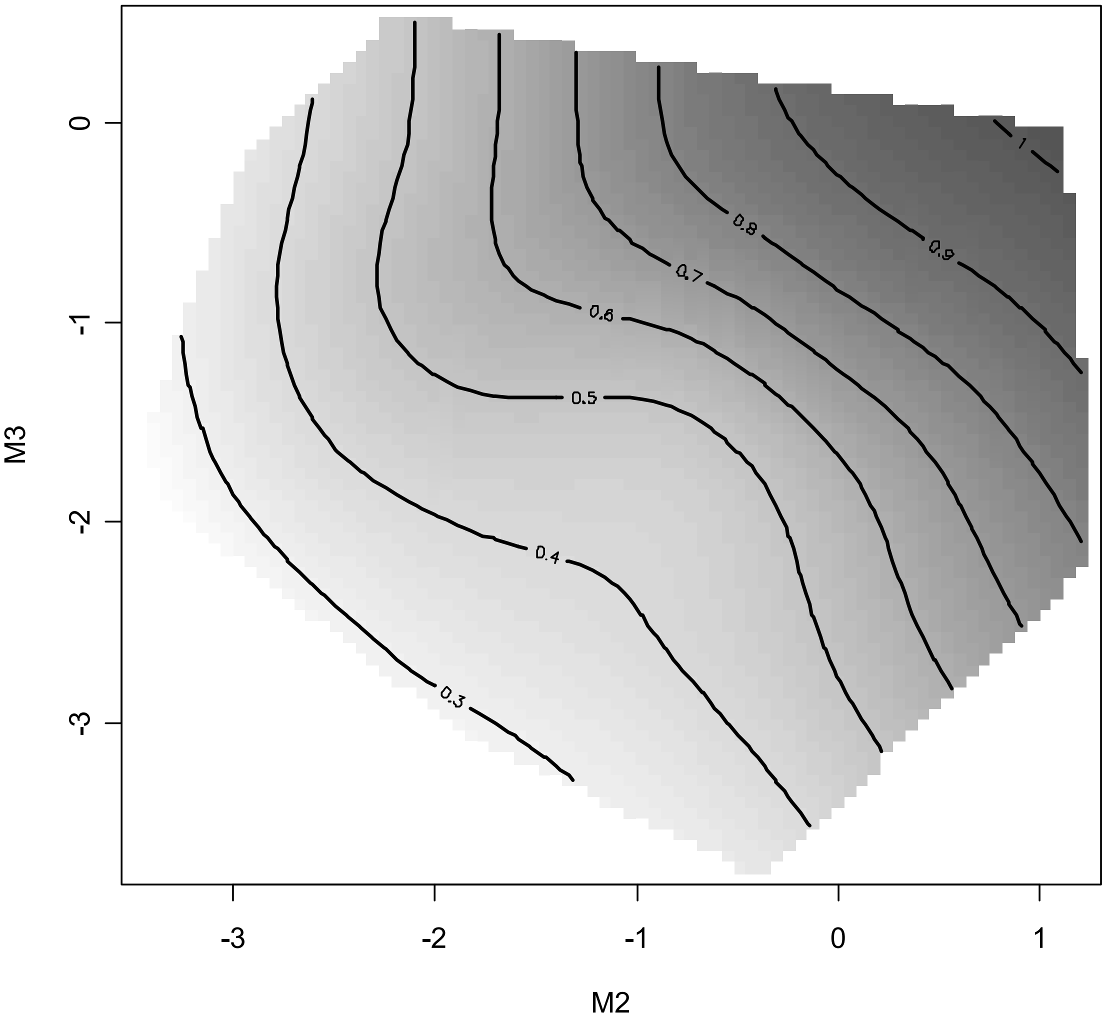
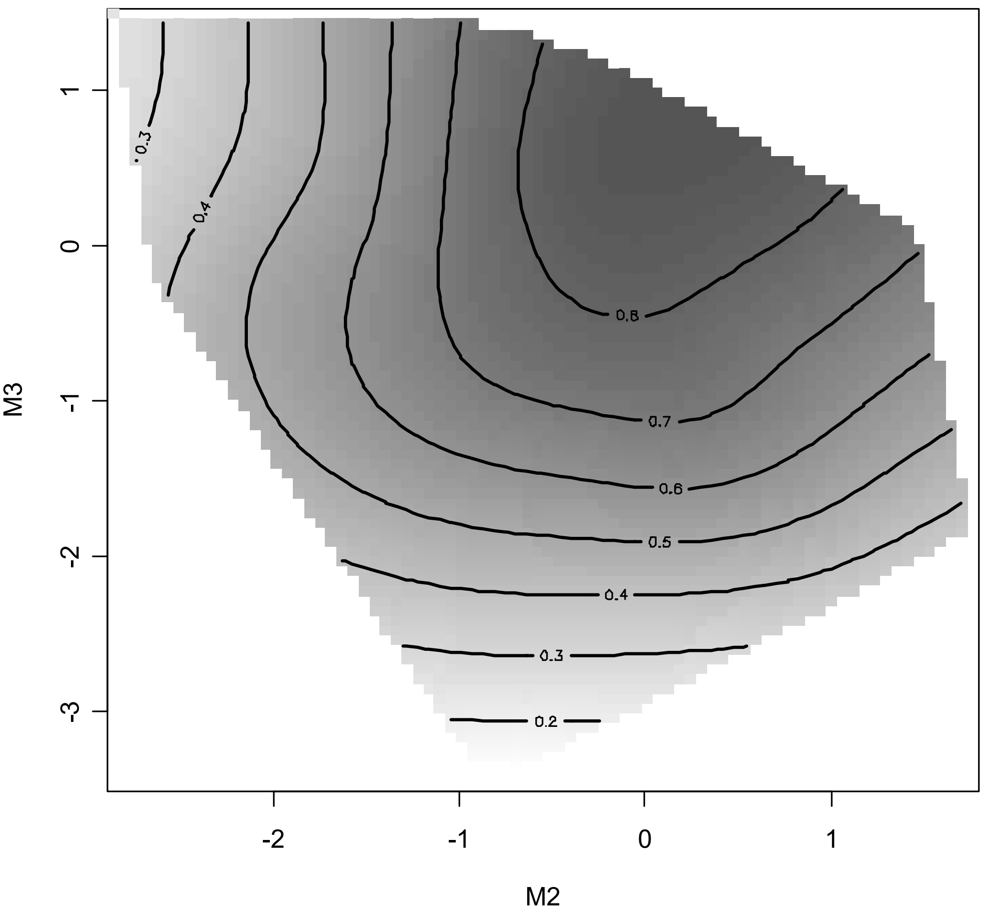


Figure 7.2 Three dimensional perspective and contour plot of thin plate splines showing selection along axes m_2 and m_3 following canonical analysis of Dataset B.

Figure 7.3 Three dimensional contour plots showing selection along axes m_2 and m_3 for the seven separate studies that comprised Dataset B.

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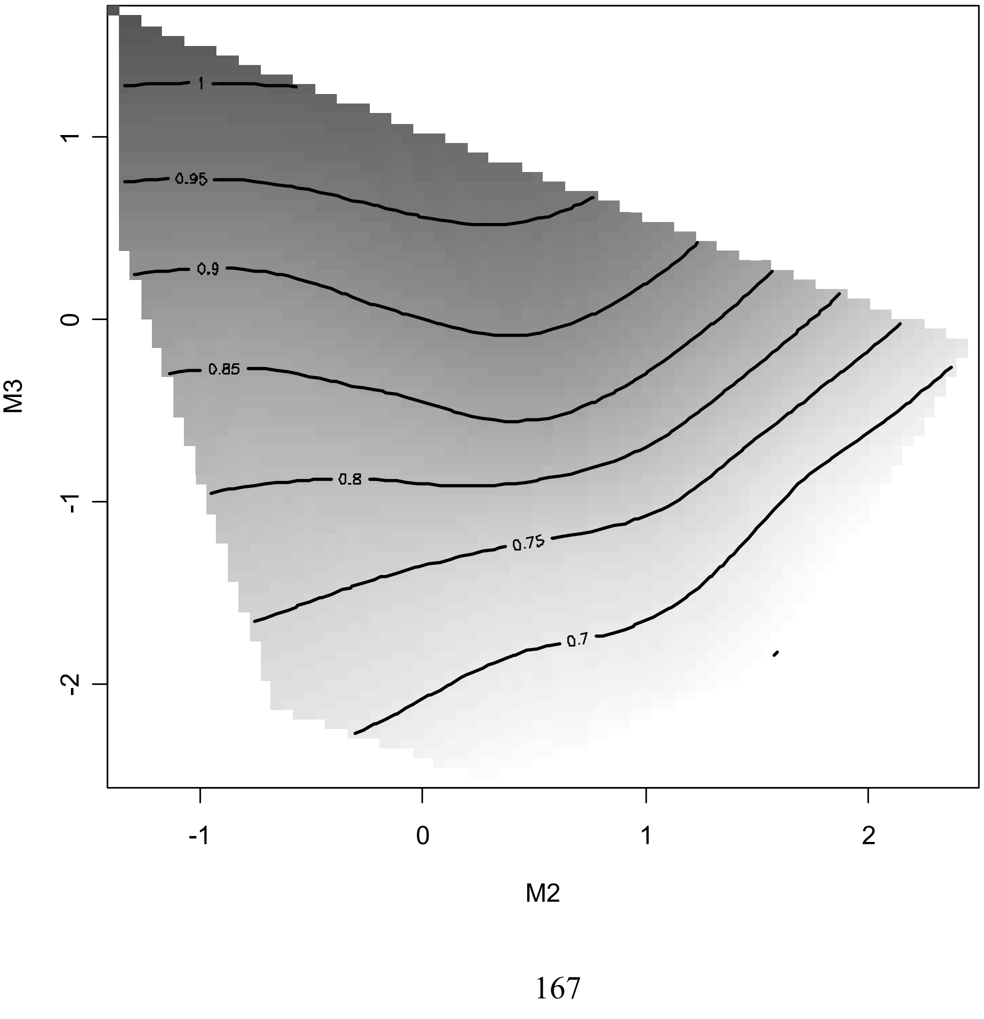
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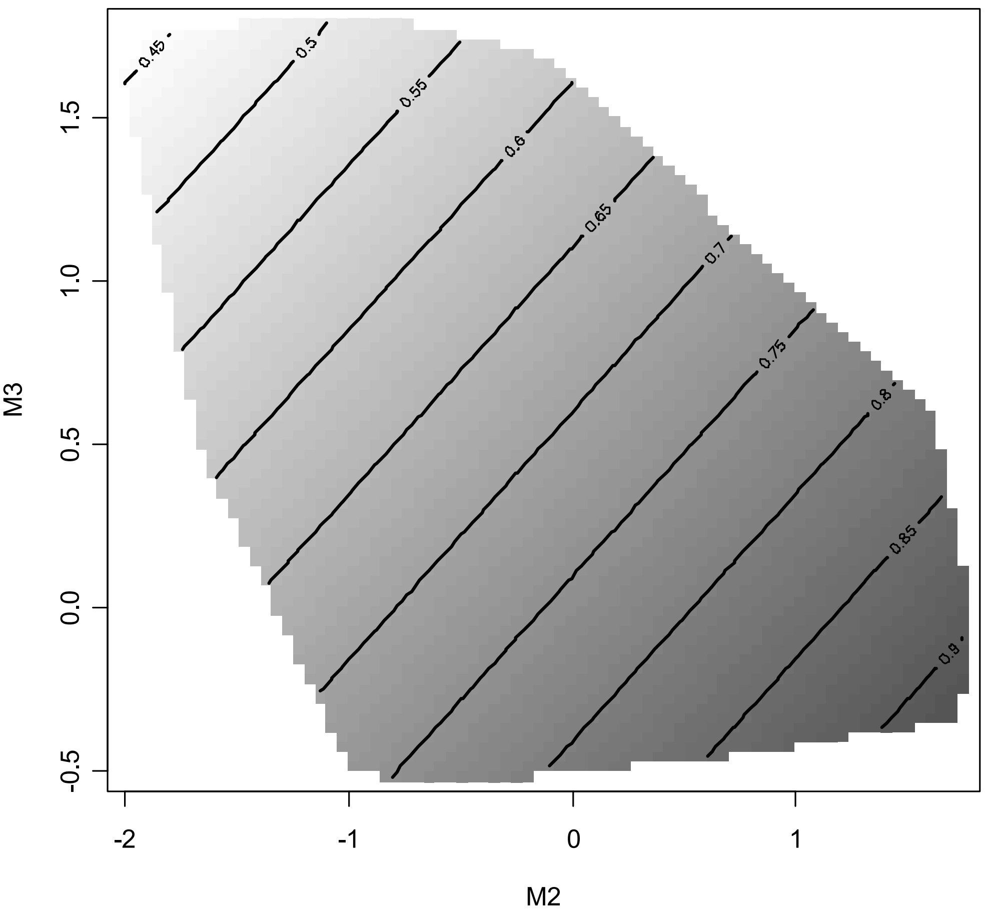
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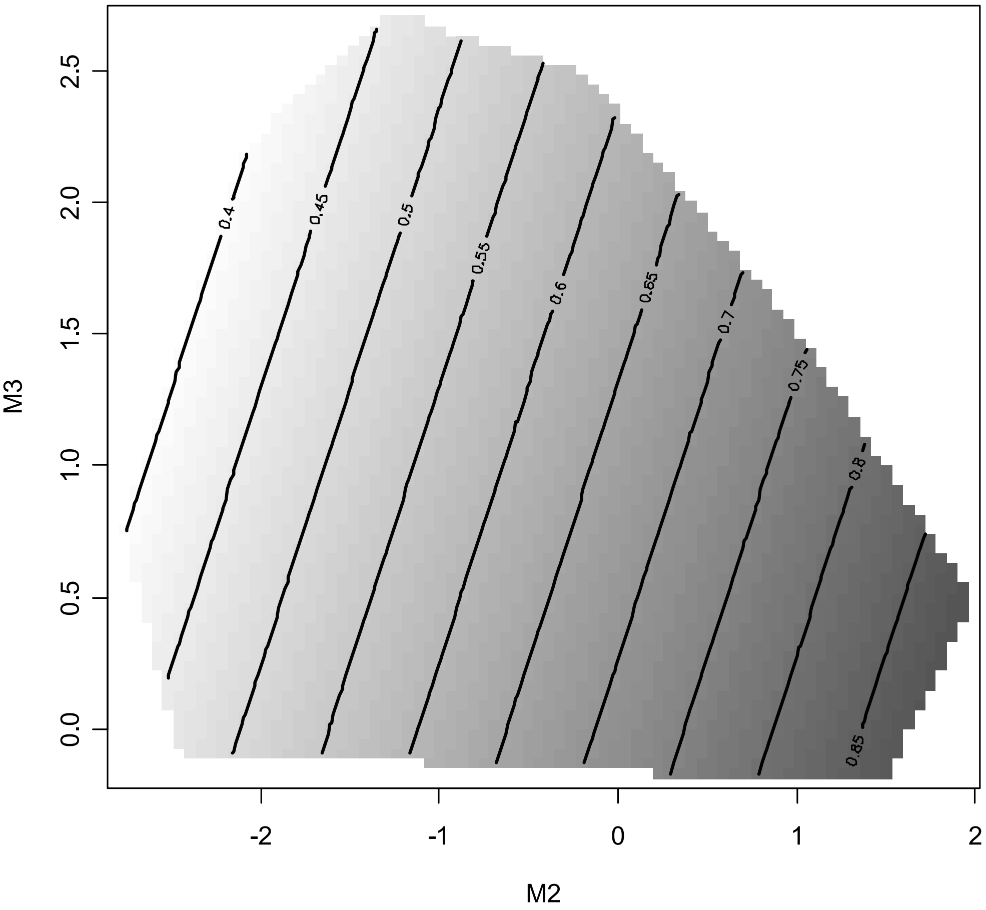
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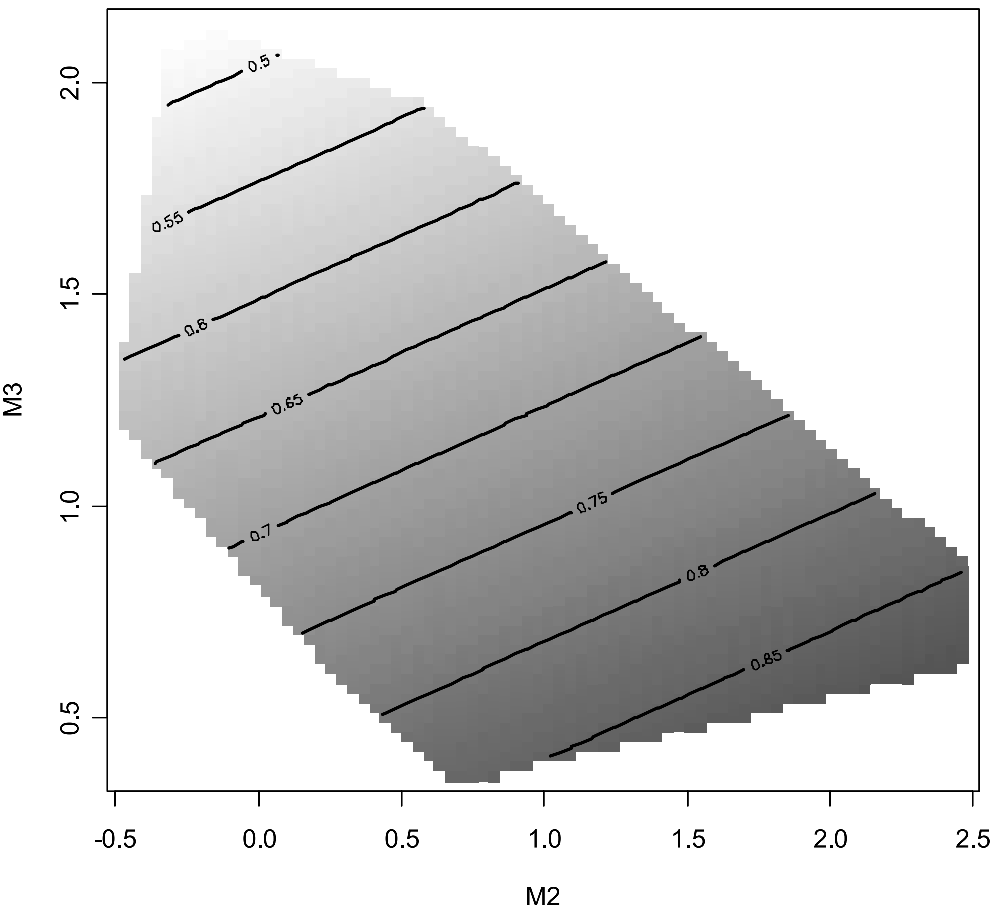
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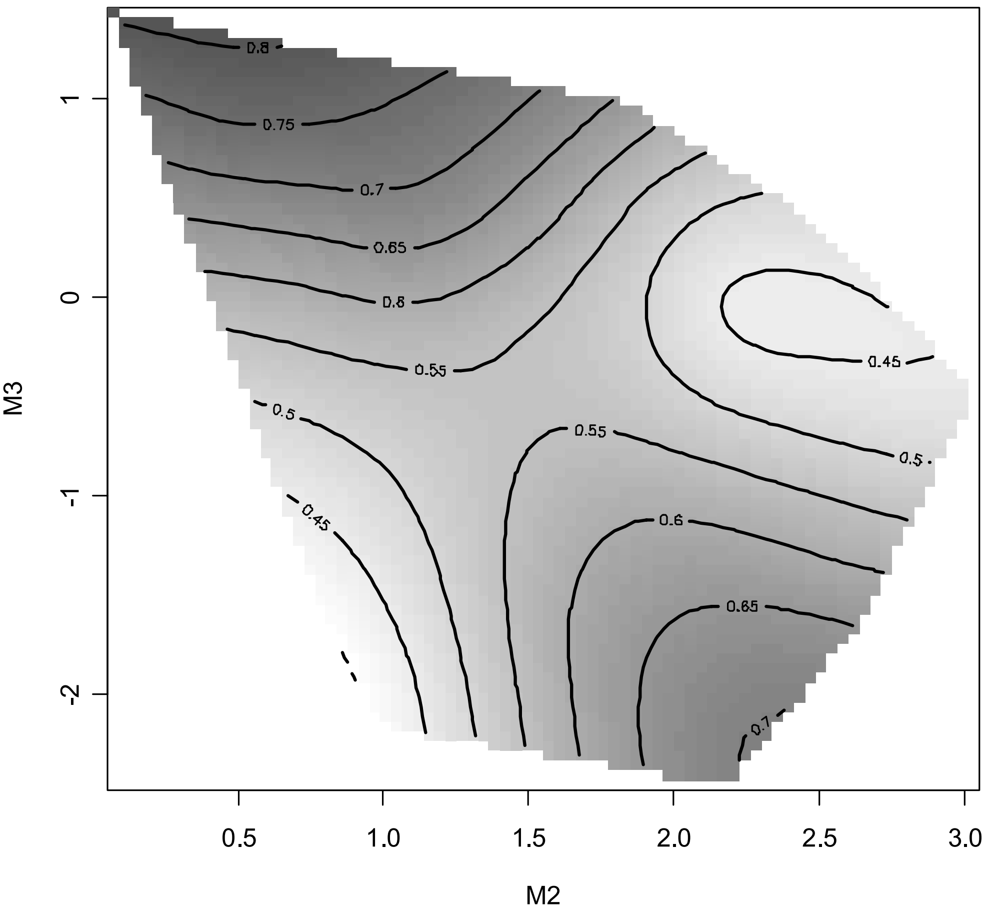
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7.4 Discussion

Non-linear and multivariate selection in the seaweed fly has been overlooked through more than two decades of behavioural studies. Selection for large male seaweed flies has been identified on numerous occasions (Gilburn et al. 1992, 1993; Crean et al. 2000) yet the influence of other male traits correlated with size has not been examined. The results of this analysis demonstrate significant stabilising selection for both male size and struggle duration in each of the datasets. Canonical analysis of each dataset further demonstrates significant multivariate selection and different patterns of selection across environmental conditions.

The results of Dataset A demonstrate that selection is dependent upon the algae in which larvae develop. By accepting an alpha level of 0.05 this relationship is only found following canonical rotation of the data. Though the rotated axes approximate closely to original trait space this result demonstrates that selection is multivariate. Previous work has shown that adult *C frigida* developing in *Laminaria* are of improved condition (Chapter 6). Not only does the condition of *Fucus* and *Laminaria* reared females differ, but also the pattern of sexual selection imposed upon males; a difference that is principally observed in selection for male size. Until very recently, all laboratory studies of seaweed fly behaviour were conducted following culture on *Fucus* algae. It is now apparent that this practice restricts variation in the development of flies and can affect the outcome of selection studies.

The results of the second dataset yield a diverse account of sexual selection in *C. frigida*. Though data was not obtained exclusively for this analysis this approach has the advantage of providing a large and diverse dataset. Results demonstrate that within this data the most significant selection does not act on traits in isolation but upon multivariate composites of the original traits. Variation in multivariate selection across environmental conditions represents a novel finding in the study of seaweed flies. The extent of diversity is also greater than may have been anticipated.

The ease of mass culture and ability to control both developmental and adult conditions has contributed to the success of *C. frigida* as a model species for the study of sexual conflict. Unfortunately, incomplete knowledge of the diverse effects of environmental variation and a limited statistical approach has led to an incomplete interpretation of results. The data used in this analysis varied in both sample size and environmental conditions. Results demonstrate that the best way to improve clarity of results may not always be solely through more numerous data.

Varying patterns of sexual selection within a species may be a fundamental component to evolutionary processes that are not yet fully understood. For example, it has been demonstrated that interpopulation variation in sexual conflict and the strength of sexual selection may contribute to reproductive isolation (Lande 1981; Parker & Partridge 1998; Arnqvist et al. 2000; Gavrilets 2000; Martin & Hosken 2003b). A greater diversity of interpopulation variation in selection demonstrated in *C. frigida* may enhance this process. In addition, greater variation in sexual selection may assist in maintaining genetic variance in male traits. Genetic variance and heritability of selected traits form an elemental component of sexual selection theory (Andersson 1994).

The findings of this latest analysis formally demonstrate different selection across environmental conditions in *C. frigida*. Greater emphasis needs to be placed upon addressing this variation in future studies. Adopting techniques such as canonical analysis to address multivariate selection will further assist in more accurate presentation of results.

Chapter 8 - Longevity of *C. frigida*

8.1 Introduction

Existence is a costly endeavour. There is a limit to the resources that any individual may acquire during a lifetime and fitness can be measured through the success of different strategies in allocating these assets (Stearns 1976; Partridge & Harvey 1988; Roff 1992; Stearns 1992). The decision to invest in any particular venture will ultimately force a trade-off as resources are diverted from other life history traits. A fundamental component of fitness is reproductive success and so it is unsurprising that the costs and trade offs associated with reproduction have attracted substantial interest (Williams 1966; Reznick 1985; Partridge & Harvey 1985; Reznick 1992; Koivula et al. 2003). Crucially, an increase in current investment in reproduction can limit future reproductive prospects and survival (Williams 1966; Reznick 1985).

The costs of reproduction are diverse - from the production of sex organs and cells, time and energy invested in mate searching and copulation through to energy invested in raising offspring (Bell & Koufopanou 1986; Roff 1992; Stearns 1992). Though it has historically been perceived that these costs are borne through mutual cooperation of the sexes, this view is now superseded by substantial evidence to the contrary (Arnqvist & Rowe 2005). Sexual conflict results from a divergence in the evolutionary interests of the sexes driven by maximisation of individual, rather than group, fitness (Parker 1979). Asymmetry in reproductive interests means that the costs of reproduction can no longer be considered independent of sex. Furthermore, the evolutionary outcomes of sexual conflict cannot be fully understood without a comprehensive understanding of the costs involved (Andersson 1994; Arnqvist &

Nilsson 2000; Johnstone & Keller 2000). For sexually antagonistic coevolution to operate both males and females must incur significant costs of mating that will drive the evolution of adaptive traits (Parker 1979; Rowe et al. 1994; Holland & Rice 1998; Gavrilets et al. 2001). Traits that have minimal influence on the costs of reproduction in the opposite sex will consequently have minimal influence in the evolution of a response. Identifying sexual conflict may be considered only the first step in describing this evolutionary process and should be accompanied by identification of the costs involved.

The costs of sexual conflict have been quantified in a number of model species and include harm caused by components of the male ejaculate, physical damage resulting from multiple copulations, energy and time invested in multiple mating attempts, greater risk of predation and reduced foraging efficiency (Magnhagen 1991; Rowe et al. 1994; Chapman et al. 1995; Stockley 1997; Crudgington & Siva-Jothy 2000). A frequently adopted measure of these costs has been longevity following reproduction. Reduced longevity in females resulting from sexual conflict has been recorded in the fruit fly, *Drosophila melanogaster* (Fowler & Partridge 1989; Wigby & Chapman 2004), the bean weevil, *Callosobruchus maculatus* (Crudgington & Siva-Jothy 2000), the tsetse fly, *Glossina morsitans,* (Clutton-Brock & Langley 1997), the ground cricket, *Allonemobius socius* (Fedorka & Mousseau 2002), the seed bug, *Lygaeus equestris* (Shuker et al. 2006), the yellow dung fly, *Scatophaga stercoraria* (Martin et al. 2004) and the dung fly, *Sepsis cynipsea* (Blanckenhorn et al. 2002; Martin & Hosken 2003b). Costs of mating may be confused with a simultaneous increase in female oviposition rate, though a number of studies have found the effects of mating on female lifespan to be independent of oviposition (Fowler & Partridge 1989; Chapman et al. 1998; Blanckenhorn et al. 2002).

In both *S. cynipsea* and *C. maculatus* longevity costs are incurred through genital damage caused by the male intromittent organ (Crudgington & Siva-Jothy 2000; Blanckenhorn et al. 2002). In *D. melanogaster* these costs can be attributed to male accessory gland products (Chapman et al. 1995). In tsetse flies, female mortality is increased as a result of physical attributes of mating rather than components of the ejaculate (Clutton-Brock & Langley 1997). Importantly, it has been demonstrated that females can adapt to these costs after a number of generations reared under high conflict conditions (Martin & Hosken 2003b; Wigby & Chapman 2004). In *C. maculatus*, mating costs are reduced by female kicking, an adaptation that reduces copulation duration and genital damage (Crudgington & Siva-Jothy 2000).

The cost of reproduction to males remains relatively understudied, perhaps due to the historical perspective that males invest only in cheaply manufactured sperm (Dewsbury 1982). Nevertheless, a number of studies have recognized a cost of mating to male longevity in fruit flies, wolf spiders, seed beetles, dung beetles and dung flies (Partridge & Farquhar 1981; Cordts & Partridge 1996; Mappes et al. 1996; Clutton-Brock & Langley 1997; Kotiaho 2000; Kotiaho & Simmons 2003; Martin & Hosken 2004; Paukku & Kotiaho 2005). It is then not surprising that the evolution of male mate choice is attracting increased attention (Bonduriansky 2001; Wedell et al. 2002).

A number of studies have considered factors affecting longevity in *C. frigida*; principally the effects of exposure to algae and repeated matings. Both Shuker (1998) and Dunn et al. (2002) found few apparent costs to longevity of multiple mating in *C. frigida*, though neither study considered the effect of mating on male longevity. Dunn et al. (2002) did identify a reduction in female longevity coinciding with increased oviposition rate when exposed to algae; however these effects were independent of multiple mating. Though no cost of mating had been identified in these studies their relevance may be limited by the number of mating interactions considered; either a single interaction (Dunn et al. 2002) or being left with up to 5 males for 48 hours (Shuker 1998). These conditions may underestimate natural levels of conflict as seaweed flies are known to be highly promiscuous and females will potentially be harassed by many hundreds of males during a 3-4 week lifespan (Blyth & Gilburn 2006). Nevertheless, in more recent work Dunn et al. (2005) did find an oviposition independent reduction in longevity for females after only four matings. In a more recent study of seaweed fly longevity that included extended exposure to the opposite sex Meader & Gilburn (in press) found an impact on longevity resulting from both exposure to seaweed and sexual conflict. The sexes showed different mean longevities, females surviving up to 53.6 days in the absence of seaweed, outliving males by more than 7 days. However, the effect of sexual conflict on longevity was asymmetrical. A reduction in female longevity was recorded when paired with a male yet there was no significant effect on male longevity when paired with a female (Meader & Gilburn, in press). This result suggests that the costs of sexual conflict are borne primarily by females.

Having established asymmetrical reproductive interests and costs of reproduction driving sexually antagonistic coevolution, it is variation in these processes that leads to diversity. Variation in the costs of sexual conflict may derive from ecological differences between populations. Sex ratios, population density, predation risk and food availability may all influence the relative costs of reproductive interactions (Magnhagen 1991; Rowe et al. 1994; Hardling & Kaitala 2005). Indeed, it has recently been demonstrated that the negative effects of multiple mating vary between different populations of the seed bug, *L. equestris* (Shuker et al. 2006). It is suggested that future studies need to consider a wider range of ecological variation to accurately determine the costs of sexual conflict.

Though evidence of sexual conflict in seaweed flies is abundant (Day & Gilburn 1997; Crean & Gilburn 1998) there are conflicting reports of the costs of sexual conflict (Shuker (1998); Dunn et al. 2002, 2005; Meader & Gilburn, in press) and nothing is known about how these costs may vary under different ecological conditions. It is now understood that female oviposition preferences, male mating behaviour and adult condition can vary when exposed to different algae (Philips et al. 1995b; Chapters 4, 5 & 6). It is therefore predicted that the costs of sexual conflict will also differ between algal environments. As the level of sexual conflict is increased when adult *C. frigida* are exposed to *Laminaria* algae (Chapter 4) it is predicted that a corresponding increase in the costs of sexual conflict will also be realised. In this study the costs of sexual conflict in *C. frigida* are addressed by determining the longevity of i) both male and female flies, ii) flies exposed to realistic levels of sexual conflict and iii) flies experiencing different ecological conditions. Male and female *C. frigida* are paired with members of the opposite sex in the presence of either *Fucus* or *Laminaria* algae for the lifetime of each individual.

8.2 Methods

8.2.1 Experimental procedure

Wild *C. frigida* larvae were collected from wrack beds at Crail, Fife and cultured using *Laminaria* algae. Virgin male and female flies were placed individually into small containers (55mm diameter by 75mm) containing a small sponge square soaked in 5% sucrose solution and a small amount of either *Laminaria* or *Fucus* algae. In addition to these focal individuals, either none, one or three flies of the opposite sex were also introduced into each container. Containers were stored in a controlled temperature room set at 25°C on a 12h:12h light dark cycle and checked twice daily. When the focal individual died this was recorded and the wing length of the fly was measured. If a fly other than the focal individual died then the body was removed and replaced with another randomly selected fly of the same sex. Twice each week the contents of containers were replaced with fresh algae and the sponge pad replenished with sucrose solution. Observations continued until all focal individuals had died.

8.2.2 Statistical analysis

All data were checked for normality and transformed where necessary. Longevity was cube root transformed. The effects on longevity of sex, wing length, exposure to different algae and different levels of sexual conflict plus all two and three way interactions were analysed in General Linear Models using SPSS v12.0.2 (SPSS Inc. Chicago IL, USA). In addition, separate General Linear Models were constructed for exposure to each alga and for each sex. Maximal models were chosen based upon the Akaike Information Criterion calculated using R 2.5.1 (R Development Core Team 2007), non-significant terms being removed.

8.3 Results

8.3.1 Summary of results

The longevity of 473 flies was recorded; 235 female and 238 male. Consistent with previous studies, males were larger and showed greater variation in size than females. An initial model comparing exposure to either one or three members of the opposite sex showed no significant effect of sexual conflict on longevity ($F_{(1,268)} = 1.295, p = 0.256$). All further analyses considered sexual conflict as either the presence or absence of members of the opposite sex.

A model including all individuals showed significant effects of sex ($F_{(1,463)} = 5.883, p = 0.016$), algae ($F_{(1,463)} = 50.101, p < 0.001$) and conflict ($F_{(1,463)} = 107.979, p < 0.001$) on longevity. The direction of these effects become confused due to interactive effects between sex and conflict ($F_{(1,463)} = 12.965, p = 0.001$), algae and conflict ($F_{(1,463)} = 7.115, p = 0.008$) and a near significant three way interaction between sex, conflict and algae ($F_{(1,463)} = 3.679, p = 0.056$). Size did have an effect on longevity, though this was dependent on an individuals sex ($F_{(1,463)} = 6.522, p = 0.011$). Greater clarity of results is achieved through simplification of the model using subsets of the data.

8.3.2 Effect of algal exposure

When exposed to *Fucus*, sexual conflict caused a decrease in longevity ($F_{(1,229)} = 27.363, p < 0.001$), though the magnitude of this decrease was asymmetrical between the sexes ($F_{(1,229)} = 12.603, p < 0.001$). The reduction in longevity resulting from sexual conflict was greater for females (Fig 8.1). Sexual conflict also caused a reduction in longevity when exposed to *Laminaria*, ($F_{(1,233)} = 98.810, p < 0.001$), though the decrease was similar for both sexes ($F_{(1,232)} = 1.845, p = 0.176$; Fig 8.1).

8.3.3 Effect of sex

Male and female longevity were both significantly influenced by exposure to different species of algae and levels of sexual conflict. However, only for males was there an interactive effect between these two factors (Table 8.1). The reduction in longevity resulting from sexual conflict was greater for males when exposed to *Laminaria* than *Fucus* (Fig. 8.1).

Male longevity was influenced by wing length ($F_{(1,232)}$ = 7.725, p = 0.006), though this was dependent on the level of sexual conflict ($F_{(1,232)}$ = 6.247, p = 0.013; Fig 8.2). Female longevity was not influenced by wing length ($F_{(1,231)}$ = 1.763, p = 0.186).

Table 8.1 Results of General Linear Models showing the effects of different algae and sexual conflict on male and female longevity.

Sex	Factor	d.f.	F-statistic	P
Male	Algae	1, 232	23.275	.000
	Sexual Conflict	1, 232	9.702	.002
	Algae * Conflict	1, 232	11.836	.001
Female	Algae	1, 232	31.023	.000
	Sexual Conflict	1, 232	85.889	.000
	Algae * Conflict	1, 231	0.279	.598

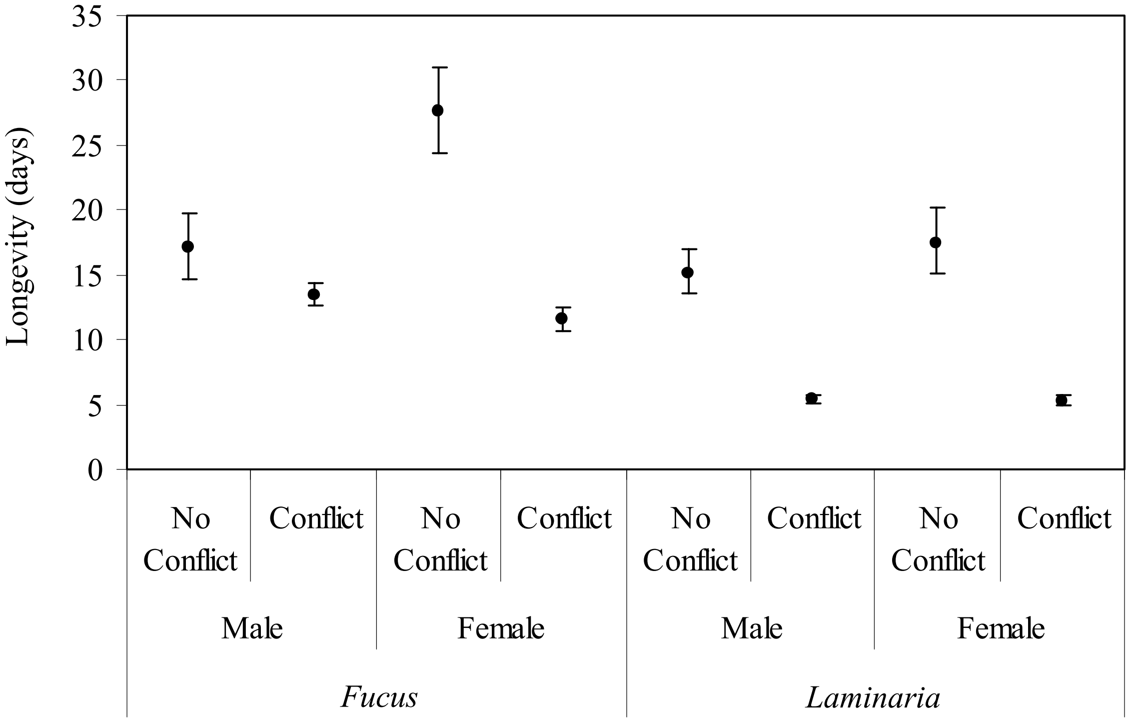

Figure 8.1 Male and female longevity when exposed to either *Laminaria* or *Fucus* algae and in the presence or absence of members of the opposite sex. (Error bars show standard errors.)

Figure 8.2 The relationship between male size and longevity with (Δ) and without (□) sexual conflict.

8.4 Discussion

For *C. frigida*, reproduction is typified not only by high promiscuity (Blyth & Gilburn 2006) but a visibly energetic mating interaction (Day et al. 1990). The time and energy invested must therefore be considerable for both males and females. It is anticipated that these costs will be offset against other life history traits. The results of previous work that found no effect of mating on longevity is inconsistent with expectations (Shuker 1998; Dunn et al. 2002). One explanation for this disparity is the number of mating opportunities presented to flies. Individual flies may have hundreds of sexual partners over a lifetime. Assuming a cumulative effect on longevity the reduction in lifespan after only a few matings will be relatively minor. Where a cost to longevity has been found in previous studies the magnitude of this reduction has been small in comparison to that reported here. This illustrates the importance of recreating natural levels of sexual conflict when measuring the costs and may explain why other studies have not identified a reduction in longevity.

A trade off between reproduction and longevity is evident from the results of this study and others. The high costs of sexual conflict in this mating system are consistent with maintenance of female preference through convenience polyandry (Thornhill & Alcock 1983; Rowe et al. 1994; Crean & Gilburn 1998; Meader & Gilburn, in press). The results of this study are also in direct accord with those of Meader & Gilburn (in press). In both cases it is demonstrated that sexual conflict when exposed to *Fucus* algae has an effect on longevity that is greater for females than males. Further to this finding, this study is the first to examine longevity of *C. frigida* when exposed to *Laminaria* and finds a contrasting symmetric effect of sexual conflict.

When exposed to algae an elevation in male mating behaviour is observed, which is likely to include mate searching behaviour. In the absence of females males may still experience a cost of mate searching though no costs of mating itself. The close similarity between male longevities on each alga in the absence of females suggests that there is little difference in the costs of mate searching when exposed to different algae. The increase in costs when females are present is instead expected to be the result of physiological costs of male harassment (Meader & Gilburn, in press). Previous work has found that male harassment is increased when males are exposed to *Laminaria* rather than *Fucus* (Chapters 4 & 5). This led to the prediction that sexual conflict would be greater in a *Laminaria* rich environment. Reduced longevity of males in the presence of females and *Laminaria* algae is consistent with increased physiological costs of elevated levels of sexual conflict.

In the absence of sexual conflict there is a greater relative cost of exposure to *Laminaria* than *Fucus* for females, but not for males. Females are not predicted to engage in mate searching behaviour, however oviposition is stimulated when exposed to algae and is independent of mating (Dunn et al. 2002). The greater reduction in longevity when exposed to *Laminaria* is consistent with higher oviposition rates on this alga (Phillips et al. 1995b; Dunn 2001; though see Chapter 6) and a previously reported effect of oviposition on longevity (Dunn et al. 2002). The reduction in female longevity resulting from sexual conflict is expected to be due to physiological costs of pre-copulatory conflict and not post copulatory effects of chemicals that may have been passed to the female (Meader & Gilburn, in press).

A cost of conflict to male *C. frigida* represents the selective pressure exerted by the female rejection response. The identification of these costs is important in describing sexually antagonistic coevolution as sexual conflict has a cost for both sexes. Male adaptations to maintain reproductive fitness in spite of these costs would be advantageous. Though there was an advantage of greater longevity to smaller males this was reduced during sexual conflict (Fig. 8.3). This is consistent with a larger size of male being able to resist costs of mating imposed by the female rejection response and the resultant large male mating advantage. In addition to attaining greater size, males could maintain reproductive fitness through mate choice. The benefits of male mate choice are predicted to increase as mating becomes more costly (Bonduriansky 2001). A cost to longevity for males forces a trade off between current and future reproductive opportunities. A decision to limit investment in poor quality females may prove beneficial if resources can be reserved for future matings with high quality females. Male mate choice in *C. frigida* has received mixed support in studies carried out to date (Pitafi et al. 1990, 1995; Dunn et al. 1999). The finding that the costs of sexual conflict are dependent on algal exposure presents new opportunities to compare evidence for male mate choice in different populations of *C. frigida*.

Investigating the processes and outcomes of sexual conflict has been dependent on the manipulation of conflict and comparison of responses. Previous studies have achieved this through altering sex ratios (Clutton-Brock & Langley 1997; Wigby & Chapman 2004), mating frequency (Fowler & Partridge 1989; Blanckenhorn et al. 2002; Kolodziejczyk & Radwan 2003) and constraining evolution by enforcing monogamy and polyandry (Martin et al. 2004; Martin & Hosken 2003b). Whilst intuitive, such manipulations may provide unrealistic comparisons. Wrack bed composition is known to be variable and provides an opportunity to indirectly alter the costs of sexual conflict within naturally occurring parameters without directly affecting the mating system. It is anticipated that manipulation of the local environment should play an important part in future studies of sexual conflict; not only in *C. frigida*.

An important caveat to the interpretation of these results is that the relationship between how the costs of mating influencing longevity and overall reproductive fitness may be different. Martin & Hosken (2003b) showed that despite a cost to longevity resulting from sexual conflict in *Sepsis cynipsea* there is no overall difference in reproductive success. Though sexual conflict when exposed to *Laminaria* may lead to a greater reduction in longevity for *C. frigida* this may be offset by benefits of development in this alga. Individual condition, fecundity, survival rate of larvae and size at eclosion are known to be greater following development on this alga (Culture 6). Indirect benefits through offspring may justify greater investment by parents. The true costs of reproduction, particularly in different ecological settings, may only truly be understood following a more complex assessment of progeny fitness and survival.

Chapter 9 - Stable isotopes and larval diets

9.1 Introduction

The successful development of any individual is undeniably influenced by the quality of the surrounding environment. Consequently, the successful ability to locate and utilise the most suitable conditions for development are fundamental life history traits. Within the invertebrates this is typified by the close relationship to be found between the oviposition preferences of females and offspring development (Thompson, 1988; Poore & Steinberg, 1999; Awmack & Leather, 2002). In addition to locating the most nutritious site for oviposition, identifying other factors such as enemy-free space, competition-free space and avoiding hosts that produce inhibitory substances also contribute to the decision making process (Björkman et al., 1997; Ballabeni et al., 2001).

Oviposition preferences are of particular significance due to the often limited mobility of larvae and the subsequent requirement to complete development within a limited range (Björkman et al. 1997). Larval stages capable of increased mobility may be associated with relaxed oviposition preferences, particularly if significant costs result from the preference (Björkman & Larsson, 1991; Poore & Steinberg, 1999). Dipteran larvae have been shown to identify the most nutritious diet dependent upon the physiological requirements of their age (Waldbauer et al., 1984; Zucoloto 1987, 1991). For example, oranges that comprise the diet of the medfly *Ceratitis capitata* can vary in quality within individual fruits though adult females show no preference for more nutritious regions (Fernandes-da-Silva & Zucoloto 1993). Following oviposition individual larvae locate and migrate towards more profitable parts of the fruit. The ability of both adults and larvae to identify profitable environments at different spatial and temporal scales further enhances individual developmental success (Zucoloto 1991; Fernandes-da-Silva & Zucoloto 1993).

The two British species of coelopid, *C. frigida* and *C. pilipes*, are often found to inhabit the same beaches but there is now substantial evidence to suggest preferences towards different seaweed species. Despite the abundance of information concerning adult preferences very little is understood about the behaviour of coelopid larvae. In addition to the potential benefits of enhanced development in the most nutritious macroalgae there may also be substantial gains through reduced competition whilst adopting different development strategies (Leggett et al. 1996). Wrack beds constitute a highly heterogeneous environment in which coelopid larvae are relatively mobile so that spatial separation resulting from female oviposition preferences are expected to be short lived without additional mechanisms of segregation (Phillips et al. 1995a). Larval dietary preferences that mirror maternal preferences may be expected to significantly contribute to developmental success, either through maintaining species segregation or identifying profitable resources. Alternatively, larval preferences that act contrary to maternal preferences may suggest alternative pressures leading to oviposition bias or the inability of larvae to differentiate between resources in the wrack bed environment. Patterns of larval dietary preferences are thus fundamental in our understanding of co-evolutionary processes of both intraspecific behaviours and interspecific interactions. Furthermore, wrack beds constitute an understudied ecosystem despite being recognised as a site of significant secondary productivity (Koop & Field 1980) in which resident macrofauna make significant contributions to decomposition rates (Harrison 1977;

Robertson & Mann 1980; Koop & Griffiths 1982; Cullen et al. 1987). Patterns of larval preferences are therefore also of significance in a wider context.

The nature of coelopid foraging within wrack beds effectively precludes the ability to make accurate direct observation of dietary preferences; thus necessitating indirect assessment. Stable isotope analysis has previously been applied in studies of trophic relationships in the marine environment (Minagawa & Wada, 1984; Riera & Richard, 1996; Riera, 1998; Fredriksen, 2003) including the study of marine oalgae (Bustamante & Branch 1996; Riera & Richard 1996; Adin & Riera 2003). This technique has also been successfully applied in the identification of alternative dietary patterns in a number of invertebrate species (Markow et al. 2000; Adin & Riera 2003; Koyama et al. 2003). A number of assumptions have been identified that require to be met in order for stable isotope analysis to be applied successfully in animal ecology (Gannes et al. 1997). Most notably there can be significant variation in fractionation processes between trophic levels (DeNiro & Epstein 1978, 1981; Stephenson et al. 1984; Post 2002; Spence & Rosenheim 2005). To account for this variation comparative laboratory experiments are recommended for correct interpretation of results (Gannes et al.1997, Ostrom et al. 1997).

The objectives of this study are two fold. First, it is predicted that coelopid larvae will exhibit algal specific dietary preferences. This hypothesis is addressed by obtaining stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for wild caught coelopids and macroalgae. These values are then compared to the stable isotope ratios of coelopids developing in laboratory cultures. Secondly, from these results we examine the effectiveness of stable isotope analysis to answer questions related to animal food webs, in particular considering the use of laboratory cultures.

9.2 Methods

9.2.1 Collection of algae and coelopids

Initial collections of algae and coelopids were made during June and July 2005 from Roome Bay, Crail, Fife (NO 6192 0782). This site was selected for analysis due to the presence of both *Fucus* and *Laminaria* algae in substantial proportions and both coelopid species in significant numbers. Collection of samples was carried out on two occasions at the start and end of a tidal cycle. On the first visit algae were collected as live specimens directly from the shore at low tide so that there should be no effect of decomposition on stable isotope ratios. Samples of *Fucus* and *Laminaria* algae were taken at random intervals from the entire length of the beach (approximately 200m) and in sufficient quantities for both stable isotope analysis and laboratory cultures. On the second visit samples of adult *C. frigida* and *C. pilipes* were collected. This delay was intended so that wild caught flies will have developed on similar algae to that previously collected, thus minimising any effects of temporal variation in stable isotope ratios. Sufficient quantities of wild coelopids were collected for both stable isotope analysis and initiation of laboratory cultures. In response to stable isotope ratios obtained from initial sampling, a further collection of red algae, *Ceramium virgatum* and *Palmaria palmata*, was conducted in July 2006 from the same study site.

9.2.2 Laboratory cultures

Within 24 hours of collection algae were sorted, epiphytes removed, frozen and coarsely minced to assist decomposition. Four monocultures were generated; *C. frigida* raised on *Fucus*, *C. frigida* raised on *Laminaria*, *C. pilipes* raised on *Fucus* and *C. pilipes* raised on *Laminaria*. In addition, a fifth culture of *C. frigida* was generated using an equal mix (by wet mass) of *Laminaria* and *Fucus* algae. In order to remove any influence of female oviposition preference in this fifth culture the following procedure was adapted from Leggett et al. (1996). 100 male and 100 female *C. frigida* were placed in a 15 litre box lined with strips of *Laminaria* and left for 16 hours at 25°C to allow fertilisation and oviposition to take place. *Laminaria* was chosen as this alga is known to increase oviposition rates (Phillips et al. 1995b; Dunn 2001). Flies were removed from the box and eggs collected by washing the algae in a 3% saline solution. Eggs were separated by shaking and filtered onto strips of cloth which were placed into 15 litre culture boxes containing a mixture of *Laminaria* and *Fucus* algae. This procedure could not be replicated with *C. pilipes* owing to delayed oviposition behaviour inhibiting the collection of large numbers of eggs prior to hatching. All cultures were stored in controlled temperature rooms at 25°C on a 12h:12h light-dark cycle. Laboratory reared adult coelopids were collected from each culture daily and stored at 5°C until no further adults eclosed. A randomly selected sample of flies from each culture was then selected for analysis of stable isotope ratios.

9.2.3 Stable isotope analysis

Wild caught *C. pilipes* and *C. frigida*, laboratory reared flies from each of the five cultures and samples of each alga were analysed to determine stable isotope ratios of carbon and nitrogen. All samples were freeze dried and weighed into tin capsules (5mm by 3.5mm) prior to analysis. Flies were analysed individually, using only the head, wings and legs to achieve a mass of between 0.4 and 0.8 mg. Samples of seaweed were selected at random following mincing and a mass of between 0.8 and 1.1 mg selected after drying for analysis.

Stable isotope ratios were determined using continuous-flow isotope ratio mass spectrometry, with a Costech ECS 4010 (Milan) elemental analyser interfaced with a Thermo Electron (Bremen) Delta XP Plus mass spectrometer. Isotope ratios are reported using the delta notation, where

$$\delta = 1000\ \frac{R_x - R_{std}}{R_{std}}\ ‰$$

where R_x = sample isotope ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$; R_{std} = isotope ratio of international standard AIR (for $\delta^{15}N$) or PDB (for $\delta^{13}C$). Units are expressed as ‰ (permil). Replicate (n=82) $\delta^{15}N$ and $\delta^{13}C$ analyses of an internal gelatine standard analysed over the duration of the sample isotope analyses had SD of 0.24 ‰ and 0.06 ‰ respectively.

9.2.4 Statistical analysis

Stable isotope ratios of ^{15}N and ^{13}C were analysed separately using SPSS v12.0.2 (SPSS Inc. Chicago IL, USA). General Linear Modelling was adopted to account for variation that may have resulted due to different sexes and weights of samples. Separate models were generated for each isotope analysing results for samples of algae, *C. frigida* and *C. pilipes*. Maximal models were chosen based upon the Akaike Information Criterion calculated using R 2.5.1 (R Development Core Team 2007) and pairwise comparisons conducted using Tukey's HSD.

9.3 Results

A total of 40 samples of brown algae, 38 samples of red algae, 118 laboratory reared coelopids and 111 wild caught coelopids were analysed. Algae showed a wide range of $\delta^{13}C$ values but could be differentiated from coelopids through lower $\delta^{15}N$.

Coelopids had broadly similar nitrogen isotopic compositions; however those that were wild caught could be differentiated due to lower $\delta^{13}C$ values. Wild coelopids had $\delta^{13}C$ values lower than those of brown algae, whilst laboratory coelopids had higher values (Fig. 9.1).

Considerable differences were evident between red and brown algae (Table 9.1). *Ceramium* differed from all other algae in both carbon and nitrogen stable isotopes whilst *Palmaria* differed from *Laminaria* and *Fucus* in $\delta^{13}C$. *Laminaria* and *Fucus* did not differ significantly in either $\delta^{13}C$ or $\delta^{15}N$. Samples of *C. frigida* could not be distinguished through $\delta^{15}N$ but showed far greater variation in $\delta^{13}C$. Notably, there was no apparent difference in $\delta^{13}C$ between *C. frigida* reared on either a *Laminaria* or mixed culture. In comparison, there were no similarities evident between *C. pilipes* samples.

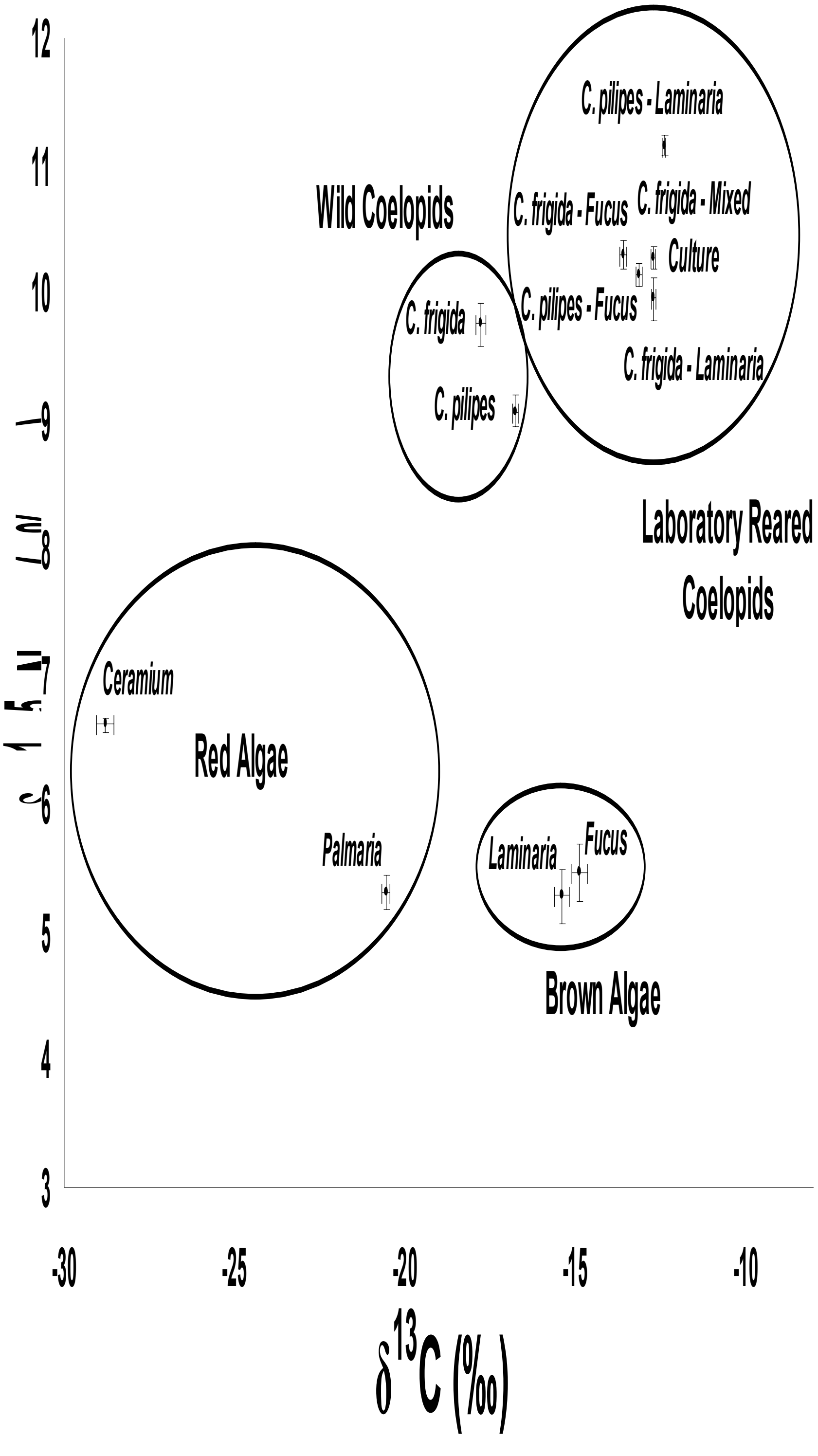

Figure 9.1 $\delta^{13}C$ against $\delta^{15}N$ for wild flies, laboratory reared flies, brown and red algae. (Error bars show standard errors.)

Table 9.1 Results of General Linear Models examining variation in the stable isotope ratios of carbon and nitrogen within algae, *C. frigida* and *C. pilipes*. Below the results for each model are shown the significance values for pairwise comparisons following adjustment using Tukey's HSD.

			$\delta^{13}C$		$\delta^{15}N$	
			Mean Difference	Sig.	Mean Difference	Sig.
Algae			$F_{(3,74)} = 900.33$, $p < 0.001$		$F_{(3,74)} = 14.69$, $p < 0.001$	
	Fucus	*Laminaria*	0.51	**0.350**	0.18	**0.868**
		Ceramium	13.93	<0.001	1.16	<0.001
		Palmaria	5.68	<0.001	0.17	**0.901**
	Laminaria	*Fucus*	0.51	**0.350**	0.18	**0.868**
		Ceramium	13.42	<0.001	1.34	<0.001
		Palmaria	5.17	<0.001	0.01	**>0.999**
	Ceramium	*Fucus*	13.93	<0.001	1.16	<0.001
		Laminaria	13.42	<0.001	1.34	<0.001
		Palmaria	8.25	<0.001	1.33	<0.001
	Palmaria	*Fucus*	5.68	<0.001	0.17	**0.901**
		Laminaria	5.17	<0.001	0.01	**>0.999**
		Ceramium	8.25	<0.001	1.33	<0.001
C. frigida			$F_{(3,119)} = 441.94$, $p < 0.001$		$F_{(3,116)} = 5.11$, $p = 0.002$	
	Wild	*Fucus*	4.13	<0.001	0.42	**0.206**
		Laminaria	4.98	<0.001	0.01	**>0.999**
		Mixed	4.88	<0.001	0.40	**0.105**
	Fucus	Wild	4.13	<0.001	0.42	**0.206**
		Laminaria	0.85	0.001	0.41	**0.404**
		Mixed	0.75	0.001	0.02	**>0.999**
	Laminaria	Wild	4.98	<0.001	0.01	**>0.999**
		Fucus	0.85	0.001	0.41	**0.404**
		Mixed	0.10	**0.956**	0.39	**0.335**
	Mixed	Wild	4.88	<0.001	0.40	**0.105**
		Fucus	0.75	0.001	0.02	**>0.999**
		Laminaria	0.10	**0.956**	0.39	**0.335**
C. pilipes			$F_{(2,94)} = 853.55$, $p < 0.001$		$F_{(2,94)} = 53.65$, $p < 0.001$	
	Wild	*Fucus*	3.63	<0.001	0.94	<0.001
		Laminaria	4.36	<0.001	1.99	<0.001
	Fucus	Wild	3.63	<0.001	0.94	<0.001
		Laminaria	0.73	<0.001	1.06	<0.001
	Laminaria	*Wild*	4.36	<0.001	1.99	<0.001
		Fucus	0.73	<0.001	1.06	<0.001

9.4 Discussion

Rather than resembling the stable isotope ratio of their diet, enrichment of ^{13}C and ^{15}N is commonly observed in consumers. An increase of 0.4‰ (S.D. ±1.3‰) in δ^{13}C and 3.4‰ (S.D. ±1‰) in δ^{15}N has been estimated between trophic levels (DeNiro & Epstein 1978; Rau et al. 1983; Minagawa & Wada 1984; Post 2002). In view of these estimations, the results of this study suggest more than one trophic level between algae and laboratory reared coelopids. This is consistent with previous evidence that coelopid larvae are consuming bacteria rather than the algae direct (Cullen et al. 1987). These results also demonstrate different metabolic processes between coelopids both in the laboratory and in the wild. Despite inhabiting the same environment wild *C. frigida* and *C. pilipes* have significantly different isotopic signatures, indicative of differences in their development. Even under the controlled conditions of laboratory monoculture these two species incorporate algal material into tissues by different processes. In the absence of specific mechanistic knowledge, it is proposed that this is indirect evidence of adaptations to avoid direct competition.

It has been suggested that stable isotope analysis is most suited to the determination of diet in those circumstances where isotope ratios display greatest variation between alternative resources. For example in comparing terrestrial and aquatic diets or consumption of C3 and C4 plants (DeNiro & Epstein 1978, 1981). The ratio of carbon stable isotopes within algal species is known to be highly variable, even within individual plants, preventing discrimination. This has led to suggestions that this isotope is of limited use in the study of marine algal food webs (Stephenson et al. 1984; Boon & Bunn 1994). The lack of a significant difference between brown algae in this and a similar study (Adin & Riera 2003) support, in part at least, this suggestion. Of greater interest though is that the lack of significant variation between brown algae has not restricted variation in $\delta^{13}C$ and $\delta^{15}N$ in laboratory monocultures of both *C. frigida* and *C. pilipes*. It is demonstrated that the lack of difference in potential diets should not be an absolute barrier to the application of this technique.

Comparative laboratory studies have been recommended in the analysis of stable isotopes in animal ecology because of variation in the fractionation of isotopes and efficiency in which animals incorporate components of the diet (Gannes et al. 1997). It is known that there can be significant variation in isotope enrichment between different consumers (Spence & Rosenheim 2005). This has been aptly demonstrated in coelopids as two closely related species with very similar life histories and consuming identical resources can produce distinct isotopic signatures. The use of a laboratory comparison to control for differential fractionation has the potential to utilise this property of a food web to overcome the problems associated with indistinguishable diets. The use of selectively reared controls as benchmarks for the analysis of wild flies also eliminates inaccuracies that may arise through back calculation of expected stable isotope ratios of the consumer from alleged diets (McCutcheon et al. 2003; Focken 2004). Having established the utility of this approach, the confounding issue then becomes the ability to accurately recreate natural conditions.

A main objective of this study was to determine the diet of wild larvae of *C. frigida* and *C. pilipes*. The difference between laboratory reared flies and wild caught flies plainly demonstrates that natural conditions have not been recreated. Whilst the most obvious explanation for this inconsistency would be that wild coelopids have an alternate food source to those predicted, this needs to be considered within the context of what is already known of the life history of the seaweed fly. The dietary requirements of *C. frigida* have been examined in detail (Rowell 1969; Cullen et al. 1987) revealing that bacterial cells in combination with some component of marine algae, possibly a secondary metabolite, are essential to larval development. Coelopids are only found to breed within wrack beds in the wild (Dobson 1974a). Brown algae are the most abundant algae found in wrack beds containing coelopids and coelopids are patently able to develop upon these algae, it therefore seems highly unlikely that brown algae do not constitute a high proportion of the natural diet. Nonetheless, a mixed diet cannot be discounted, hence the additional analysis of red algae, being the next most abundant algae at the study site. Given the very low $\delta^{13}C$ of these algae it is apparent that only a relatively small proportion may be required in the diet of wild flies to explain the differences observed. The contribution of red algae in coelopid development has thus far gone unnoticed. These results suggest that greater consideration be made in the role of this alternative food in future studies.

As coelopid larvae do not feed directly upon the algae it is possible that differences in the intermediary trophic level may also contribute to differences in stable isotope ratios between laboratory and wild populations. Coelopids are able to consume a number of bacterial species, some with greater success than others (Cullen et al. 1987). The marine algal microflora has been comprehensively studied though the majority of this work has sampled living specimens rather than decaying deposits (Chan & McManus 1969; Laycock 1974; Shiba & Taga 1980; Hollohan et al. 1986; Bolinches et al. 1988). Whilst the wrack bed microfauna will initially be derived from the marine community, the change in environment from sea bed to sea shore is likely to provide the circumstances for a change in bacterial community. Other factors that may also have influenced bacterial communities and assimilation of isotopes include precipitation, temperature variation and accumulation of pollutants. If this change is significant then it is increasingly unlikely that laboratory conditions will be able to reflect this accurately. Such natural modification of the environment has similarly been overlooked in the past.

In the absence of accurately recreated laboratory conditions, the most useful insight into the dietary preferences of coelopid larvae instead comes from the laboratory culture of *C. frigida* using a mixture of *Fucus* and *Laminaria* algae. As there is no variation in δ^{15}N between any of the *C. frigida* sources it is not surprising that there is also no variation between *Laminaria* monoculture of *C. frigida* and mixed cultured of *C. frigida*. There is however much greater variation between δ^{13}C for *Laminaria* and *Fucus* cultures, so that the close similarity between δ^{13}C for *Laminaria* monoculture and mixed cultured flies is all the more significant. This similarity is consistent with predictions based upon observations of oviposition preference (Phillips et al. 1995b; Dunn 2001), male mating preference (Chapter 4 & 5) and larval development (Dobson 1974b; Phillips et al. 1995b; Chapter 6). Unfortunately, the lack of a comparison with *C. pilipes* and knowledge that laboratory culture is not representative of the natural situation weakens this finding.

The results of this study demonstrate the potential of stable isotope analysis to determine dietary preferences even in situations where the potential diets may be isotopically similar. Results are not inconsistent with previous observations of algal preferences in coelopids though inability to accurately recreate natural conditions within the laboratory environment has limited conclusions that may be drawn. It is suggested that stable isotope analysis is of greater value than previously recognised in the study of animal food webs.

Chapter 10 - AFLP

10.1 Introduction

The use of molecular techniques in animal ecology is becoming increasingly widespread, accessible and applicable to a wide variety of questions (DeYoung & Honeycutt 2005). In particular, the use of molecular tools to determine parentage has played a central role in revolutionising our understanding of evolutionary and behavioural ecology (Avise 1994; Hughes 1998).

To date, a molecular approach to the study of coelopid ecology has proved sporadic and a limiting factor in the utility of seaweed flies as a model species. Intraspecific variation in mitochondrial cytochrome oxidase II gene has been used to compare variation between British and Swedish populations (MacDonald & Brookfield 2002) and interspecific variation in the sequence of 16S rDNA and EF1-α used to resolve phylogenies (Meier & Wiegmann 2002). The inversion polymorphism on chromosome I has been studied extensively in respect of life history traits in *C. frigida* (Butlin et al. 1982a; Butlin et al. 1984; Butlin & Day 1985; Chapter 1) but has been of limited use in determining parentage. Polymorphism at the Adh locus limits the number of male partners that may be resolved to just five; a figure that is woefully inadequate considering natural levels of promiscuity (Blyth & Gilburn 2005).

The ability to determine parentage in experiments using females mated to a realistic number of males would answer important questions related to female preferences, last male sperm precedence, sperm competition, multiple paternity and cryptic female mate choice. A method of determining parentage is vital to advance research in this species. The requirement is for a cheap, easy to use and reliable method that requires no prior knowledge of the *C. frigida* genome.

Amplified Fragment Length Polymorphism (ALFP) is a multilocus DNA fingerprinting technique based upon the selective amplification of fragments from a total digest of genomic DNA (Vos et al. 1995). The AFLP procedure assesses genome wide variation to produce a unique reproducible profile for each individual. A principle advantage of this technique is that it requires no prior knowledge of the molecular ecology of the organism and can be applied to genomic DNA of any origin or complexity. Furthermore, AFLP is a relatively cheap, easy, fast and reliable method of DNA fingerprinting (Vos et al. 1995; Mueller & Wolfenbarger 1999; Bensch & Akesson 2005).

The process of AFLP comprises five steps – digestion of genomic DNA, ligation of adapters, pre-selective amplification, selective amplification and fragment analysis. Genomic DNA is digested using a combination of a rare cutting restriction endonuclease (usually *Eco*RI) and a frequent cutting restriction endonuclease (usually *Mse*I) that leave overhanging ends. Double stranded adapters corresponding to the overhanging ends are then ligated. Ligation of adapters provides a known sequence to which primers can anneal in subsequent polymerase chain reactions (PCR). During pre-selective amplification primers are chosen that anneal to the adapter sequence but with the addition of a single nucleotide to increase the selectivity of fragments that are amplified. By convention this is the addition of adenine at the *Eco*RI adapter site and cytosine at the *Mse*I adapter site (Vos et al. 1995). During selective amplification this selectivity is further increased by the addition of two more nucleotides. By altering the last two nucleotides added to primers during selective amplification it is possible to create up to 256 primer pair combinations, each yielding different fragment patterns. In the two amplification steps, primers are designed to amplify only from *Eco*RI restriction sites at the 5’ end of a fragment and *Mse*I restriction sites at the 3’ end of a fragment. For this reason only the primers annealing at the *Eco*RI primer sites require labelling. Fragments can then be visualised by agarose gel electrophoresis. This is usually carried out using high throughput capillary electrophoresis to improve the accuracy and speed of obtaining results.

One of the main disadvantages of AFLP markers is that they are dominant, so cannot distinguish between individuals with either one or two copies of the allele (Mueller & Wolfenbarger 1999). In this respect, co-dominant markers such as microsatellites or allozymes provide more detailed information. Whilst AFLP markers are individually less informative, greater statistical power can be derived through the number and range of markers available (Gerber et al. 2000; Bensch & Akesson 2005; Meudt & Clarke 2007). AFLP is useful in this respect as it can produce a higher percentage of polymorphic bands per analysis than related procedures such as Restriction Fragment Length Polymorphism (RFLP) or Random Amplified Polymorphic DNA (RAPD; Lin et al. 1996).

Though the original report of this technique demonstrated its use in a variety of organisms from bacteria to humans (Vos et al. 1995) AFLP has been comparatively underused for animals when compared to plants, fungi and bacteria (Bensch & Akesson 2005). AFLP markers have been used in a wide variety of contexts including parentage analysis, studies of genetic diversity, population structure, hybridisation and phylogenetic studies (Mueller & Wolfenbarger 1999; Bensch & Akesson 2005). Examples of the use of AFLP in insects include distinguishing between sister species of the dung beetle *Onthophagus* spp. (Pizzo et al. 2006), identifying resistance loci in the Colorado potato beetle *Leptinotarsa decemlineata* (Hawthorne 2001), measuring genetic diversity in the mountain pine beetle, *Dendroctonus ponderosae* (Mock et al. 2007) and studying the genetic basis of behaviours (reviewed in Behura 2006). Following comparison with microsatellites it has been determined that AFLP can be successfully used to determine parentage (Gerber et al. 2000). Parentage studies adopting AFLP have included plants (Krauss & Peakall 1998; Krauss 1999; Gerber et al. 2000), vertebrates (Questiau et al. 1999) and insects (García-González et al. 2003, 2005; Simmons et al. 2004). The aim of this study is to investigate the use of AFLP for the study of parentage in *C. frigida*.

10.2 Methods

10.2.1 Preparation

Four male and four female virgin adult *C. frigida* were selected at random from a first generation laboratory culture. Flies were paired at random in small containers (55mm diameter by 75mm) half filled with *Laminaria* algae and containing a sponge pad soaked in 5% sucrose solution. Containers were stored in a controlled temperature room at 25°C on a 12h:12h light-dark cycle. Once larvae were observed in a container parental flies were removed and stored at -20°C. Larvae were allowed to develop, additional algae being added to encourage growth and survival. Eclosing progeny were collected as virgins and stored at -20°C. Progeny were collected until none had eclosed for at least 48 hours. A third party selected eight progeny at random that included at least one individual from each of the four parental groups. AFLP analysis was conducted on the eight parents and eight selected progeny to determine parentage. The identity of samples referred to are detailed in Table 10.1.

Table 10.1 The sex and identity of the four sets of parents and eight progeny alongside sample number.

Sample Number	Identity	Sex
1	Parents B	Male
2		Female
3	Parents C	Male
4		Female
5	Parents F	Male
6		Female
7	Parents G	Male
8		Female
9	Progeny F	Female
10	Progeny F	Male
11	Progeny B	Female
12	Progeny G	Female
13	Progeny C	Male
14	Progeny G	Male
15	Progeny B	Female
16	Progeny C	Female

10.2.2 DNA extraction

DNA was extracted from whole flies using the Qiagen DNeasy® Blood & Tissue Kit (Qiagen Inc, Valencia CA, USA). The manufacturer's instructions were followed with the following modifications. Samples were homogenised using the cut end of a pipette tip in a 1.5ml microcentrifuge tube and incubated at 56°C overnight following addition of proteinase K. 4µl RNaseA (100mg/ml) was added following proteinase digestion. Samples were eluted in 100µl to increase the final concentration of DNA. The concentration of DNA obtained was estimated at 10-15ng per µl using a Nanodrop Spectrophotometer ND-1000 (NanoDrop Technologies Inc, Wilmington, DE, USA).

10.2.3 Restriction & ligation of adapters

Restriction and ligation of samples was conducted using the Invitrogen AFLP® Core Reagent Kit (Invitrogen Life Technologies, Paisley, UK) that contained the restriction enzymes EcoRI and MseI. The manufacturers instructions were followed with the exception that the final ligation mixture was not diluted prior to pre-selective amplification.

10.2.4 Amplification of fragments

Ligation mixtures were amplified in a DNA Engine Tetrad 2 Peltier Thermal Cycler (MJ Research, Waltham MA, USA) using pre-selective and selective primers as detailed in Table 10.2.

Table 10.2 Primer sequences used in the pre-selective and selective amplification of fragments.

	***Eco*R1**	***Mse*I**
Universal Primer (P)	5’ - GACTGCGTACCAATTC	5’ - GATGAGTCCTGAGTAA
Pre-Selective Primer	5’ - P - A	5’ - P - C
Selective Primers	5’ - P - AAG 5’ - P - AAC	5’ - P - CAT 5’ - P - CAG

Primers were chosen following successful use for paternity analysis in the golden egg bug, *Phyllomorpha laciniata* (Garcia-Gonzalez et al. 2005). Conditions for the polymerase chain reaction (PCR) were optimised following trials of alternative primer concentrations, template concentrations, polymerase concentrations, annealing temperatures and length of the denaturation step (Table 10.3a-b). PCR reagents were taken from the Taq PCR Core Kit (Qiagen Inc, Valencia CA, USA) and all primers obtained from Sigma-Proligo (The Woodlands TX, USA). Selective amplification was carried out in four separate reactions for each primer pair combination. A 1:10 dilution in TE buffer was carried out for the PCR product obtained following pre-amplification prior to use in selective amplification. DNA extractions and PCR products were visualised in 1% agarose gels stained with ethidium bromide. 3µl of product was electrophoresed for 45 minutes at 100V.

Table 10.3 Composition and conditions for PCR reactions during (a) pre-selective amplification and (b) selective amplification of fragments.

Table 10.3a

Constituent	**Volume**	
10X Reaction Buffer	1 µl	
Q-Solution	2 µl	
dNTP Mix	0.2 µl	Each dNTP being at 10mM to give a final concentration of 0.2mM each
Taq DNA Polymerase	0.1 µl	At 5 units/µl to give a final concentration of 0.05 units/µl
Pre-Selective Primers	1 µl	At 5µM each to give a final concentration of 0.5µM each
Template DNA	1 µl	
Distilled Water	4.7 µl	

Total Reaction Volume 10 µl

Denaturation	94°C	30 seconds	
Annealing	48°C	1 minute	25 cycles
Extension	72°C	2 minutes	

Followed by 72°C for 2 minutes then 60°C for 30 minutes

Table 10.3b

Constituent	**Volume**	
10X Reaction Buffer	1 µl	
Q-Solution	2 µl	
dNTP Mix	0.2 µl	Each dNTP at 10mM to give a final concentration of 0.2mM each
Taq DNA Polymerase	0.1 µl	At 5 units/µl to give a final concentration of 0.05 units/µl
Selective Primers	1 µl	At 2.5µM each to give a final concentration of 0.25µM each
Template DNA	1 µl	
Distilled Water	4.7 µl	

Total Reaction Volume 10 µl

Denaturation	94°C	30 seconds	
Annealing	65°C	30 seconds	Annealing temperature reduced by 0.7°C each cycle for 12 cycles
Extension	72°C	2 minutes	
Denaturation	94°C	30 seconds	
Annealing	56°C	30 seconds	25 cycles
Extension	72°C	2 minutes	

Then 60°C for 30 minutes

10.2.5 Fragment analysis

The selective primers annealing to adapters at the *Eco*R1 restriction site were labelled with WellRED fluorescent dyes D3 and D4 (Beckman Coulter Inc, Fullerton, CA, USA). Though four primer pair combinations were used in selective PCR, primer pairs using different *Eco*R1 primers could be distinguished during fragment analysis. These primer pairs were combined prior to electrophoresis.

Fragments were analysed using a multi-channel capillary electrophoresis system, Beckman CEQ 8800 (Beckman Coulter Inc, Fullerton, CA, USA). 0.5 µl of sample was loaded with 30 µl of sample loading solution and 0.5µl of DNA Size Standard (600). These conditions were adopted following the trial of different volumes of size standard (0.25µl, 0.5µl, 0.625µl, 0.75µl & 0.875µl) and sample (0.5µl, 0.75µl & 1µl). AFLP profiles were scored for the presence/absence of fragments using Beckman CEQ 8800 Genetic Analysis System Software v9.0.25 (Beckman Coulter Inc, Fullerton, CA, USA). Two peaks were considered the same size if they differed by less than 0.5 base pairs.

To determine repeatability of this technique all steps post DNA extraction were conducted twice for each sample. Repeated analyses of re-extracted DNA from the same individual are recommended as a control (Bensch & Akesson 2005), however multiple DNA extractions was not possible with the kit adopted and size of samples.

10.3 Results

Agarose gel electropherograms of extracted DNA are shown in Figure 10.1. Three samples from the second cycle of AFLP were not successfully analysed during electrophoresis so are excluded from further analysis. (Sample 3 primer pairs *Mse*I – ACAT/*Eco*RI – CAAG & *Mse*I – ACAT/*Eco*RI – CAAC, sample 4 primer pairs *Mse*I – ACAG/*Eco*RI – CAAG & *Mse*I – ACAG/*Eco*RI – CAAC and sample 7 primer pairs *Mse*I – ACAT/*Eco*RI – CAAG & *Mse*I – ACAT/*Eco*RI – CAAC).

Only fragments between 60 and 460bp were included in analysis as this range of fragments was found to be most accurately determined. Within this range all fragments of the size standard had been identified in all remaining samples. The total number of fragments found across all 16 samples for each primer pair combination is given in Table 10.4. All of these fragments were polymorphic.

Table 10.4 The number of fragments identified for each primer pair.

*Mse*I – ACAG *Eco*RI – CAAG	*Mse*I – ACAG *Eco*RI – CAAC	*Mse*I – ACAT *Eco*RI – CAAG	*Mse*I – ACAT *Eco*RI – CAAC	All Primer Pairs
100	142	79	239	560

The number of markers found for each individual was highly variable both between individuals and for the same individual on different runs (Fig 10.2). In all but four of the individuals analysed, less fragments were identified on the second run.

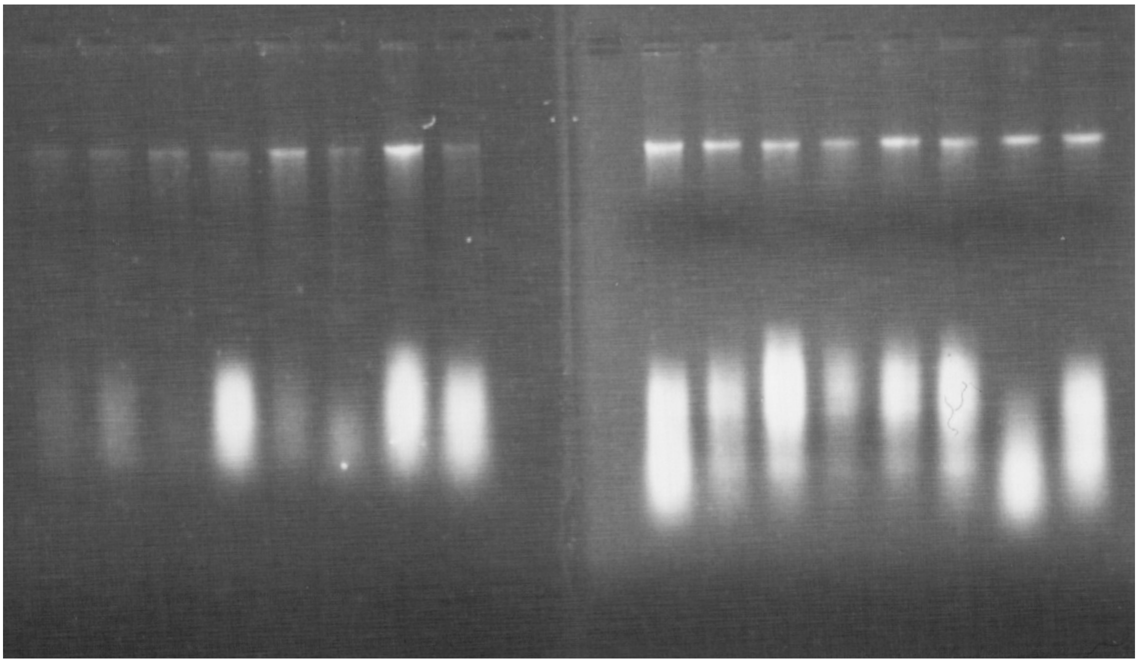

Figure 10.1 Agarose gel electropherogram of extracted DNA. Samples are in order from left to right.

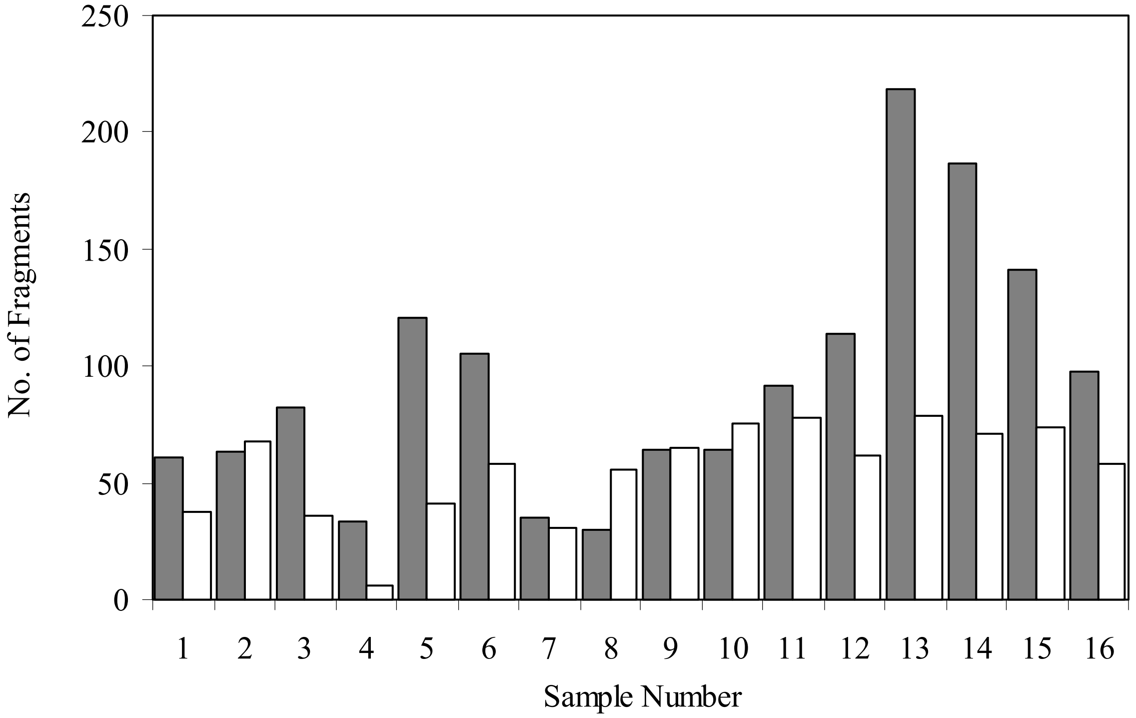

Figure 10.2 The number of fragments for each sample identified on the first cycle (filled bars) and second cycle (empty bars) of AFLP.

The repeatability of AFLP fingerprints was determined as the proportion of fragments found in both cycles of the procedure for the same sample divided by the total number of fragments found in the least productive cycle. Repeatability was highly variable, but overall was greatest for the *Mse*I – ACAT / EcoRI – CAAG primer pair (Fig. 10.3). To improve estimates of the extent of polymorphism and the number of markers identified a subset of the data was considered that included only reactions giving repeatabilities greater than 80%. Of these reactions only fragments found in both cycles of AFLP were considered accurate. The number of samples meeting these criteria for each primer pair are detailed in Table 10.5.

The lack of repeatability, particularly for parental samples, precluded accurate assessment of parentage so no further analysis was conducted.

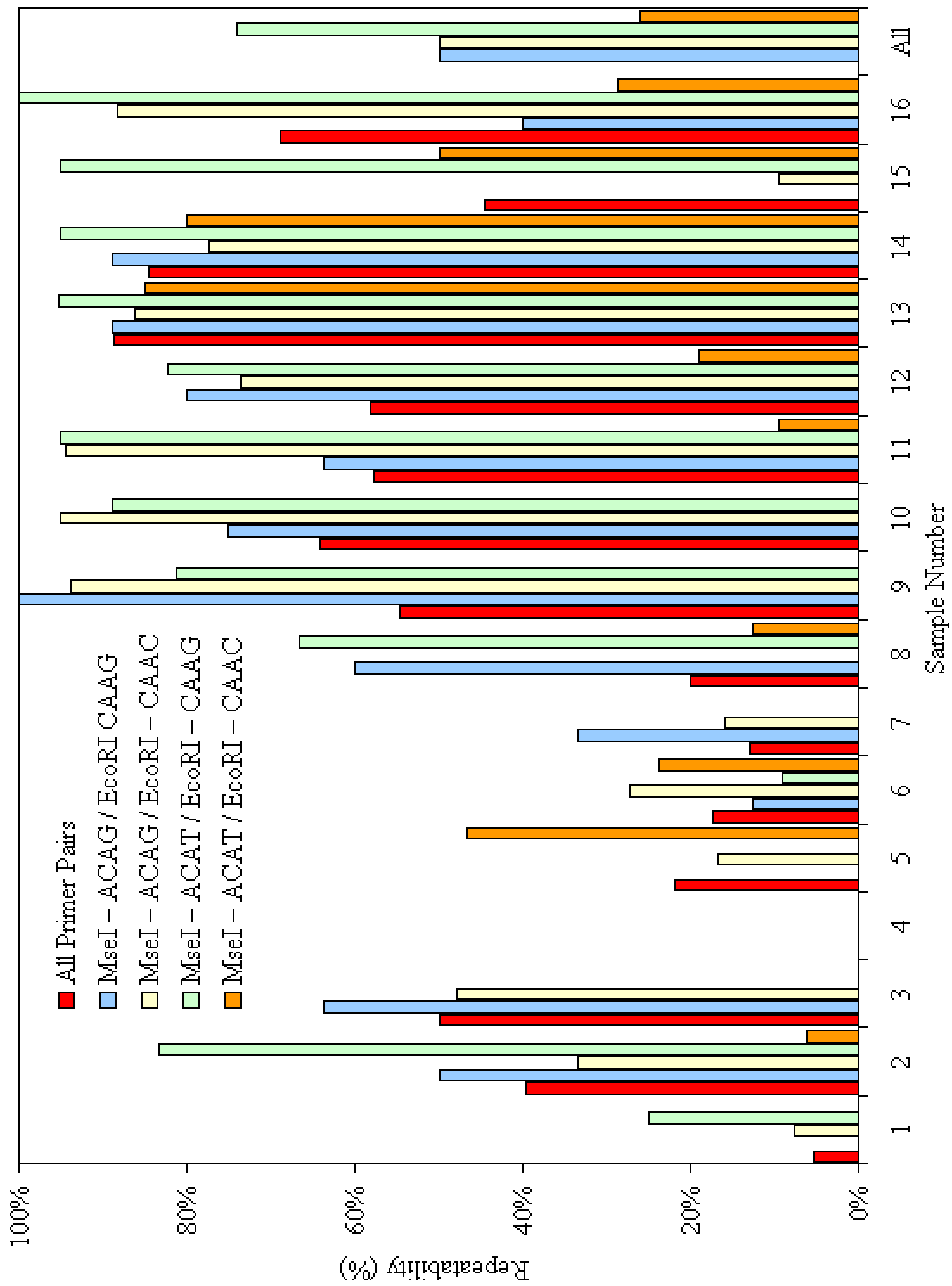

Figure 10.3 Repeatability of fragments found following AFLP for different primer pairs, for each sample and for fragments from all samples.

Table 10.5 The number of samples for each primer pair that gave repeatabilities in excess of 80%. The number of different fragments found in these reactions and polymorphism of these fragments is also detailed.

Primer Pair	No. of samples over 80% repeatability	No. of fragments found in both cycles	% polymorphism of these fragments
*Mse*I – ACAG *Eco*RI – CAAG	4	13	76.9%
*Mse*I – ACAG *Eco*RI – CAAC	5	37	81.1%
*Mse*I – ACAT *Eco*RI – CAAG	9	27	70.4%
*Mse*I – ACAT *Eco*RI – CAAC	2	21	42.9%
All Primer Pairs		98	69.4%

10.4 Discussion

Results of this study clearly demonstrate that a substantial amount of work is still required for AFLP to prove an effective technique for the study of parentage in *C. frigida*. The high degree of unrepeatable results and lack of non-polymorphic fragments is evidence of a failure in the procedure. The proportion of polymorphic fragments found in this study is most likely due to the lack of identification of fragments rather than true absence of a fragment. Though 100% repeatability is not expected, over 95% repeatability has been achieved in a previous study using AFLP in a parentage study of insects (Garcia-Gonzalez et al. 2005). At this stage, the inability to reliably identify fragments precludes the use of this technique in parentage studies.

Nevertheless, a number of positive findings may be drawn from these results. The proportion of polymorphic fragments is expected to vary both between species and with different primer pair combinations. As a guide, previous studies using AFLP in dung beetles, *Onthophagus* spp., and the golden egg bug, *Phyllomorpha laciniata*, report proportions of polymorphic fragments between 60 and 95% (Simmons et al. 2004; Garcia-Gonzalez et al. 2005; Pizzo et al. 2006). In considering those samples whose fingerprints were determined most accurately (Table 10.5) the proportion of polymorphic fragments is within realistic expectations and the identification of non-polymorphic fragments suggest data are not entirely random. Given the proportion of polymorphic fragments found in this subset of results there is potential for these primer pairs to be suitable for parentage analyses if the repeatability of results can be improved.

Given the relationship between the quality of DNA extracts and repeatability of results it is evident that this part of the protocol is undermining the procedure. AFLP requires high molecular weight and high quality DNA. Use of small quantities or degraded DNA can lead to poor quality profiles with low reproducibility (Bensch & Akesson 2005). AFLP is more sensitive to DNA quality and presence of inhibitors in the extract than other standard PCR-based applications (Bensch & Akesson 2005). For this reason commercially available kits, such as Qiagen DNeasy used in this study, are recommended to give the better quality DNA needed for this kind of work (Benjak et al. 2006). The first logical step for further improvement of this procedure would therefore be to trial other kits of this type. It should be noted however that following different extraction methods AFLP profiles can differ (Benjak et al. 2006). The repeatability of results within different extraction protocols rather than between protocols should be examined.

Other possible modifications that may be made to the procedure include the use of different restriction enzymes and different primer sets (Bensch & Akesson 2005). Using alternative restriction enzymes, such as *Taq*I or *Pst*I in place of *Eco*RI, may increase or decrease the number of fragments available for selective amplification. In addition, four primer pairs have been trialled so far out of the 256 commercially available combinations. The possible number of primer pairs available is even greater considering non-commercial sequences and also the possible addition of a fourth selective base (Bensch & Akesson 2005). The quality of profiles is known to vary with different primer combinations because of the base compositions (Bensch & Akesson 2005). Though there is great scope in using different restriction enzymes and/or primers this may prove a false economy before DNA extraction has been optimised. There is no way to predict which primer pair combinations may work best and the effort required to optimise PCR and screen even a small proportion would be worthless given the potential of the primer pairs used so far. Alternatively it may be more profitable to focus on those primer pairs used in this study that show the greatest potential (for example *Mse*I – ACAT / EcoRI – CAAG).

Other improvements worthy of further examination include optimising PCR for specific primer pairs, optimising PCR according to the results of fragment analysis and optimising fragment analysis through changes in the amount of PCR product and size standard used. Following improvement of DNA extractions these steps are expected to improve results without the need for fundamental changes to the protocol.

Despite evident shortcomings at this stage of development, AFLP has the potential to provide valuable information about the coelopid mating system. Given the expense required to develop alternative molecular methods, such as microsatellite markers, the relatively simple steps suggested to develop the use of this technique are recommended to be most cost-effective based upon the results generated so far. Given the valuable information that may be achieved through molecular analyses of the *C. frigida* mating system this endeavour is certainly worthwhile.

Chapter 11 - General discussion

This thesis represents the most comprehensive study of ecological variation affecting *C. frigida*. The results of this work have significantly contributed to our understanding of both the life history and mating system of this species. Results show that males increase mating activity when they are either exposed to or are cultured on *Laminaria*. These behavioural changes coincide with increased oviposition of females when exposed to (Phillips et al. 1995b; Dunn 2001; though not found in this study) and when cultured on the same algae. *Laminaria* clearly promotes reproduction in this species.

The proximal mechanisms driving this adaptation may be explained by enhanced development on *Laminaria*, which confers increased size and greater egg to adult viability. Patterns of development on different algae are diverse, so that different karyotypes are favoured in different environments. A further advantage of development in *Laminaria* is that algal specificities may reduce interspecific competition with other coelopids.

The culture of *C. frigida* on two different genera of algae results in flies of differing condition. This property has been used to demonstrate associations between adult condition and changes in the mating system. Females cultured using *Laminaria* show an apparent reduction in sensitivity to male persistence traits. Female insensitivity is predicted to limit a co-evolutionary arms race between the sexes, alleviating the possible extent of sexual conflict. There is also strong evidence to suggest stabilising selection for male traits, particularly male size. Strong positive selection for male size reported in numerous previous studies is found only when males are cultured on *Fucus*. Results suggest that large males cultured on *Laminaria* are effectively “overweight” and have a reduced ability to achieve copulations. This will further contribute to a limit for sexually antagonistic coevolution. The mechanisms maintaining assortative mating may similarly have been misunderstood in past studies that only adopted *Fucus* as the culture algae. Homokaryotype advantage following culture on *Laminaria* increases the potential for good genes sexual selection to favour assortative mating.

These key findings demonstrate alternative outcomes for sexual conflict dependent upon the environment adopted for replication in the laboratory and highlight the importance of conducting experiments under varied conditions. Finally, the costs of sexual conflict for *C. frigida* may have been underestimated in the past. It is convincingly demonstrated that multiple mating contributes to a significant reduction in adult lifespan. The high costs of sexual conflict imply strong selective pressures driving the evolution of the female rejection response.

Whilst it can be said with certainty that patterns of mating and development on the two algae are very different; it is more difficult to state definitively whether one alga constitutes the “best” environment. This question is chiefly hypothetical as naturally occurring monocultures are expected to be in the minority. Indeed, the apparent unhealthy size of large males when cultured on *Laminaria* in the laboratory may be the result of a lack of experience of this environment in the wild. *Laminaria* can certainly provide greater resources to increase the condition of adults yet this is at a cost of development time which may be expensive if wrack beds are unpredictable. Rather than selection to breed exclusively within *Laminaria* it is anticipated that behavioural responses have evolved merely to take advantage of this alga when it is available. Stochastic patterns of individual success are expected to maintain adoption of a varied diet.

The discoveries of this report reveal exciting opportunities for further research. Perhaps the most interesting possibility is to observe evolution in real time. With a short life cycle and ability to rear large numbers of flies cheaply and easily it would be relatively straightforward to send populations down separate evolutionary trajectories on the basis of culture algae. Population crosses could then demonstrate how mating systems adapt to different environments. It would also be of great interest to discover the outcome for different forms of the chromosomal inversion. This kind of work need not only focus on laboratory populations. The distribution of algae is highly variable, even over short distances dependent upon beach topography. Thus it should be possible to carry out crosses using natural populations.

It is imperative that all future work on *C. frigida* is conducted in consideration of the potential variation that may be caused through using different species of algae. In some respects it may be considered that this thesis has focussed on two rarely encountered extremes of a variable. A principle suggestion for future work would therefore be to conduct experiments using a mixed algal environment so that results may be placed within a workable context for application to the natural situation.

There is, and rightly so, a strong scientific ethic for conducting research that is rigorously controlled and repeatable. A negative consequence of this approach has been highlighted in this thesis as crucial variables can easily be omitted to simplify the model if we are ignorant of their effects. Even if it is theoretically possible to conduct studies with a multitude of variables this is often practically limited by time and financial constraints as much as the ability to analyse results in a meaningful way. The real challenge is not to incorporate all aspects of variation but to be aware of what variables there are, which are most pertinent and acknowledge the potential influences.

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Appendix 1 - Chapter 3

Columns are (left to right) geographical area (1 = north east coast of England, 2 = Norway, 3 = Orkney Isles, 4 = Scottish Mainland, 5 = Outer Hebrides, 6 = Shetland Isles, 7 = south coast of England, 8 = Sweden), site name, latitude, longitude, date sampled, sample size and proportion of sample that was *C. pilipes*.

1	Beadnell	55.55364	-1.62728	Feb-1967	28	0.89
1	Craster	55.47280	-1.59337	Feb-1967	146	0.11
1	Beadnell	55.55364	-1.62728	Feb-1967	214	0.12
1	Boulmer	55.41919	-1.58171	Feb-1967	306	0.81
1	Craster	55.47280	-1.59337	Feb-1967	261	0.06
1	Beadnell	55.55364	-1.62728	Aug-1967	149	0.01
1	Craster	55.47280	-1.59337	Aug-1967	277	0.01
1	Dunstanburg	55.49432	-1.60712	Aug-1967	149	0.01
1	Beadnell	55.55364	-1.62728	Sep-1967	185	1.00
1	Boulmer	55.41919	-1.58171	Sep-1967	113	0.94
1	Craster	55.47280	-1.59337	Sep-1967	29	0.07
1	Beadnell	55.55364	-1.62728	Oct-1967	80	0.66
1	Boulmer	55.41919	-1.58171	Oct-1967	37	0.54
1	Dunstanburg	55.49432	-1.60712	Oct-1967	9	0.00
1	Beadnell	55.55364	-1.62728	Oct-1967	256	0.92
1	Boulmer	55.41919	-1.58171	Oct-1967	23	1.00
1	Boulmer	55.41919	-1.58171	Nov-1967	141	0.99
1	Dunstanburg	55.49432	-1.60712	Nov-1967	160	0.11
1	Beadnell	55.55364	-1.62728	Jan-1968	321	0.20
1	Boulmer	55.41919	-1.58171	Jan-1968	34	0.97
1	Craster	55.47280	-1.59337	Jan-1968	255	0.08
1	Dunstanburg	55.49432	-1.60712	Jan-1968	33	0.06
1	SMI	55.07009	-1.45424	Mar-1981	288	0.00
1	Whitburn	54.94678	-1.36055	Mar-1981	287	0.67
1	SMI	55.07009	-1.45424	Apr-1981	285	0.01
1	SMI	55.07009	-1.45424	May-1981	273	0.00
1	SMI	55.07009	-1.45424	May-1981	280	0.00
1	Whitburn	54.94678	-1.36055	May-1981	41	0.95
1	SMI	55.07009	-1.45424	Jun-1981	217	0.15
1	Whitburn	54.94678	-1.36055	Jun-1981	144	0.19
1	Robin Hoods Bay	54.43012	-0.53014	Jul-1981	126	0.00
1	SMI	55.07009	-1.45424	Jul-1981	277	0.02
1	SMI	55.07009	-1.45424	Jul-1981	319	0.06
1	Whitburn	54.94678	-1.36055	Jul-1981	144	0.16
1	Whitburn	54.94678	-1.36055	Jul-1981	144	0.01
1	SMI	55.07009	-1.45424	Aug-1981	283	0.57
1	Whitburn	54.94678	-1.36055	Aug-1981	143	0.16
1	Whitburn	54.94678	-1.36055	Oct-1981	189	0.89
1	SMI	55.07009	-1.45424	Nov-1981	288	0.02
1	Whitburn	54.94678	-1.36055	Nov-1981	135	0.00
1	Boulmer	55.41919	-1.58171	Mar-2005	15	1.00
1	Beadnell	55.55364	-1.62728	Jul-2005	10	0.50

1	Boulmer	55.41919	-1.58171	Jul-2005	18	0.72
1	Craster	55.47280	-1.59337	Jul-2005	161	0.09
1	SMI	55.07009	-1.45424	Jul-2005	77	0.65
1	Whitburn	54.94678	-1.36055	Jul-2005	41	0.76
1	Beadnell	55.55364	-1.62728	Aug-2005	169	0.24
1	Boulmer	55.41919	-1.58171	Aug-2005	458	0.68
1	Craster	55.47280	-1.59337	Aug-2005	321	0.38
1	Dunstanburg	55.49432	-1.60712	Aug-2005	1524	0.10
1	SMI	55.07009	-1.45424	Aug-2005	2230	0.95
2	Kvassheimhamna	58.54608	5.68133	Dec-2005	436	0.00
2	Stavstø	58.12700	6.59131	Dec-2005	83	0.00
2	Osthasselstrand	58.07158	6.64742	Dec-2005	367	0.00
2	Verdalen	58.77082	5.51553	Jul-2006	573	0.00
2	Osthasselstrand	58.07158	6.64742	Jul-2006	155	0.00
2	Stavern	58.98141	10.02581	Jul-2006	302	0.00
3	Ayre of Westermill	58.84338	-2.90254	Jun-2005	59	0.10
3	Backaskaill Bay	59.23811	-2.62606	Jun-2005	88	0.52
3	Bay of Lopness	59.28293	-2.44860	Jun-2005	101	0.00
3	Bay of Pierowall	59.32548	-2.98382	Jun-2005	100	0.68
3	Bay of Skaill	59.34766	-2.95422	Jun-2005	100	0.24
3	Grobust	59.32679	-3.00804	Jun-2005	219	0.00
3	Mill Sand	58.95344	-2.84685	Jun-2005	2	0.00
3	Pool Bay	59.22348	-2.67378	Jun-2005	100	0.01
3	Sand of Wright	58.82303	-2.99727	Jun-2005	53	0.02
3	Sands Of Evie	59.12010	-3.09969	Jun-2005	126	1.00
3	Sandwick	58.78689	-2.97970	Jun-2005	68	0.00
3	Sty Wick	59.23353	-2.56931	Jun-2005	229	0.00
3	The Ouse	59.24112	-2.58175	Jun-2005	100	0.08
3	Whitemill Bay	59.30486	-2.55176	Jun-2005	153	1.00
4	Whitesands	55.98905	-2.46546	Aug-2004	243	0.97
4	Barn's Ness	55.98831	-2.44637	Oct-2004	314	1.00
4	Whitesands	55.98905	-2.46546	Oct-2004	184	0.93
4	Cellardyke	56.22505	-2.68338	Oct-2004	1458	0.65
4	Crail	56.26206	-2.61520	Oct-2004	1736	0.72
4	Elie	56.18640	-2.81174	Oct-2004	180	0.99
4	St.Monans	56.20166	-2.77400	Feb-2005	188	1.00
4	Cellardyke	56.22505	-2.68338	Mar-2005	238	0.27
4	Crail	56.26206	-2.61520	Mar-2005	262	0.64
4	St.Monans	56.20166	-2.77400	Mar-2005	2332	1.00
5	Caolas	56.94516	-7.55053	Aug-2004	100	0.30
5	Cliaid	57.01410	-7.48734	Aug-2004	35	0.00
5	Bayh a Tuath	56.99606	-7.39858	Aug-2004	695	0.03
5	Cuinabunay, Benbecula			Aug-2004	37	1.00
5	Trolaisgeir	57.21942	-7.43623	Aug-2004	105	0.11
5	Eilean na Rubja, North Uist			Aug-2004	65	0.58
5	Siorrabharg			Aug-2004	173	1.00
6	Brae	60.39435	-1.35588	Aug-2005		0.00
6	Dales Voe	60.19140	-1.18675	Aug-2005		0.00
6	East Voe	60.27200	-1.15221	Aug-2005		0.00
6	Lower Voe	60.34813	-1.27163	Aug-2005		0.00
6	Lunna Voe	60.50560	-1.33729	Aug-2005		0.00
6	Nibon	60.44415	-1.44939	Aug-2005		0.00
6	Ollaberry Bay	60.50560	-1.33729	Aug-2005		0.00
6	Scalloway	60.13269	-1.28180	Aug-2005		0.00

6	South Nesting Bay	60.29164	-1.13542	Aug-2005		0.00
7	Rustington	50.80253	-0.52303	Apr-1981	287	0.00
7	Rustington	50.80253	-0.52303	May-1981	25	0.00
7	Rustington	50.80253	-0.52303	Jul-1981	141	0.00
7	Portland	50.57717	-2.46967	Aug-1981	141	0.01
7	Beer	50.69814	-3.08625	Sep-1981	141	0.10
7	Beer	50.69814	-3.08625	Aug-2005	57	0.00
7	Osmington	50.63428	-2.37617	Aug-2005	1263	1.00
7	Rustington	50.80253	-0.52303	Aug-2005	593	1.00
8	Lerkil	57.45600	11.91600	Oct-1990	364	0.00
8	Bua	57.23700	12.12200	Oct-1990	52	0.13
8	Glommen	56.93100	12.36300	Oct-1990	66	0.00
8	Molle	56.28300	12.49900	Oct-1990	303	0.56
8	Steninge	56.77200	12.62500	Oct-1990	111	0.00
8	Torekov	56.42500	12.62900	Oct-1990	265	0.28
8	Traslovslage	57.05400	12.27800	Oct-1990	167	0.00
8	Vejbystrand	56.31500	12.76900	Oct-1990	72	0.00
8	Viken	56.15300	12.56900	Oct-1990	247	0.00
8	Gislovslage	55.35600	13.23800	Oct-1990	87	0.00
8	Horte	55.38330	13.55000	Oct-1990	241	0.00
8	Kampinge	55.40500	12.98300	Oct-1990	255	0.00
8	Kaseberga	55.38600	14.06500	Oct-1990	60	0.00
8	Smygehuk	55.34300	13.37300	Oct-1990	78	0.00
8	Ystad	55.43100	13.82800	Oct-1990	195	0.00
8	Glommen	56.93152	12.35202	Sep-2005	223	1.00
8	Molle	56.28398	12.49468	Sep-2005	11	1.00
8	Steninge	56.76596	12.63060	Sep-2005	638	0.14
8	Traslovslage	57.05600	12.27168	Sep-2005	564	1.00
8	Vejbystrand	56.31737	12.76198	Sep-2005	439	0.00
8	Viken	56.14268	12.57456	Sep-2005	202	0.99
8	Gislovslage	55.35653	13.22761	Sep-2005	139	0.00
8	Kaseberga	55.38372	14.06365	Sep-2005	974	0.00

Appendix 2 – Chapter 4

Columns are (left to right) species of coelopid (*C. **frigida*** / *C. **pilipes***), treatment (A = exposure to *Fucus serratus*, B = exposure to *Fucus vesiculosus*, C = exposure to *Laminaria digitata*, D = exposure to sucrose only control), length of exposure to treatment (days), male harassment (0 = no mount observed, 1 = mount observed), result of mount (0 = no copulation, 1 = copulation), male wing length (mm) and female wing length (mm).

F	A	1	1	0	5.7	4.5
F	A	1	0		4.2	5.1
F	A	1	0		5.1	4.9
F	A	1	0		5.9	5.4
F	A	1	0		5.7	4.7
F	A	1	1	0	5.8	5.2
F	A	1	1	1	5.9	4.6
F	A	1	1	1	6.1	4.6
F	A	1	0		4.0	6.0
F	A	1	1	0	6.3	4.6
F	A	1	1	1	6.2	5.4
F	A	1	1	1	6.4	5.3
F	A	1	0		6.7	4.7
F	A	1	0		4.3	5.0
F	A	1	1	0	6.2	4.8
F	A	1	1	1	6.0	4.8
F	A	1	1	1	4.8	5.0
F	A	1	1	1	6.1	4.9
F	A	1	1	0	5.6	5.0
F	A	1	1	1	5.4	4.6
F	A	1	1	1	4.9	5.0
F	A	1	0		5.4	4.9
F	A	1	0		6.3	5.0
F	A	2	1	1	4.5	4.2
F	A	2	1	1	5.3	4.8
F	A	2	0		5.1	5.3
F	A	2	0		4.4	4.7
F	A	2	0		5.9	4.8
F	A	2	1	0	5.0	5.3
F	A	2	1	0	6.1	4.0
F	A	2	1	1	4.1	5.1
F	A	2	1	1	5.9	5.4
F	A	2	0		4.0	4.9
F	A	2	1	0	4.1	4.8
F	A	2	1	1	6.8	5.5
F	A	2	1	1	5.9	5.3
F	A	2	0		6.6	5.0
F	A	2	0		4.2	5.2
P	A	1	0		5.3	4.8
P	A	1	0		3.8	4.7
P	A	1	0		5.2	5.2
P	A	1	0		4.8	4.7
P	A	1	0		4.7	4.6
P	A	1	0		5.1	5.2
P	A	1	0		4.7	4.7
P	A	1	0		5.4	5.1
P	A	1	0		4.9	4.7
P	A	1	0		4.6	5.1
P	A	1	0		4.4	4.5
P	A	1	0		4.6	5.0
P	A	1	0		5.1	5.1
P	A	1	0		4.7	5.5
P	A	1	0		5.2	4.9
P	A	1	0		5.5	5.0
P	A	1	0		5.5	4.8
P	A	1	0		4.7	5.3
P	A	1	0		5.1	5.0
P	A	1	0		5.2	5.1
P	A	2	1	0	4.8	4.8
P	A	2	1	0	4.6	4.6
P	A	2	1	1	3.9	4.5
P	A	2	1	1	4.6	4.5
P	A	2	0		4.4	4.7
P	A	2	0		4.0	4.4
P	A	2	0		4.7	4.6
P	A	2	0		4.6	4.6
P	A	2	1	0	5.6	5.5
P	A	2	1	1	5.6	5.2
P	A	2	1	1	5.7	5.7
P	A	2	1	1	5.8	5.8
P	A	2	0		5.6	5.3
P	A	2	0		5.9	5.5
P	A	2	0		4.6	5.1
P	A	2	0		6.0	5.4
P	A	2	1	0	5.6	5.1
P	A	2	1	0	5.1	5.3

F	A	2	0		6.1	4.9	P	A	2	0		4.9	5.3
F	A	2	1	0	4.8	4.6	P	A	2	0		5.3	5.4
F	A	2	1	1	6.6	4.9	P	A	2	0		5.5	4.8
F	A	2	1	1	6.1	5.2	P	A	2	0		5.7	4.8
F	A	2	0		6.1	5.2	P	A	2	0		5.7	5.3
F	A	2	0		6.0	5.0	P	A	2	0		5.7	5.6
F	A	2	1	1	6.3	5.3	P	A	2	1	1	4.9	4.9
F	A	2	1	1	6.1	5.0	P	A	2	0		5.5	5.0
F	A	2	0		4.5	4.9	P	A	2	0		5.3	4.9
F	A	3	1	0	4.2	5.0	P	A	3	1	0	4.5	4.4
F	A	3	1	1	4.2	4.4	P	A	3	1	0	4.8	4.5
F	A	3	0		5.0	4.9	P	A	3	0		4.7	4.7
F	A	3	1	0	3.9	4.8	P	A	3	0		4.8	5.1
F	A	3	1	1	5.3	5.2	P	A	3	0		5.0	4.5
F	A	3	1	1	5.4	4.1	P	A	3	0		4.4	4.6
F	A	3	0		5.1	4.6	P	A	3	0		5.0	4.8
F	A	3	0		6.2	4.5	P	A	3	0		4.8	4.6
F	A	3	1	1	4.8	5.2	P	A	3	1	0	5.5	5.7
F	A	3	1	1	6.1	4.8	P	A	3	1	1	5.4	5.4
F	A	3	0		4.3	4.8	P	A	3	0		5.4	5.3
F	A	3	0		6.5	5.1	P	A	3	0		5.7	5.6
F	A	3	0		6.3	4.9	P	A	3	0		5.8	5.2
F	A	3	1	0	4.5	5.3	P	A	3	0		6.0	5.2
F	A	3	1	1	6.4	5.5	P	A	3	1	0	6.0	5.3
F	A	3	1	1	6.3	5.1	P	A	3	1	0	5.7	5.1
F	A	3	0		6.3	5.0	P	A	3	1	1	5.7	5.1
F	A	3	1	0	5.5	5.4	P	A	3	1	1	5.5	5.3
F	A	3	1	1	6.1	5.4	P	A	3	1	1	5.0	5.4
F	A	3	1	1	4.5	4.8	P	A	3	0		5.7	5.0
F	A	3	1	1	4.4	5.0	P	A	3	1	0	4.0	4.9
F	A	3	0		6.1	4.5	P	A	3	1	0	5.0	5.1
F	B	1	1	1	4.4	5.3	P	A	3	1	1	5.5	5.0
F	B	1	0		4.1	4.8	P	A	3	0		5.9	4.9
F	B	1	0		4.5	4.4	P	A	3	0		4.8	5.0
F	B	1	0		5.8	4.9	P	B	1	1	0	4.7	5.1
F	B	1	1	0	5.6	4.8	P	B	1	0		4.8	4.7
F	B	1	1	0	4.9	4.4	P	B	1	0		5.2	4.7
F	B	1	1	0	4.9	5.3	P	B	1	0		4.7	4.8
F	B	1	1	0	5.0	4.6	P	B	1	0		4.5	5.5
F	B	1	1	1	6.1	5.4	P	B	1	0		4.5	4.8
F	B	1	0		5.9	5.0	P	B	1	0		4.6	4.7
F	B	1	0		5.9	4.9	P	B	1	0		4.7	4.5
F	B	1	0		6.5	5.0	P	B	1	0		5.4	4.8
F	B	1	0		6.6	4.8	P	B	1	0		4.3	4.6
F	B	1	1	0	6.3	5.3	P	B	1	0		4.6	4.6
F	B	1	1	0	4.7	5.2	P	B	1	0		4.8	5.1
F	B	1	1	1	6.8	4.8	P	B	1	0		5.1	4.5
F	B	1	0		6.4	4.8	P	B	1	1	0	5.0	4.9
F	B	1	1	0	5.9	4.9	P	B	1	1	0	5.6	4.6
F	B	1	1	1	5.8	5.0	P	B	1	1	1	5.3	4.8
F	B	1	1	1	4.7	5.1	P	B	1	0		5.8	5.1
F	B	1	0		5.4	4.9	P	B	1	0		4.3	5.1
F	B	1	0		4.2	4.6	P	B	1	0		5.2	5.2
F	B	1	0		5.4	4.8	P	B	1	0		5.3	5.3

F	B	2	1	0	4.3	4.6
F	B	2	1	0	4.8	5.3
F	B	2	1	0	4.7	4.6
F	B	2	0		5.4	4.9
F	B	2	0		4.3	4.5
F	B	2	1	0	5.6	4.4
F	B	2	1	1	5.4	5.0
F	B	2	1	1	5.5	5.1
F	B	2	0		4.0	5.1
F	B	2	1	0	4.3	5.3
F	B	2	1	0	6.2	4.9
F	B	2	1	1	6.7	5.4
F	B	2	0		6.4	4.8
F	B	2	0		6.8	4.7
F	B	2	0		6.5	5.1
F	B	2	1	0	6.3	5.3
F	B	2	1	0	4.6	5.3
F	B	2	1	1	5.9	5.2
F	B	2	0		4.6	4.4
F	B	2	0		5.1	5.2
F	B	2	1	0	5.1	5.4
F	B	2	1	1	5.2	5.1
F	B	2	1	1	5.9	4.8
F	B	3	1	0	4.3	4.6
F	B	3	1	1	5.1	4.6
F	B	3	1	1	5.6	5.1
F	B	3	0		5.6	5.4
F	B	3	0		4.2	5.0
F	B	3	1	0	6.1	4.2
F	B	3	1	1	5.6	4.4
F	B	3	1	1	5.1	4.5
F	B	3	0		4.4	4.6
F	B	3	0		5.4	5.0
F	B	3	0		5.8	5.1
F	B	3	1	1	6.6	5.5
F	B	3	1	1	4.4	4.7
F	B	3	0		6.2	4.9
F	B	3	0		6.4	4.8
F	B	3	0		6.3	5.2
F	B	3	1	0	5.6	4.8
F	B	3	1	1	4.6	4.8
F	B	3	1	1	6.4	4.9
F	B	3	1	1	4.6	4.9
F	B	3	1	0	5.5	5.1
F	B	3	1	1	4.7	5.0
F	B	3	0		5.9	5.0
F	C	1	1	1	4.5	4.1
F	C	1	1	1	4.7	4.9
F	C	1	1	1	4.3	4.9
F	C	1	1	1	5.7	5.1
F	C	1	0		4.5	5.0
F	C	1	1	0	3.9	4.1
F	C	1	1	1	5.7	4.8
F	C	1	1	1	5.9	5.2
P	B	2	1	0	5.0	4.8
P	B	2	1	0	4.3	4.6
P	B	2	0		4.1	4.8
P	B	2	0		4.7	4.6
P	B	2	0		4.8	4.7
P	B	2	0		4.5	4.6
P	B	2	0		4.7	4.8
P	B	2	0		4.5	4.5
P	B	2	1	0	6.3	5.4
P	B	2	1	0	5.5	5.1
P	B	2	1	0	6.0	5.1
P	B	2	0		5.9	5.6
P	B	2	0		6.1	5.9
P	B	2	0		5.6	5.6
P	B	2	0		5.7	5.1
P	B	2	1	0	5.6	5.4
P	B	2	1	1	5.5	5.0
P	B	2	0		5.5	4.9
P	B	2	0		4.6	5.0
P	B	2	0		5.2	4.9
P	B	2	0		5.4	5.3
P	B	2	0		5.3	5.3
P	B	2	0		5.1	5.1
P	B	2	1	1	5.0	5.1
P	B	2	1	1	5.4	5.0
P	B	2	0		5.8	4.8
P	B	3	1	1	4.5	4.6
P	B	3	0		4.6	4.6
P	B	3	0		4.6	4.5
P	B	3	0		4.2	4.9
P	B	3	0		4.6	4.8
P	B	3	1	0	6.1	5.5
P	B	3	1	0	5.4	5.3
P	B	3	0		5.6	4.9
P	B	3	0		5.7	5.4
P	B	3	0		5.7	5.2
P	B	3	0		5.0	5.4
P	B	3	1	1	5.1	4.7
P	B	3	1	1	5.3	5.1
P	B	3	1	1	5.5	5.4
P	B	3	1	1	5.7	5.1
P	B	3	1	1	5.6	5.1
P	B	3	0		4.8	5.0
P	B	3	0		5.3	5.1
P	B	3	0		5.6	5.2
P	B	3	1	0	4.9	5.0
P	B	3	1	0	5.3	4.8
P	B	3	1	1	5.2	5.0
P	B	3	0		5.3	4.8
P	C	1	1	0	5.4	4.6
P	C	1	0		4.4	4.6
P	C	1	0		4.5	4.8
P	C	1	0		5.2	4.6
P	C	1	0		5.0	4.8

F	C	1	0		5.6	4.4
F	C	1	1	1	6.3	4.9
F	C	1	1	1	6.6	5.1
F	C	1	0		5.2	4.9
F	C	1	0		5.8	5.1
F	C	1	0		6.2	4.9
F	C	1	1	0	4.7	5.0
F	C	1	1	1	6.6	5.1
F	C	1	1	1	4.5	4.8
F	C	1	1	0	5.0	5.2
F	C	1	1	0	6.0	4.8
F	C	1	1	1	5.1	4.5
F	C	1	1	1	4.9	4.8
F	C	1	1	1	5.1	4.9
F	C	1	1	1	4.1	4.7
F	C	1	1	1	5.1	4.9
F	C	2	1	1	5.1	5.1
F	C	2	1	1	5.1	5.2
F	C	2	1	1	4.5	4.8
F	C	2	1	1	5.2	4.9
F	C	2	0		5.2	5.1
F	C	2	1	0	5.7	5.1
F	C	2	1	0	4.4	4.5
F	C	2	1	1	5.1	4.4
F	C	2	1	1	6.1	5.1
F	C	2	1	1	5.7	5.0
F	C	2	1	0	4.4	4.9
F	C	2	1	0	6.7	4.6
F	C	2	1	1	6.1	5.0
F	C	2	0		6.4	5.0
F	C	2	0		6.6	5.1
F	C	2	0		4.5	5.2
F	C	2	1	0	6.4	4.6
F	C	2	1	0	4.6	4.8
F	C	2	1	1	5.4	5.0
F	C	2	1	1	6.2	5.1
F	C	2	1	1	5.9	4.5
F	C	2	1	0	5.9	5.1
F	C	2	1	1	6.2	5.1
F	C	2	1	1	5.6	4.9
F	C	2	1	1	4.6	5.1
F	C	3	1	0	6.0	4.7
F	C	3	1	1	5.1	4.9
F	C	3	1	1	4.8	5.0
F	C	3	0		4.1	5.0
F	C	3	0		5.9	5.1
F	C	3	1	0	4.6	4.4
F	C	3	1	0	6.1	4.6
F	C	3	1	1	5.7	4.6
F	C	3	1	1	5.4	5.4
F	C	3	1	1	4.5	5.0
F	C	3	1	0	4.6	4.8
F	C	3	1	1	6.2	4.9
F	C	3	0		4.6	5.2
P	C	1	0		5.1	4.8
P	C	1	0		4.4	5.0
P	C	1	0		5.6	4.6
P	C	1	0		4.8	4.6
P	C	1	0		4.7	4.6
P	C	1	0		4.8	4.6
P	C	1	0		4.7	4.9
P	C	1	0		5.3	4.8
P	C	1	0		5.6	5.0
P	C	1	0		4.7	4.8
P	C	1	1	1	5.2	5.3
P	C	1	0		5.6	5.0
P	C	1	0		4.4	5.1
P	C	1	0		5.9	5.0
P	C	1	0		5.6	4.8
P	C	1	0		4.9	4.9
P	C	1	0		5.0	4.9
P	C	1	0		5.6	5.4
P	C	2	1	0	4.7	4.5
P	C	2	0		4.7	4.4
P	C	2	0		4.1	4.6
P	C	2	0		5.0	4.9
P	C	2	0		4.1	5.0
P	C	2	0		5.0	4.8
P	C	2	1	0	5.5	5.4
P	C	2	1	0	5.5	5.4
P	C	2	1	1	5.8	5.3
P	C	2	1	1	4.6	5.3
P	C	2	0		5.9	5.4
P	C	2	0		5.7	5.5
P	C	2	0		5.5	5.6
P	C	2	0		5.4	5.4
P	C	2	1	0	5.2	5.1
P	C	2	1	0	5.2	5.3
P	C	2	1	0	5.5	5.2
P	C	2	1	0	5.0	5.0
P	C	2	0		5.6	5.2
P	C	2	0		5.3	4.8
P	C	2	0		5.6	4.8
P	C	2	0		5.4	5.0
P	C	2	0		4.7	5.0
P	C	3	1	1	4.4	4.7
P	C	3	1	1	4.7	4.8
P	C	3	1	1	4.7	4.8
P	C	3	1	1	4.5	5.0
P	C	3	0		4.4	4.8
P	C	3	0		4.0	4.9
P	C	3	0		4.9	4.5
P	C	3	0		4.5	4.6
P	C	3	1	0	5.6	5.3
P	C	3	1	0	5.5	5.0
P	C	3	1	0	6.2	5.2
P	C	3	0		5.8	5.8
P	C	3	0		6.1	5.6

F	C	3	0		6.4	5.1
F	C	3	0		6.2	5.3
F	C	3	1	1	6.4	4.8
F	C	3	1	1	6.2	5.3
F	C	3	0		4.5	5.2
F	C	3	0		6.2	5.0
F	C	3	1	0	4.9	4.6
F	C	3	1	1	6.2	4.8
F	C	3	0		4.8	5.2
F	D	1	1	0	5.3	5.1
F	D	1	1	0	4.9	4.9
F	D	1	0		4.3	4.6
F	D	1	0		5.4	5.3
F	D	1	1	0	4.5	3.9
F	D	1	1	1	5.6	4.7
F	D	1	0		3.8	4.7
F	D	1	0		6.5	4.9
F	D	1	0		3.7	4.3
F	D	1	1	0	6.1	5.0
F	D	1	1	0	6.7	5.3
F	D	1	0		6.2	4.8
F	D	1	0		6.5	4.7
F	D	1	0		6.1	4.8
F	D	1	1	0	4.7	5.0
F	D	1	0		4.9	5.2
F	D	1	0		6.3	4.4
F	D	1	0		6.4	5.2
F	D	1	1	0	4.9	5.2
F	D	1	1	1	4.7	4.8
F	D	1	0		4.2	4.6
F	D	1	0		5.2	5.1
F	D	1	0		6.0	4.8
F	D	1	0		5.5	5.0
F	D	2	1	0	4.5	4.6
F	D	2	1	0	4.0	5.4
F	D	2	1	0	5.0	5.4
F	D	2	0		4.5	5.5
F	D	2	0		5.5	4.6
F	D	2	1	0	6.2	4.7
F	D	2	1	1	6.4	5.3
F	D	2	1	1	6.1	4.9
F	D	2	0		5.0	4.9
F	D	2	0		5.4	5.2
F	D	2	1	1	6.4	5.3
F	D	2	1	1	6.2	5.0
F	D	2	0		4.6	5.0
F	D	2	0		6.5	4.8
F	D	2	0		6.3	4.8
F	D	2	0		6.0	4.7
F	D	2	1	0	4.5	5.0
F	D	2	1	0	4.4	4.9
F	D	2	1	1	6.3	5.0
F	D	2	0		6.1	4.6
F	D	2	0		4.5	5.0
P	C	3	0		5.4	5.4
P	C	3	1	0	5.4	5.1
P	C	3	1	0	5.7	5.1
P	C	3	1	1	5.2	5.1
P	C	3	1	1	5.5	5.1
P	C	3	1	1	5.8	5.2
P	C	3	0		5.3	5.0
P	C	3	0		5.7	5.3
P	C	3	1	0	5.1	4.9
P	C	3	1	1	5.1	5.1
P	C	3	1	1	5.1	5.3
P	C	3	1	1	5.6	5.3
P	C	3	0		5.6	5.0
P	D	1	0		5.2	5.2
P	D	1	0		4.9	4.7
P	D	1	0		4.7	4.6
P	D	1	0		4.7	4.8
P	D	1	0		4.9	4.8
P	D	1	0		5.1	4.7
P	D	1	0		4.9	4.8
P	D	1	0		4.4	4.6
P	D	1	0		4.8	4.6
P	D	1	0		4.9	4.6
P	D	1	0		4.8	4.6
P	D	1	0		4.6	5.2
P	D	1	0		4.6	5.1
P	D	1	0		5.0	4.7
P	D	1	1	0	5.3	5.3
P	D	1	0		5.7	5.6
P	D	1	0		5.2	5.0
P	D	1	0		5.6	4.9
P	D	1	0		4.9	5.3
P	D	1	0		5.2	5.1
P	D	1	0		4.8	4.9
P	D	2	0		4.3	4.6
P	D	2	0		4.8	4.7
P	D	2	0		4.8	4.8
P	D	2	0		5.0	4.9
P	D	2	0		4.8	4.9
P	D	2	0		4.7	4.7
P	D	2	0		4.7	4.8
P	D	2	0		4.5	4.8
P	D	2	1	0	5.3	5.0
P	D	2	1	1	5.6	5.6
P	D	2	1	1	5.9	5.4
P	D	2	0		6.0	5.3
P	D	2	0		5.9	5.4
P	D	2	0		5.7	5.3
P	D	2	0		5.6	5.5
P	D	2	1	0	5.4	5.0
P	D	2	1	0	6.0	5.2
P	D	2	1	1	5.4	4.9
P	D	2	0		5.6	5.2
P	D	2	0		5.5	5.0

F	D	2	1	1	6.0	5.0
F	D	2	0		4.7	4.7
F	D	2	0		5.4	4.7
F	D	3	1	0	6.2	4.7
F	D	3	1	0	4.4	5.4
F	D	3	1	1	5.6	5.3
F	D	3	0		4.1	4.1
F	D	3	0		4.2	5.0
F	D	3	1	0	4.3	4.6
F	D	3	1	1	6.0	5.0
F	D	3	0		3.9	5.3
F	D	3	0		4.0	5.0
F	D	3	0		4.0	4.8
F	D	3	1	0	4.9	4.9
F	D	3	1	1	4.8	5.1
F	D	3	0		4.2	4.7
F	D	3	0		4.4	5.0
F	D	3	1	0	4.5	5.2
F	D	3	1	0	4.4	5.2
F	D	3	1	0	4.6	4.9
F	D	3	1	1	6.3	5.0
F	D	3	0		4.8	5.3
F	D	3	1	0	5.6	4.8
F	D	3	1	0	5.4	5.1
F	D	3	1	1	5.1	5.1
F	D	3	1	1	5.6	5.1
F	D	3	1	1	4.8	5.0
P	A	1	1	0	5.0	4.4
P	A	1	0		5.8	4.8
P	A	1	0		4.9	4.9
P	D	2	0		5.7	5.2
P	D	2	0		5.9	5.1
P	D	2	0		5.4	4.9
P	D	2	0		5.0	4.9
P	D	2	0		5.5	5.5
P	D	3	1	1	4.8	4.9
P	D	3	1	1	4.9	4.8
P	D	3	1	1	4.9	4.6
P	D	3	0		4.1	4.2
P	D	3	0		4.9	4.9
P	D	3	0		4.4	4.3
P	D	3	0		4.6	5.0
P	D	3	1	0	5.6	5.3
P	D	3	1	0	5.6	5.2
P	D	3	1	0	5.7	5.5
P	D	3	1	1	5.7	5.5
P	D	3	0		6.0	5.3
P	D	3	0		5.8	5.1
P	D	3	1	0	4.5	5.2
P	D	3	0		5.5	5.4
P	D	3	0		5.4	5.1
P	D	3	0		5.3	5.2
P	D	3	0		6.1	5.0
P	D	3	0		5.3	5.5
P	D	3	0		4.3	4.8
P	D	3	0		5.1	5.1
P	D	3	0		5.2	5.0
P	D	3	0		5.6	5.2
P	D	3	0		5.7	5.3

Appendix 3 – Chapter 5

Columns are (left to right) days since preparation, treatment (L = exposure to *Laminaria*, F = exposure to *Fucus*, S = exposure to sucrose only control), male harassment (0 = no mount observed, 1 = mount observed), struggle duration (seconds), result of mount (0 = no copulation, 1 = copulation), copulation duration (seconds), male wing length (mm) and female wing length (mm).

2	L	0				3.9	4.9
2	L	0				4.1	5.4
2	L	1	10	1	290	5.0	5.5
2	L	1	1	1	239	6.2	4.7
2	L	1	13	1	27	4.8	5.1
2	L	1	23	1	67	5.1	4.8
2	L	1	59	1	146	4.2	5.5
2	L	1	1	1	59	5.1	5.6
2	L	1	420	1	120	6.5	5.5
2	L	1	4	0		5.1	5.4
2	L	1	174	1	136	5.6	5.2
2	L	1	15	1	45	4.9	4.6
2	L	1	7	0		4.9	4.9
2	L	1	540	1	240	6.4	5.1
2	L	1	8	1	127	6.1	5.4
2	L	1	60	1	155	6.6	4.0
2	L	1	6	1	375	6.4	5.1
2	L	1	20	0		5.1	5.3
2	L	1	292	1	315	6.5	5.5
2	L	1	40	0		5.0	5.0
2	L	1	2	1	198	6.6	4.8
2	L	1	176	1	110	4.8	5.5
2	L	1	2	1	168	5.2	5.1
2	L	1	6	1	189	4.9	5.2
2	L	1	13	1	240	7.0	5.7
2	L	1	14	1	55	4.7	5.4
2	L	1	5	1	109	6.8	5.2
2	L	1	109	1	158	4.9	4.9
2	L	1	13	1	38	6.5	5.5
2	L	1	466	1	154	5.4	5.5
2	L	1	67	1	340	4.8	5.2
2	L	1	4	1	94	5.2	5.2
2	L	1	501	1	289	5.1	5.4
2	L	1	76	1	255	6.4	5.9
2	L	1	60	1	324	5.1	5.7
2	L	1	26	1	347	4.6	5.3
2	L	1	532	1	315	5.1	5.3
2	L	1	22	1	165	5.2	5.9
2	S	0				7.0	5.2
2	S	0				6.0	5.4
2	S	0				6.0	5.2
2	S	0				5.2	4.9
2	S	0				5.3	5.5
2	S	1	25	1	198	6.2	4.9
2	S	1	4	1	63	6.1	5.3
2	S	1	59	1	83	6.7	5.5
2	S	1	130	0		5.2	5.1
2	S	1	6	1	80	6.6	5.9
2	S	1	51	1	180	5.0	5.0
3	S	1	2	0		4.8	5.1
3	S	1	90	1	50	6.8	5.0
3	S	1	7	1	80	7.1	5.0
3	S	0				5.0	5.2
3	S	0				4.7	5.6
3	S	0				5.0	5.3
3	S	0				5.1	5.5
3	S	0				6.9	5.5
3	S	0				4.9	5.0
3	S	0				6.0	4.9
3	S	0				4.6	4.7
3	S	0				6.0	4.9
3	S	0				6.0	5.3
3	S	0				6.3	5.5
3	S	1	7	1	100	5.2	5.1
3	S	1	8	1	59	6.2	5.1
3	S	1	13	1	79	6.2	5.0
3	S	1	8	1	17	5.5	5.5
3	S	1	4	1	86	5.9	5.0
3	S	1	15	1	115	5.5	5.6
3	S	1	5	0		5.1	5.2
3	S	1	15	1	125	4.9	5.2
3	S	0				4.6	5.1
3	S	0				5.4	5.8
3	S	0				6.5	5.5
3	S	0				5.9	5.7
3	S	0				4.9	5.3

2	L	1	377	1	415	5.8	5.3
2	L	1	32	0		5.2	5.1
3	L	1	220	1	200	4.0	5.3
3	L	0				5.3	5.4
3	L	0				6.0	5.3
3	L	1	120	1	125	5.5	5.0
3	L	1	416	1	186	4.7	5.2
3	L	1	977	1	223	4.2	5.3
3	L	1	3	0		6.1	5.8
3	L	1	795	1	120	6.9	5.2
3	L	1	3	0		5.0	5.0
3	L	1	733	1	110	7.1	5.4
3	L	1	28	1	92	6.6	5.1
3	L	1	251	1	96	7.0	5.0
3	L	1	121	1	112	6.1	4.9
3	L	1	802	1	125	6.1	5.1
3	L	1	180	1	160	4.9	5.3
3	L	1	28	0		4.7	5.2
3	L	1	5	1	120	6.3	5.0
3	L	1	133	1	50	5.0	5.5
3	L	1	441	1	246	5.0	5.3
3	L	0				5.1	5.3
3	L	1	106	1	245	5.0	5.6
3	L	1	5	1	209	5.3	5.0
3	L	1	105	1	299	6.0	5.2
3	L	1	136	1	180	6.5	5.5
3	L	1	31	0		5.5	4.9
3	L	1	528	1	265	5.3	5.0
3	L	1	139	1	169	4.9	4.9
3	L	1	509	1	245	5.2	5.3
3	L	1	217	1	113	6.7	5.5
3	L	1	20	1	241	4.9	5.1
3	L	1	55	1	93	5.7	5.2
3	L	1	55	1	246	6.3	5.5
3	L	1	184	1	233	4.9	5.1
3	L	1	10	0		6.7	5.6
3	L	1	15	0		6.1	5.6
3	L	1	56	1	145	5.3	4.9
3	L	1	21	0		4.5	4.9
3	L	1	500	1	255	6.0	6.0
2	L	1	39	1	89	5.0	4.8
2	L	1	180	1	190	5.5	5.5
2	L	1	446	1	227	7.1	5.7
2	L	1	133	1	139	5.5	6.0
2	L	1	347	1	260	6.1	5.0
2	L	1	22	1	98	7.0	5.4
2	L	0				5.0	6.2
2	L	0				6.4	5.1
2	L	0				6.5	5.5
2	L	0				5.9	4.9
2	L	0				7.1	5.1
3	S	0				5.5	6.0
3	S	0				5.2	5.4
3	S	0				6.1	4.9
3	S	0				6.1	5.3
3	S	1	11	1	199	5.6	5.6
3	S	1	91	1	129	6.7	5.0
3	S	1	24	1	60	5.0	5.8
3	S	1	3	0		5.5	5.6
3	S	1	3	0		6.3	5.3
3	S	1	3	0		5.2	5.5
3	S	1	8	1	57	5.1	6.0
3	S	1	8	1	59	6.1	5.2
3	S	0				4.7	5.0
3	S	0				6.9	5.3
3	S	0				4.1	5.1
3	S	0				6.4	4.9
3	S	0				7.0	5.3
3	S	0				6.6	5.1
3	S	0				6.3	4.9
3	S	1	45	1	98	5.9	5.2
3	S	1	8	1	122	5.8	5.3
3	S	1	54	1	79	6.3	5.0
3	S	1	6	1	30	6.1	5.4
3	S	1	34	1	60	6.1	5.0
3	S	1	34	0		5.1	5.1
3	S	1	45	0		4.8	5.1
3	S	0				5.1	5.1
3	S	0				6.4	5.4
3	S	0				5.5	6.1
3	S	0				5.5	5.0
3	S	0				7.1	5.1
3	S	0				5.5	5.4
3	S	0				7.0	5.3
3	S	1	100	1	50	7.0	4.6
3	S	1	26	1	77	5.9	5.2
3	S	1	27	1	39	6.1	5.0
3	S	1	22	0		6.5	5.5
3	S	1	11	1	259	6.4	5.3
3	S	1	4	0		4.5	5.4
3	S	1	4	0		7.0	5.9
3	S	1	148	1	57	5.9	5.5
3	S	1	5	1	100	5.4	5.5
3	S	1	18	1	17	7.1	5.6
3	S	1	5	1	132	6.7	5.8
4	S	1	1	0		5.0	5.1
4	S	1	1	0		6.3	5.2
4	S	1	2	1	55	5.9	5.1
4	S	0				5.0	4.2
4	S	0				4.2	5.1
4	S	1	17	1	183	6.2	5.0
4	S	0				6.4	5.2

2	L	1	29	0		6.0	5.6
2	L	1	3	0		6.0	5.5
2	L	1	305	1	140	6.0	6.1
2	L	1	191	1	140	7.0	5.4
2	L	1	6	0		6.7	5.3
2	L	1	8	0		6.5	5.0
2	L	1	418	1	192	4.5	4.6
2	L	1	149	1	174	5.9	5.5
2	L	1	650	1	244	6.1	5.3
2	L	1	7	1	87	4.9	4.7
2	L	1	5	1	19	6.0	4.6
2	L	1	68	1	104	4.9	5.3
2	L	1	4	1	170	4.6	4.8
2	L	1	36	1	201	4.2	4.8
2	L	1	2	1	83	5.4	4.7
2	L	1	256	1	194	5.9	5.4
2	L	0				4.9	4.7
2	L	0				5.5	4.7
2	L	1	256	1	201	5.3	4.9
2	L	1	421	1	190	6.0	5.1
2	L	1	11	1	64	4.6	5.1
2	L	1	4	0		5.3	5.5
2	L	1	7	0		4.9	5.0
2	L	1	6	0		5.7	5.1
2	L	1	316	1	204	5.1	5.6
2	L	1	926	1	179	4.6	5.6
2	L	1	50	1	93	6.5	5.7
2	L	1	460	1	154	6.0	5.7
2	L	1	2	0		5.5	5.7
2	L	1	8	1	127	6.8	5.8
2	L	1	4	0		6.2	5.3
2	L	1	399	1	148	5.9	6.1
2	L	1	191	1	140	6.7	5.3
2	L	0				6.1	4.7
2	L	0				6.3	5.3
2	L	0				6.5	5.3
2	L	0				7.0	5.3
2	L	1	72	1	153	6.6	5.6
2	L	1	446	1	111	5.5	5.4
2	L	1	4	1	66	6.1	5.3
2	L	1	293	1	187	5.3	5.5
2	L	1	5	0		6.6	5.6
2	L	1	6	0		6.6	5.6
2	L	1	541	1	120	5.0	5.4
2	L	1	86	1	98	4.8	5.0
2	L	1	2	1	60	6.6	5.3
2	L	1	117	1	134	4.5	4.5
2	L	1	115	1	115	5.0	5.1
2	L	1	323	1	139	5.6	5.2
2	L	1	401	1	151	5.8	5.4
2	L	1	471	1	214	5.3	5.4

4	S	0				6.3	5.4
4	S	0				7.0	5.3
4	S	1	9	1	50	5.0	5.3
4	S	1	4	1	211	6.0	5.0
4	S	1	4	0		5.4	5.6
4	S	1	154	1	67	6.0	5.2
4	S	1	5	0		4.7	5.4
4	S	1	7	0		4.5	5.2
4	S	1	70	1	30	4.8	5.3
4	S	1	30	1	40	4.9	5.1
4	S	1	14	1	79	5.0	5.7
4	S	1	11	1	57	4.6	6.3
4	S	1	5	1	39	6.4	4.9
4	S	1	19	1	94	6.3	5.0
4	S	1	18	1	49	6.0	5.4
4	S	0				6.6	5.8
4	S	0				6.7	5.2
4	S	0				4.9	5.1
4	S	0				4.6	5.3
4	S	0				7.2	5.9
4	S	0				7.1	5.1
4	S	0				7.2	5.4
4	S	0				5.3	5.1
4	S	0				6.2	5.5
4	S	0				7.1	5.2
4	S	0				5.6	5.0
4	S	1	4	0		6.7	5.1
4	S	1	8	0		5.2	5.6
4	S	1	11	0		5.2	5.2
2	S	1	32	1	66	6.0	5.1
2	S	1	8	1	162	7.2	6.1
2	S	1	55	1	185	6.6	4.9
2	S	1	4	1	63	6.5	5.5
2	S	0				6.0	5.3
2	S	0				5.9	5.5
2	S	0				7.0	5.3
2	S	0				6.0	5.1
2	S	0				6.1	5.4
2	S	0				6.2	5.0
2	S	0				6.3	5.3
2	S	0				6.6	5.0
2	S	0				5.0	5.0
2	S	0				5.5	4.9
2	S	1	20	1	117	5.6	5.0
2	S	1	8	0		6.3	5.3
2	S	1	27	0		6.6	5.1
2	S	1	1	0		6.4	5.0
2	S	1	1	0		5.9	5.2
2	S	1	2	0		6.2	5.0
2	S	1	8	1	59	6.1	5.2
2	S	0				6.0	5.4

2	L	1	5	1	114	6.6	5.0	2	S	0				5.5	5.1
2	L	1	172	1	195	5.5	5.0	2	S	0				5.0	4.7
2	L	1	249	1	264	6.8	4.9	2	S	0				6.0	4.7
2	L	1	632	1	215	5.2	4.8	2	S	0				5.9	5.2
2	L	1	7	0		4.9	5.2	2	S	0				5.2	4.6
2	L	1	3	0		6.3	4.3	2	S	0				6.0	5.5
2	L	0				6.8	4.5	2	S	0				4.9	5.4
2	L	0				5.9	5.2	2	S	1	4	1	50	6.0	5.1
2	L	0				6.8	5.2	2	S	1	10	1	65	6.1	5.3
2	L	0				7.0	5.3	2	S	1	29	1	38	4.2	4.7
2	L	1	416	1	114	4.5	5.0	2	S	1	20	1	200	5.9	5.0
2	L	1	8	0		6.0	5.8	2	S	1	5	0		5.2	5.2
4	L	1	26	1	122	5.0	4.8	2	S	1	17	0		6.0	5.4
4	L	1	9	0		6.9	4.7	2	S	1	13	1	61	4.7	5.5
4	L	1	294	1	262	5.5	5.4	2	S	1	100	1	50	7.0	4.6
4	L	1	188	1	112	5.4	5.5	2	S	1	179	1	74	6.7	5.7
4	L	1	114	1	276	5.0	6.0	2	S	1	307	1	120	6.7	6.0
4	L	1	127	1	210	5.0	5.2	2	S	1	38	1	54	5.9	5.2
4	L	1	105	1	190	5.1	4.9	2	S	1	3	1	60	6.7	5.2
4	L	1	136	1	250	5.5	5.3	2	S	1	197	1	114	6.2	5.1
4	L	1	19	0		5.0	5.2	2	S	1	16	1	76	6.6	5.5
4	L	1	385	1	185	5.5	4.9	2	S	1	10	1	64	6.6	5.5
4	L	1	21	1	60	6.7	4.9	2	S	0				6.7	5.6
4	L	1	266	1	224	6.1	5.0	2	S	0				6.5	5.6
4	L	1	188	1	192	5.5	5.0	2	S	0				7.1	5.7
4	L	1	8	1	119	6.1	5.3	2	S	0				5.3	5.1
4	L	1	46	1	122	6.8	5.2	2	S	0				5.7	5.7
4	L	0				4.9	5.5	2	S	0				7.1	5.3
4	L	0				7.2	5.5	2	S	0				5.8	5.2
4	L	1	17	0		6.6	5.0	2	S	0				6.1	5.6
4	L	1	195	1	192	6.3	4.9	2	S	0				5.1	5.3
4	L	1	440	1	101	4.3	5.2	2	S	0				6.1	5.5
4	L	1	13	1	140	5.7	5.2	2	S	0				6.1	5.7
4	L	1	235	1	230	6.3	4.5	2	S	0				7.0	5.3
4	L	1	72	1	64	5.2	5.8	2	S	1	3	1	35	5.5	5.5
4	L	1	803	1	224	5.0	4.5	2	S	1	5	1	140	4.5	4.7
4	L	1	16	1	62	6.1	5.3	2	S	1	2	0		5.1	5.5
4	L	1	110	1	184	5.0	5.5	2	S	1	8	1	157	6.1	5.0
4	L	1	2	0		6.3	5.0	2	S	1	2	0		5.1	5.1
4	L	1	1539	1	381	5.0	4.7	2	S	0				6.0	5.0
4	L	1	20	1	276	5.5	4.8	2	S	0				4.9	4.9
4	L	1	188	1	192	5.2	5.0	2	S	0				6.4	5.2
4	L	1	62	1	234	5.2	5.0	2	S	0				6.2	5.7
4	L	1	8	1	147	6.0	4.1	2	S	0				6.9	5.1
4	L	0				4.5	4.9	2	S	0				4.7	4.5
4	L	0				6.0	5.0	2	S	0				5.4	5.4
4	L	1	5	0		6.2	4.7	2	S	0				4.9	5.5
4	L	1	381	1	175	6.1	4.8	2	S	0				4.1	4.6
4	L	1	9	0		5.7	4.4	2	S	0				5.5	5.5
5	L	1	5	1	55	6.1	4.5	2	S	0				5.8	5.0
5	L	1	75	1	30	6.0	4.8	2	S	0				3.5	4.7

5	L	1	1	0		6.1	4.9	2	S	1	3	0		5.2	5.0
5	L	1	5	1	40	5.5	5.0	4	S	1	5	0		5.8	5.2
5	L	1	6	1	40	5.3	5.1	4	S	1	38	1	98	5.0	5.3
5	L	1	43	1	271	5.4	5.4	4	S	1	16	1	47	5.0	5.2
5	L	1	346	1	114	5.2	5.3	4	S	1	33	1	183	5.2	4.8
5	L	1	307	1	210	5.2	5.3	4	S	1	5	1	90	5.8	4.9
5	L	1	156	1	104	5.3	5.1	4	S	1	3	0		5.3	5.0
5	L	1	2	0		5.9	5.4	4	S	1	15	1	130	5.5	5.5
5	L	1	331	1	149	5.5	4.7	4	S	1	3	0		6.5	4.2
5	L	1	7	1	120	6.2	5.0	4	S	0				7.0	6.0
5	L	1	3	1	47	5.1	4.5	4	S	0				7.1	5.2
5	L	1	5	0		6.0	5.4	4	S	0				6.5	4.3
5	L	1	36	1	275	6.1	4.6	4	S	0				6.1	5.0
5	L	1	4	1	105	6.3	5.5	4	S	0				5.0	4.0
5	L	1	771	1	146	5.8	5.6	4	S	0				5.0	5.2
5	L	1	70	1	34	5.5	5.0	4	S	0				5.0	4.6
5	L	1	6	1	54	5.8	4.9	4	S	0				6.1	4.9
5	L	0				6.4	5.0	4	S	0				4.9	5.3
5	L	1	186	1	178	4.8	5.1	4	S	1	24	1	172	4.5	5.3
5	L	1	154	1	153	5.5	5.3	4	S	1	10	1	45	5.0	5.0
5	L	1	175	1	215	6.6	5.3	4	S	1	2	0		4.6	4.3
5	L	1	286	1	110	6.3	5.0	4	S	1	2	0		4.3	5.0
5	L	1	6	1	264	6.0	5.7	4	S	1	6	1	69	4.3	4.1
5	L	1	7	1	87	6.2	5.1	4	S	1	40	1	101	6.1	4.9
5	L	1	2	0		4.6	4.7	4	S	1	3	0		5.0	4.8
5	L	1	350	1	90	6.4	5.1	4	S	0				6.5	4.5
5	L	1	3	0		6.5	5.3	4	S	0				6.1	5.1
5	L	1	3	1	153	5.4	5.5	4	S	0				6.1	5.0
5	L	1	119	1	165	5.9	5.0	4	S	0				6.1	4.9
5	L	1	164	1	178	6.0	5.0	4	S	0				4.8	4.5
5	L	1	25	1	160	7.0	4.4	4	S	0				4.8	5.0
5	L	1	7	0		4.9	5.1	4	S	0				5.0	5.0
5	L	0				6.3	5.1	4	S	0				4.6	4.3
5	L	0				6.8	5.0	4	S	0				5.0	5.5
5	L	1	59	1	64	6.1	5.1	4	S	0				4.1	5.5
5	L	1	598	1	137	6.0	5.2	4	S	0				5.0	5.4
2	F	1	9	1	160	4.5	4.6	4	S	0				7.0	5.2
2	F	1	24	1	191	5.7	4.4	5	S	1	2	1	95	6.5	5.0
2	F	1	1	0		4.6	3.7	5	S	1	16	1	59	4.9	5.5
2	F	1	440	1	124	5.9	4.5	5	S	1	230	0		4.6	4.7
2	F	1	12	1	15	5.8	4.5	5	S	1	35	1	15	5.4	4.5
2	F	1	1	0		4.7	4.4	5	S	1	1	0		6.0	5.0
2	F	1	285	1	305	3.9	5.1	5	S	1	3	1	91	6.5	5.1
2	F	1	46	1	101	4.6	4.5	5	S	1	271	1	60	4.5	4.6
2	F	1	65	1	258	5.1	4.4	5	S	1	2	0		6.0	4.5
2	F	1	3	0		4.5	4.0	5	S	1	44	1	60	4.9	5.4
2	F	1	44	1	53	6.0	4.9	5	S	1	3	0		6.5	5.5
2	F	1	8	1	104	4.8	4.7	5	S	0				5.6	5.2
2	F	1	212	1	252	5.6	4.4	5	S	0				5.6	4.8
2	F	1	144	0		6.0	5.2	5	S	0				5.3	4.6
2	F	1	4	0		5.0	4.1	5	S	0				6.0	5.0

2	F	1	64	1	92	5.1	4.6
2	F	1	38	1	136	5.3	4.2
2	F	1	27	0		5.0	4.3
2	F	1	406	1	184	5.0	5.4
2	F	1	38	1	146	3.9	4.6
2	F	1	21	1	115	5.4	4.8
2	F	0				5.6	5.2
2	F	1	57	1	10	5.6	4.4
2	F	1	49	1	207	5.0	4.2
2	F	1	136	1	180	5.3	4.6
2	F	1	78	1	18	5.7	5.2
2	F	1	17	1	189	5.9	4.1
2	F	1	3	0		5.6	4.2
2	F	0				4.8	5.4
2	F	1	17	0		4.2	3.7
2	F	1	4	0		4.3	4.5
2	F	0				5.4	4.4
2	F	0				5.4	4.6
2	F	1	168	1	194	6.2	5.1
2	F	1	7	1	81	5.5	4.8
2	F	1	2	0		5.9	5.0
2	F	1	4	0		5.6	4.3
3	F	1	88	1	213	4.7	4.0
3	F	1	4	1	174	6.0	4.1
3	F	1	6	1	70	6.3	5.1
3	F	0				5.0	4.8
3	F	1	31	1	14	4.8	4.5
3	F	0				5.7	4.2
3	F	1	107	1	90	5.2	4.6
3	F	1	2	1	91	5.2	4.3
3	F	1	425	1	228	5.8	5.1
3	F	1	32	1	50	5.6	4.9
3	F	1	152	1	83	4.5	4.2
3	F	0				5.1	4.0
3	F	1	10	1	132	5.5	4.4
3	F	0				5.4	4.4
3	F	1	3	0		4.9	5.2
3	F	1	64	0		5.5	4.2
3	F	1	72	1	214	5.6	4.1
3	F	0				4.7	4.2
3	F	0				5.2	4.2
3	F	1	3	0		5.8	5.3
3	F	1	7	1	169	5.8	4.9
3	F	0				5.6	5.1
3	F	1	156	1	164	5.1	4.4
3	F	1	8	1	74	4.4	4.4
3	F	1	66	1	114	4.4	4.5
3	F	1	26	1	81	5.3	3.9
3	F	1	114	1	238	4.5	5.2
3	F	1	2	0		5.5	4.8
3	F	1	101	1	134	6.0	4.4

5	S	0				4.6	4.6
5	S	0				4.6	4.8
5	S	0				5.3	4.9
5	S	0				5.6	4.8
5	S	0				5.2	5.3
5	S	0				6.0	5.0
5	S	1	2	0		5.2	5.2
5	S	1	8	1	47	5.0	4.3
5	S	1	4	0		4.0	5.0
5	S	0				6.2	4.5
5	S	0				6.2	5.1
5	S	0				6.2	4.3
5	S	0				4.7	5.0
5	S	0				4.8	4.9
5	S	0				6.0	4.4
5	S	0				6.1	4.5
5	S	0				6.0	5.3
5	S	0				4.6	5.0
5	S	0				6.5	5.0
5	S	0				5.6	4.6
5	S	0				4.7	5.3
5	S	0				5.2	4.9
5	S	0				5.7	5.5
5	S	1	6	0		3.8	5.0
2	S	0				6.1	4.5
2	S	0				5.1	4.7
2	S	0				3.9	4.0
2	S	1	3	0		4.3	4.5
2	S	1	15	0		4.9	5.2
2	S	0				4.7	4.3
2	S	1	32	1	25	6.0	4.8
2	S	0				5.2	4.8
2	S	0				5.6	5.3
2	S	0				4.8	4.6
2	S	1	38	1	34	6.1	4.4
2	S	0				5.4	4.2
2	S	1	52	1	17	6.0	5.1
2	S	1	3	0		4.8	4.9
2	S	0				5.8	5.0
2	S	0				3.8	4.9
2	S	0				5.8	4.3
2	S	0				5.9	3.8
2	S	0				4.6	4.2
2	S	1	93	0		5.2	4.6
2	S	0				4.5	4.0
2	S	0				5.0	4.5
2	S	0				5.4	4.2
2	S	0				5.7	4.7
2	S	0				5.1	4.6
2	S	1	5	0		6.3	4.6
2	S	0				5.5	5.2

3	F	1	69	1	66	5.4	4.8
3	F	0				5.2	4.3
4	F	0				6.1	5.1
4	F	1	15	1	35	5.2	4.0
4	F	0				5.5	4.7
4	F	1	31	1	105	4.8	4.8
4	F	1	4	1	55	5.7	4.0
4	F	1	4	1	53	5.7	4.3
4	F	0				5.3	4.8
4	F	1	15	0		4.1	4.7
4	F	1	4	1	162	4.9	4.6
4	F	1	7	0		5.9	4.3
4	F	1	6	1	106	4.2	4.3
4	F	1	281	1	119	5.4	4.9
4	F	1	19	1	79	5.1	4.7
4	F	0				5.6	4.5
4	F	0				5.4	4.6
4	F	1	21	1	221	4.5	4.6
4	F	0				4.9	5.2
4	F	0				4.1	4.4
4	F	1	15	1	64	5.2	4.5
4	F	1	6	1	69	4.9	4.2
4	F	1	6	1	103	5.5	4.1
4	F	1	4	1	159	5.6	4.3
4	F	1	16	1	46	4.9	4.7
4	F	0				5.0	4.8
4	F	1	4	1	213	5.4	4.6
4	F	0				5.9	4.5
4	F	0				4.2	4.5
4	F	1	13	1	116	5.5	5.0
4	F	1	21	0		5.1	4.3
4	F	1	217	1	175	5.7	4.0
4	F	0				5.4	4.2
4	F	1	3	0		5.0	4.7
4	F	1	3	1	68	5.1	4.3
5	F	1	11	1	23	4.7	4.9
5	F	1	10	1	53	5.4	4.7
5	F	0				4.9	4.7
5	F	1	2	0		5.3	4.5
5	F	1	1	0		5.2	4.6
5	F	0				5.1	4.1
5	F	1	6	1	119	4.6	4.4
5	F	1	1	0		5.9	5.7
5	F	0				6.0	4.8
5	F	1	5	0		5.6	4.8
5	F	0				5.3	4.0
5	F	1	37	1	73	5.5	4.4
5	F	1	4	0		5.2	4.3
5	F	1	13	1	150	5.2	4.7
5	F	0				5.0	4.3
5	F	1	36	1	101	6.1	4.9
2	S	1	25	1	59	4.8	5.1
2	S	0				5.5	5.5
2	S	1	40	1	64	6.1	4.7
3	S	1	50	1	67	4.9	4.3
3	S	1	1	0		5.5	4.8
3	S	0				5.3	4.3
3	S	0				6.1	4.9
3	S	0				5.6	4.9
3	S	0				5.5	4.2
3	S	1	4	0		5.7	5.2
3	S	0				4.5	4.4
3	S	0				4.1	4.4
3	S	0				4.6	5.0
3	S	0				5.1	4.5
3	S	0				5.2	4.7
3	S	1	21	1	61	4.5	4.6
3	S	1	3	0		5.0	4.1
3	S	1	53	1	14	5.8	4.9
3	S	0				4.9	4.4
3	S	0				6.0	4.5
3	S	0				6.1	5.1
3	S	0				5.1	4.3
3	S	1	1	0		5.0	4.6
3	S	1	6	1	110	5.4	4.2
3	S	0				4.9	4.4
3	S	0				4.2	5.4
3	S	0				4.0	4.8
3	S	1	6	0		5.5	4.9
3	S	0				5.6	5.0
3	S	0				5.0	4.3
3	S	0				5.7	4.4
3	S	1	22	1	56	4.1	4.3
3	S	0				4.8	5.2
4	S	0				5.1	4.5
4	S	1	2	0		5.0	4.1
4	S	0				5.4	4.0
4	S	0				6.2	5.2
4	S	0				6.2	5.0
4	S	0				5.6	4.8
4	S	1	6	1	40	4.4	4.2
4	S	0				5.3	4.7
4	S	1	2	0		5.4	4.3
4	S	0				4.8	4.9
4	S	0				5.5	4.9
4	S	0				6.0	4.3
4	S	1	6	1	64	5.1	4.5
4	S	0				5.3	4.4
4	S	1	52	1	62	5.5	5.0
4	S	0				5.3	4.4
4	S	0				5.0	4.0
4	S	0				5.2	4.7

5	F	1	2	0		5.0	4.7
5	F	0				5.5	4.8
5	F	1	19	1	78	5.5	4.1
5	F	0				5.5	5.0
5	F	1	27	0		5.2	5.2
5	F	1	21	1	40	4.8	4.7
5	F	1	112	0		5.6	4.3
5	F	1	26	0		5.3	4.7
5	F	0				5.0	4.9
5	F	1	2	1	84	4.8	5.0
5	F	1	18	0		4.4	4.6
5	F	1	4	0		5.6	4.5
5	F	0				5.1	5.2
5	F	0				3.9	4.5
5	F	1	116	1	129	5.1	4.5
5	F	1	1	0		5.1	5.0
5	F	1	3	0		4.7	4.5
5	F	0				4.4	5.0
2	S	0				4.9	5.1
2	S	0				4.9	5.2
2	S	0				4.5	5.0
2	S	0				5.2	5.0
2	S	0				5.9	5.3
2	S	0				6.3	5.4
2	S	0				7.0	5.3
2	S	1	48	1	189	6.2	5.2
2	S	0				5.5	5.1
2	S	0				6.0	5.5
2	S	1	15	1	125	4.9	5.2
2	S	1	27	1	113	4.9	5.0
2	S	1	3	1	56	6.0	5.1
2	S	1	5	0		5.0	5.2
2	S	1	3	0		5.4	5.5
2	S	1	5	0		5.0	5.2
2	S	1	20	0		5.1	5.1
2	S	1	24	1	60	5.0	5.8
2	S	0				5.0	5.5
2	S	1	85	1	52	7.1	4.8
2	S	0				4.6	5.2
2	S	0				5.4	5.7
2	S	1	5	1	89	6.6	5.5
2	S	0				6.5	5.3
2	S	0				6.5	5.6
4	S	0				4.9	4.1
4	S	0				6.2	4.7
4	S	0				5.3	4.5
4	S	1	9	1	33	4.7	5.0
4	S	0				5.1	4.1
4	S	0				5.3	4.1
4	S	1	2	0		5.3	4.7
4	S	0				4.7	4.7
4	S	0				5.0	4.6
4	S	0				5.9	4.9
5	S	0				5.5	4.3
5	S	0				5.0	4.2
5	S	1	1	0		5.3	4.1
5	S	1	3	0		4.8	4.6
5	S	0				4.7	4.1
5	S	0				6.2	4.5
5	S	0				5.1	4.6
5	S	1	2	0		4.7	4.8
5	S	0				5.2	4.3
5	S	1	21	1	17	5.3	4.4
5	S	1	31	1	31	5.2	4.9
5	S	0				4.7	4.6
5	S	0				4.9	4.7
5	S	0				5.6	4.9
5	S	1	26	1	31	5.2	5.0
5	S	1	2	1	56	4.2	4.1
5	S	0				5.7	4.3
5	S	0				4.4	4.9
5	S	1	14	1	140	4.6	4.6
5	S	0				5.4	4.6
5	S	0				5.4	4.7
5	S	0				4.8	4.6
5	S	1	11	1	73	5.0	4.8
5	S	0				5.0	4.7
5	S	0				5.8	4.1
5	S	0				5.3	5.6
5	S	0				4.9	4.7
5	S	0				5.8	4.6
5	S	0				6.0	5.2
5	S	1	3	0		5.9	5.1
5	S	0				4.9	5.0

Appendix 4 – Chapter 6

Part I

Columns are (left to right) male culture alga (***L****aminaria* / ***F****ucus*), male treatment (L = exposure to *Laminaria*, F = exposure to *Fucus*), female culture alga (***L****aminaria* / ***F****ucus*), male harassment (0 = no mount observed, 1 = mount observed), time preceding mount (seconds), struggle duration (seconds), result of mount (0 = no copulation, 1 = copulation), male wing length (mm) and female wing length (mm).

F	F	F	1	484	10	0	5.9	6.0
F	F	F	0				6.0	4.9
F	F	F	0				5.9	4.9
F	F	F	1	79	441	1	5.8	5.4
F	F	F	1	94	335	1	5.7	5.1
F	F	L	0				6.4	5.3
F	F	L	0				6.6	5.0
F	F	L	0				7.0	5.3
F	F	L	0				5.1	5.5
F	L	F	0				5.2	5.1
F	L	F	1	17	406	1	5.2	5.0
F	L	F	0				6.4	4.9
F	L	F	0				6.5	4.9
F	L	F	1	12	522	1	5.7	5.2
F	L	L	1	557	15	1	6.0	5.2
F	L	L	0				6.2	5.0
F	L	L	0				5.3	5.1
F	L	L	0				6.0	4.3
L	F	F	1	71	60	1	4.6	5.0
L	F	F	0				6.4	4.5
L	F	F	1	279	88	1	5.1	5.0
L	F	F	1	22	550	1	5.0	4.8
L	F	L	1	17	526	1	5.1	5.0
L	F	L	0				5.2	4.1
L	F	L	1	70	485	0	5.0	4.6
L	F	L	1	83	293	1	6.1	5.0
L	L	F	0				4.1	5.0
L	L	F	1	262	287	1	4.5	5.1
L	L	F	0				6.0	5.2
L	L	F	1	2	385	1	5.6	5.1
L	L	L	0				4.5	4.4
L	L	L	0				4.7	4.8
L	L	L	1	74	424	1	4.7	4.2
L	L	L	0				5.9	5.6
F	F	F	1	510	14	0	3.7	4.8
F	F	F	0				6.0	4.5
F	F	F	0				5.7	5.1
F	L	F	1	559	12	0	5.6	5.1

F	L	F	0				4.8	5.6
F	L	F	0				5.1	4.1
L	F	F	1	116	484	0	6.7	4.7
L	F	F	1	48	74	1	5.0	5.1
L	F	F	0				5.5	4.4
L	L	F	1	97	38	1	5.9	5.4
L	L	F	1	369	15	0	5.5	5.2
L	L	F	1	53	5	0	5.0	5.2
F	F	L	1	356	6	0	5.5	5.3
F	F	L	0				5.2	5.8
F	F	L	1	2	47	1	4.8	5.4
F	F	L	1	266	334	0	4.9	5.1
F	L	L	1	6	124	1	4.5	5.4
F	L	L	1	46	154	1	5.3	5.4
F	L	L	1	52	12	0	5.2	4.3
F	L	L	1	100	45	1	5.7	5.5
L	F	L	1	14	217	1	5.4	5.2
L	F	L	1	102	20	1	5.2	5.5
L	F	L	1	7	280	1	5.1	5.3
L	F	L	0				7.1	5.6
L	L	L	1	18	480	1	6.5	6.0
L	L	L	1	478	96	0	6.9	5.3
L	L	L	1	51	129	1	5.1	5.6
L	L	L	1	5	32	0	6.4	5.7
L	F	F	1	24	85	1	5.6	4.3
L	F	F	1	49	125	0	4.9	5.1
L	F	F	0				6.1	4.7
L	F	F	1	42	174	1	5.3	4.7
L	L	F	1	104	89	1	6.1	4.6
L	L	F	1	20	64	1	4.7	4.1
F	F	F	1	587	13	0	4.7	4.1
F	F	F	1	77	131	1	4.5	4.5
F	F	F	1	436	164	0	4.9	5.0
F	F	F	1	481	3	0	4.3	3.8
F	L	F	1	288	201	1	5.2	5.4
F	L	F	1	328	31	1	4.7	4.8
F	L	F	1	43	43	1	4.7	3.5
F	L	F	1	124	53	1	5.0	4.5
L	F	L	1	51	121	1	4.6	4.6
L	F	L	1	147	54	1	5.7	4.6
L	F	L	1	36	321	1	4.3	4.4
L	F	L	0				5.1	4.5
L	L	L	1	13	102	1	5.3	4.8
L	L	L	1	62	71	1	4.9	4.7
L	L	L	1	13	76	0	4.1	4.8
F	F	L	1	16	14	0	5.4	5.2
F	F	L	0				4.9	4.1
F	L	L	1	43	113	1	5.0	4.1
F	L	L	0				3.8	4.7
F	L	L	1	584	9	0	4.2	4.1
L	F	F	1	10	346	1	5.5	4.5
L	F	F	1	46	12	1	4.8	4.1
L	F	F	1	43	66	1	5.0	4.4
L	F	F	1	25	235	1	4.4	4.4

L	L	F	0				5.1	4.5
L	L	F	1	122	109	1	5.5	3.9
L	L	F	1	53	109	1	5.1	4.2
L	L	F	1	16	198	1	4.4	4.2
L	L	F	1	63	198	1	5.1	4.3
F	F	F	1	215	385	0	3.6	4.5
F	F	F	1	141	459	0	5.4	4.0
F	L	F	1	26	574	0	4.0	4.3
F	L	F	0				4.1	4.6
F	L	F	1	28	114	0	4.6	4.9
F	L	F	1	38	22	0	4.7	5.1
L	F	L	1	84	369	0	4.2	5.2
L	F	L	1	326	253	1	5.0	4.6
L	F	L	1	27	573	0	4.7	4.8
L	L	L	1	112	406	1	5.5	5.4
L	L	L	0				4.8	4.6
L	L	L	1	120	85	1	6.0	4.5
L	L	L	1	16	94	1	5.3	5.8
F	F	L	1	80	112	0	4.1	4.6
F	F	L	1	74	291	1	4.1	4.1
F	F	L	1	147	78	0	4.2	5.9
F	F	L	1	46	264	1	4.6	5.1
F	L	L	1	8	252	1	4.2	5.5
F	L	L	1	23	55	1	4.6	5.5
F	L	L	0				4.8	4.6
F	L	L	1	18	582	0	5.5	4.7
F	L	L	0				5.4	4.9
F	F	L	1	5	8	1	4.5	4.8
L	L	F	1	443	2	0	4.1	5.0
L	F	F	1	21	13	1	5.6	4.7
F	L	F	1	199	19	0	4.2	4.0
L	L	L	1	31	3	1	4.8	4.5
L	F	L	1	53	3	1	4.0	4.8
F	L	L	0				4.6	4.8
L	L	F	0				4.1	5.2
L	F	F	1	7	4	1	4.7	4.1
L	L	L	0				4.3	4.4
F	L	L	0				5.3	4.5
F	F	L	1	50	11	1	4.5	4.9
F	L	F	1	26	2	1	4.4	5.0
F	F	F	0				4.7	4.6
F	F	L	0				4.8	4.7
F	L	F	1	172	10	1	4.4	4.8
F	F	F	1	17	6	1	4.7	5.1
F	L	L	1	71	5	0	4.7	4.9
F	L	L	1	37	3	1	4.5	4.5
L	L	L	0				4.8	4.9
F	F	L	1	13	5	1	4.6	4.7
L	F	F	1	359	4	0	5.3	4.5
L	L	F	0				5.3	5.2
F	F	F	0				5.6	4.7
L	L	L	0				5.9	4.5
L	F	L	1	139	12	1	3.9	4.4
L	F	L	0				4.7	4.6

F	F	L	1	27	3	0	4.0	4.4
L	L	F	0				4.8	3.9
L	F	F	1	15	6	0	4.8	4.8
F	L	F	1	113	7	0	4.3	4.7
F	F	F	1	18	4	1	5.2	5.0
L	L	L	1	81	6	1	5.7	5.0
L	F	L	1	36	7	1	4.2	4.7
F	L	L	1	39	6	1	4.4	4.6
F	L	F	1	117	7	0	4.6	3.8
L	F	F	1	6	26	0	6.1	4.7
F	L	F	1	9	3	1	5.0	4.8
F	F	F	1	8	2	0	4.4	5.2
L	F	F	1	12	7	1	5.5	5.5
F	F	F	0				5.4	4.6
L	L	F	0				5.2	4.1
F	L	F	1	22	8	1	4.4	4.8
L	F	F	1	529	562	0	5.3	5.1
F	L	F	1	26	7	0	5.0	4.7
L	F	F	0				3.6	4.9
F	L	F	1	9	30	1	4.6	4.1
F	F	F	1	94	2	0	3.7	5.0
L	F	F	1	11	13	1	5.2	4.8
L	L	L	1	49	34	1	4.4	4.9
L	F	L	1	29	25	0	4.8	5.0
F	L	L	1	92	4	1	5.1	4.9
F	F	L	1	40	1	1	4.6	5.1
L	L	F	1	3	1	1	4.9	4.1
L	F	F	1	17	5	0	4.5	4.4
F	L	F	0				4.2	4.8
F	F	F	1	35	7	1	4.5	4.0
L	L	L	1	11	69	0	4.3	4.7
L	F	L	1	8	26	1	4.5	4.9
F	L	L	1	11	15	1	4.8	5.1
F	F	L	1	40	3	1	5.4	5.3
L	L	F	1	20	6	1	4.8	3.9
L	F	F	1	182	4	1	4.7	5.1
F	L	F	0				4.0	4.6
F	F	F	0				4.6	4.6
L	L	L	1	8	34	0	4.6	5.5
L	F	L	1	48	2	0	6.1	4.3
F	L	L	1	8	92	0	4.2	5.0
F	F	L	1	5	80	0	4.2	4.6
L	L	F	1	22	20	1	5.3	4.8
L	F	F	1	11	4	1	4.8	4.6
F	L	F	1	11	3	1	4.4	4.8
F	F	F	1	36	3	1	4.2	4.5
L	L	L	1	33	8	1	4.5	5.1
L	F	L	1	36	2	0	4.8	4.8
F	L	L	1	85	16	1	5.5	5.0
F	F	L	1	17	8	1	4.6	5.4
L	L	F	0				6.2	4.3
L	F	F	1	9	5	1	4.8	4.5
F	L	F	1	14	6	1	3.9	4.2
F	F	F	1	13	10	1	3.8	4.6

L	L	L	1	30	4	1	4.9	5.3
L	F	L	1	14	20	0	4.2	4.7
F	L	L	1	24	3	1	4.6	4.7
L	L	F	1	202	69	1	4.8	5.0
L	F	F	1	50	5	0	4.6	4.4
F	L	F	1	23	4	1	4.6	4.2
F	F	F	1	22	5	1	5.0	4.5
L	L	L	1	6	6	1	5.0	5.2
L	F	L	1	56	4	0	4.7	4.6
F	L	L	1	20	64	1	5.3	5.0
F	F	L	1	24	23	0	4.2	5.2
L	L	F	1	2	19	0	4.4	4.8
L	F	F	1	26	6	0	5.7	4.6
F	L	F	1	29	7	1	5.0	4.3
F	F	F	0				4.8	5.1
L	L	L	1	20	73	1	4.5	4.8
L	F	L	1	13	29	1	5.6	4.6
F	L	L	1	40	7	0	3.9	4.9
F	F	L	1	13	2	0	4.2	4.7
L	L	F	1	8	3	1	4.5	4.3
L	F	F	1	11	8	0	5.2	5.0
F	L	F	1	17	12	1	4.1	4.3
F	F	F	0				4.0	4.8
L	L	L	1	147	6	1	4.5	5.1
L	F	L	1	27	2	1	6.3	5.3
F	L	L	1	9	2	0	4.2	4.6
F	F	L	1	20	3	0	4.9	3.5
L	L	F	1	19	4	1	5.0	4.0
L	F	F	1	38	3	1	4.8	5.1
F	L	F	0				4.5	4.9
F	F	F	1	25	19	1	4.2	4.6
L	L	L	1	26	876	0	4.8	4.7
L	F	L	1	13	2	0	5.0	4.7
F	L	L	1	15	6	0	4.1	5.3
F	F	L	1	49	5	1	4.5	5.2
L	L	F	1	19	4	1	4.5	4.1
L	F	F	1	47	9	1	4.5	4.8
F	L	F	1	12	3	0	3.9	5.0
F	F	F	1	22	2	1	4.5	3.7
L	L	L	1	20	3	1	5.4	4.6
L	F	L	1	17	26	1	4.7	4.8
F	L	L	0				5.7	4.8
F	F	L	1	54	55	0	4.4	5.0
L	L	F	1	20	12	1	5.6	4.6
L	F	F	1	17	4	1	4.7	4.9
F	L	F	1	15	5	1	4.2	4.9
F	F	F	1	20	11	1	4.7	4.3
L	L	L	1	208	2	1	4.3	5.2
L	F	L	1	7	7	1	4.8	4.6
F	L	L	1	57	4	1	4.9	4.7
F	F	L	1	17	70	0	4.2	5.3
L	L	F	1	11	16	1	4.6	4.4
L	F	F	1	20	5	1	4.6	5.0
F	L	F	1	12	4	0	7.1	3.9

L	L	L	1	62	153	0	4.6	4.8
L	F	L	1	22	23	1	6.6	4.8
L	F	F	1	325	3	0	4.9	4.5
L	L	L	1	28	4	1	4.2	4.6
L	F	L	1	35	3	1	4.9	4.3
F	L	L	1	24	4	1	5.1	4.5
F	F	L	1	43	43	1	3.8	4.4
L	L	F	1	8	5	1	4.1	4.4
L	F	F	1	87	3	0	4.3	3.9
F	L	F	1	15	12	1	3.8	3.9
F	F	F	1	151	3	1	4.0	4.1
L	F	L	1	8	2	1	4.1	4.7
F	L	L	1	12	5	1	5.3	4.7
F	F	L	1	41	9	0	3.5	4.8
L	L	F	0				5.2	4.1
L	F	F	0				4.1	4.5
L	L	L	1	6	15	0	5.5	4.3
L	F	L	1	13	7	1	4.3	4.4
F	L	L	1	8	13	1	4.1	4.7
F	F	L	1	32	3	0	4.2	4.5
L	L	F	1	264	30	1	5.1	4.1
L	F	F	1	28	4	1	4.3	4.4
F	L	F	1	92	4	1	4.1	3.9
F	F	F	1	576	2	1	4.5	4.3
L	L	L	1	39	10	1	4.5	4.4
L	F	L	0				4.1	4.8
F	L	L	1	7	8	1	4.0	5.0
F	F	L	1	28	15	0	3.7	4.2
L	F	F	1	19	5	1	4.3	4.6
F	L	F	1	55	18	1	3.8	4.4
F	F	F	1	17	4	1	4.8	4.0
L	L	L	1	40	2	1	4.8	5.1
L	F	L	1	91	3	1	5.5	4.4
F	L	L	1	48	143	1	4.1	4.7
F	F	L	1	16	9	1	4.0	4.3
L	L	F	1	589	1	0	4.2	3.5
L	F	F	1	68	6	0	4.7	4.3
F	L	F	1	7	4	1	4.2	4.0
F	F	F	1	32	1	0	4.4	4.3
L	L	L	1	15	5	1	4.2	4.2
L	F	L	1	49	6	1	4.0	4.8
F	L	L	1	11	48	1	5.4	4.8
F	F	L	1	15	17	1	4.6	4.8
L	L	F	1	9	4	1	6.0	4.1
L	F	F	1	10	4	1	4.8	3.9
F	L	F	1	23	53	1	4.3	4.3
L	L	L	1	16	7	1	4.2	4.2
L	F	L	0				5.1	4.9
F	L	L	1	54	3	1	4.4	4.6
F	F	L	1	10	3	1	4.5	4.5
L	L	F	1	29	3	1	4.0	4.1
L	F	F	1	49	5	1	4.7	4.2
F	L	F	1	55	3	1	5.2	4.0
F	F	F	1	12	3	0	3.8	3.8

L	L	L	1	12	3	1	4.2	4.6
F	L	L	1	74	5	0	3.7	4.8
F	F	L	1	15	7	1	4.1	4.4
L	L	F	0				4.1	4.1
L	F	F	0				4.0	3.9
F	L	F	0				3.5	4.0
F	F	F	1	108	6	1	3.7	4.6
L	L	L	1	35	19	0	4.2	4.4
L	F	L	1	249	11	1	4.0	4.4
F	L	L	1	524	6	0	3.4	5.1
F	F	L	1	26	5	1	4.0	4.6
L	L	F	1	11	4	1	4.3	4.1
L	F	F	1	514	3	1	5.2	4.5
F	L	F	1	36	2	0	3.6	3.9
F	F	F	1	11	8	1	4.6	4.2
L	L	L	1	19	6	1	4.1	5.0
L	F	L	0				5.5	4.4
F	L	L	1	13	3	1	4.0	4.8
L	L	F	1	31	4	0	4.2	4.2
L	F	F	1	50	6	0	4.0	4.2
F	L	F	1	143	2	0	4.2	3.9
F	F	F	1	161	4	1	4.6	4.6
L	L	L	0				4.2	4.2
L	F	L	1	24	5	1	4.7	4.3
F	L	L	1	20	2	1	4.5	4.4
F	F	L	1	58	4	0	3.9	5.3
L	L	F	1	143	2	1	5.2	4.2
L	F	F	1	11	16	0	5.3	4.0
F	L	F	0				4.2	4.1
F	F	F	1	32	3	1	4.4	4.2
L	L	L	1	15	51	1	4.1	4.0
L	F	L	0				4.1	4.5
F	L	L	0				4.3	4.7
F	F	L	1	10	29	0	3.9	4.9
L	L	F	1	71	22	1	4.2	4.9
L	F	F	1	8	4	1	4.3	4.3
F	L	F	0				4.1	4.1
L	F	L	1	11	3	1	4.3	4.1
F	L	L	1	15	2	1	3.9	4.7

Part II – Flies cultured at equivalent density

Columns are (left to right) development time (days), culture algae (***Fucus*** / ***Laminaria***), sex (**m**ale / **f**emale), wing length (mm), weight (mg) and alcohol dehydrogenase allozymes (X = indeterminable).

9	F	M	5.5	-	-	10	L	F	5.4	-	-
9	F	M	5.6	-	-	10	L	F		-	-
9	F	M	6.4	-	-	10	L	F	5.5	-	-
9	F	M	5	-	-	10	L	F	5.6	-	-
9	F	M	5.4	-	-	10	L	F		-	-

9	F	M	5.1	-	-	10	L	F	5.5	-	-
9	F	M		-	-	10	L	F	5.7	-	-
9	F	M	5.3	-	-	10	L	F	5.8	-	-
9	F	M	5.4	-	-	10	L	F	6	-	-
9	F	F	5.9	-	-	10	L	F	5.4	-	-
9	F	F	5.6	-	-	10	L	F	5.6	-	-
9	F	F	5.9	-	-	10	L	F	5.2	-	-
9	F	F	5.5	-	-	10	L	F	5.2	-	-
9	F	F	5.6	-	-	10	L	F	5.4	-	-
9	F	F	5.9	-	-	10	L	F	5.4	-	-
9	F	F	4.9	-	-	10	L	F	5.4	-	-
9	F	F	5.1	-	-	10	L	F	5.5	-	-
9	F	F	5.5	-	-	10	L	F	5.1	-	-
9	F	F	5.8	-	-	10	L	F	5.1	-	-
9	F	F		-	-	10	L	F	5.7	-	-
9	F	F	5.4	-	-	10	L	F	5.8	-	-
9	F	F	5.7	-	-	10	L	F	5.8	-	-
9	F	F	5.5	-	-	10	L	F	5.5	-	-
9	F	F	6.1	-	-	10	L	F	5.4	-	-
9	F	F	5.9	-	-	10	L	F	5.5	-	-
9	F	F	5.3	-	-	10	L	F	5.6	-	-
9	F	F	5.5	-	-	10	L	F	5.7	-	-
9	F	F	5.6	-	-	10	L	F	5.7	-	-
9	F	F	5.4	-	-	10	L	F	5.5	-	-
9	F	F	5.9	-	-	10	L	F	5.2	12.4	BB
9	F	F	5.4	-	-	10	L	F	5.4	10.6	BB
9	F	F	5.3	-	-	10	L	F	5	10.9	BB
9	F	F	5.7	-	-	10	L	F	5.4	13.5	BC
9	F	F	5.5	-	-	10	L	M	5.6	13.3	BD
9	F	F	5.5	-	-	10	L	M	5.9	17	BD
9	F	F	5.6	-	-	10	L	F	5.3	13.3	BD
9	F	F	6	-	-	10	L	F	5.2	11.5	BD
9	F	F	5	-	-	10	L	M	6.2	21.2	BD
9	F	F	5.5	-	-	10	L	M	5.9	14.4	BD
9	F	F	5.7	-	-	10	L	F	5.3	11.6	BD
9	F	F	6	-	-	10	L	F	5.1	9.9	BD
9	F	F	5.5	-	-	10	L	F	5.3	10.2	BD
9	F	F	5.5	-	-	10	L	M	6.4	15.5	BD
9	F	F	5.6	-	-	10	L	M	6.4	16.9	BD
9	F	F	5.8	-	-	10	L	F	5.3	10	BD
9	F	F	5.9	-	-	10	L	F		10.8	BD
9	F	F	5.4	-	-	10	L	M	6.1	15.5	BD
9	F	F	5.6	-	-	10	L	F	5.3	10	BD
9	F	F	5.7	-	-	10	L	F	5.1	11.3	CC
9	F	F	5.3	-	-	10	L	M	5.9	14.9	CD
9	F	F	5.4	-	-	10	L	M	5.4	8.2	CD
9	F	F	5.5	-	-	10	L	F	5.1	9	CD
9	F	F	5.6	-	-	10	L	M	5.4	11.1	CD
9	F	F	5.6	-	-	10	L	M	5.1	9.1	CD
9	F	F	5.8	-	-	10	L	M	5	10.1	CD
9	F	F	5.3	-	-	10	L	F	5.2	10.8	CD
9	F	F	5.4	-	-	10	L	M	5	8.9	DD
9	F	F	5.5	-	-	10	L	F	5	8.1	DD
9	F	F	6	-	-	10	L	F	5.3	10.9	DD

9	F	F	5.5	-	-
9	F	F	5.6	-	-
9	F	F	5.8	-	-
9	F	F	5.5	-	-
9	F	F	5.7	-	-
9	F	F	5.8	-	-
9	F	F	5.4	-	-
9	F	F	5.8	-	-
9	F	F	5.9	-	-
9	F	F	5.6	-	-
9	F	F	5.6	-	-
9	F	F	5.4	-	-
9	F	F	5.5	-	-
9	F	F	5.6	-	-
9	F	F	5.6	-	-
9	F	F	5.4	-	-
9	F	F	5.5	-	-
9	F	F	5.7	-	-
9	F	F	5.1	-	-
9	F	F	5.5	-	-
9	F	F	5.4	-	-
9	F	F		-	-
9	F	M	4.9	-	-
9	F	M	6	-	-
9	F	M	6.2	-	-
9	F	M	5.7	-	-
9	F	M	6.1	-	-
9	F	M	6.4	-	-
9	F	M	5.2	-	-
9	F	M	6.2	-	-
9	F	M	6.4	-	-
9	F	M	6.3	-	-
9	F	M	6	-	-
9	F	M	5.1	-	-
9	F	M	4.8	-	-
9	F	M	5.3	-	-
9	F	M	5.7	-	-
9	F	M	5.4	-	-
9	F	M	6.5	-	-
9	F	M	5.7	-	-
9	F	M	5.2	-	-
9	F	M	5	-	-
9	F	M	6	-	-
9	F	M	4.6	-	-
9	F	M	6.3	-	-
9	F	M	6.1	-	-
9	F	M	6.2	-	-
9	F	M	6	-	-
9	F	F	5.6	-	-
9	F	F	5.4	-	-
9	F	F	5.4	-	-
9	F	F	5.9	-	-
9	F	F	5.7	-	-
9	F	F	5.5	-	-
10	L	M	5.1	8.8	DD
10	L	M	5.1	10.4	DD
10	L	M	5.1	9.2	DD
10	L	F	5.2	11.3	DD
10	L	F	5.1	11.5	DD
10	L	M	5.1	9.9	DD
10	L	M	5.3	9.7	DD
10	L	M	4.9	8.9	DD
10	L	F	5.1	10.5	DD
10	L	M	6.4	18	X
11	F	M	5.9	-	-
11	F	M	6.6	-	-
11	F	M	5	-	-
11	F	M	5.6	-	-
11	F	M	6.3	-	-
11	F	M	6.5	-	-
11	F	M		-	-
11	F	M	6.9	-	-
11	F	M	7	-	-
11	F	M	7.1	-	-
11	F	M	6.6	-	-
11	F	M	6.6	-	-
11	F	M	5.9	-	-
11	F	M	4.6	-	-
11	F	M	5.8	-	-
11	F	M	5.6	-	-
11	F	M	6.8	-	-
11	F	M	6.8	-	-
11	F	M	6	-	-
11	F	M	6.9	-	-
11	F	M	6.9	-	-
11	F	M	6.5	-	-
11	F	M	7	-	-
11	F	M	6	-	-
11	F	M	6.2	-	-
11	F	M		-	-
11	F	M	6.7	-	-
11	F	M	6.6	-	-
11	F	M	6.4	-	-
11	F	M	6.4	-	-
11	F	M	7	-	-
11	F	M	6.3	-	-
11	F	M	7.1	-	-
11	F	M	6.5	-	-
11	F	M	7.1	-	-
11	F	M	6.2	-	-
11	F	M	6.5	-	-
11	F	M	6.1	-	-
11	F	M	5.7	-	-
11	F	M	5.8	-	-
11	F	M	6.5	-	-
11	F	M	6.7	-	-
11	F	M	6.2	-	-
11	F	M	5.9	-	-

9	F	F	5.1	-	-	11	F	M	6	-	-
9	F	F	5.3	-	-	11	F	M	6.6	-	-
9	F	F	5.3	-	-	11	F	M	6.2	-	-
9	F	F	5.4	-	-	11	F	M	5.6	-	-
9	F	F	5	-	-	11	F	M	5.9	-	-
9	F	F	5.5	-	-	11	F	M	5.7	-	-
9	F	F	5.2	-	-	11	F	M	6	-	-
9	F	F	5.6	-	-	11	F	M	5	-	-
9	F	F	5.5	-	-	11	F	M	5.6	-	-
9	F	F	5.6	-	-	11	F	M	4.8	-	-
9	F	F	5.6	-	-	11	F	M	5.3	-	-
9	F	F	5.5	-	-	11	F	M	6.4	-	-
9	F	F	5.5	-	-	11	F	M	5.7	-	-
9	F	F	5	-	-	11	F	M	6.7	-	-
9	F	F	5.3	-	-	11	F	M	6.8	-	-
9	F	F	5.1	-	-	11	F	M	5.6	-	-
9	F	F	5.3	-	-	11	F	M	6.3	-	-
9	F	F	5.9	-	-	11	F	M	7.1	-	-
9	F	F	5.2	-	-	11	F	M	7.2	-	-
9	F	F	5.4	-	-	11	F	M	6.6	-	-
9	F	F	5.7	-	-	11	F	M	6.8	-	-
9	F	F	5.4	-	-	11	F	M	6.8	-	-
9	F	F	5.4	-	-	11	F	M	6.1	-	-
9	F	F	5.6	-	-	11	F	M		-	-
9	F	F	5.3	-	-	11	F	M		-	-
9	F	F	5.3	-	-	11	F	M	5.4	-	-
9	F	F	5.4	-	-	11	F	M	6.1	-	-
9	F	F	5.4	-	-	11	F	M	6	-	-
9	F	F	5.5	-	-	11	F	M	6.6	-	-
9	F	F	5.6	-	-	11	F	M	6.1	-	-
9	F	F	5.4	-	-	11	F	M	6.1	-	-
9	F	F	5.7	-	-	11	F	M	5.9	-	-
9	F	F	5.8	-	-	11	F	M	6	-	-
9	F	F	5.3	-	-	11	F	M	6.7	-	-
9	F	F	5.5	-	-	11	F	M	7.1	-	-
9	F	F	5.6	-	-	11	F	M	5.7	-	-
9	F	F	5.4	-	-	11	F	F	4.9	-	-
9	F	F	5.5	-	-	11	F	F		-	-
9	F	F	5.6	-	-	11	F	M	6.5	19.2	BB
9	F	F	5.3	-	-	11	F	M	6.2	19.8	BB
9	F	F	5.9	-	-	11	F	M	6.2	19.7	BB
9	F	F	5.5	-	-	11	F	F	5.6	16.2	BB
9	F	F	5.4	-	-	11	F	F	4.9	8.4	BB
9	F	F	5.4	-	-	11	F	F	4.9	11.2	BB
9	F	F	5.9	-	-	11	F	F	5.2	13.1	BB
9	F	F	5.6	-	-	11	F	F	5	10.2	BC
9	F	F	5.6	-	-	11	F	M	6.4	15.7	BC
9	F	F	6.1	-	-	11	F	M	6.6	24.6	BC
9	F	F	5.2	-	-	11	F	M	6	12.3	BC
9	F	F	5.9	-	-	11	F	M	6.4	16.6	BC
9	F	F	5.5	-	-	11	F	M	5.3	11	BD
9	F	F	5.6	-	-	11	F	M	5.8	16.7	BD
9	F	F	5.6	-	-	11	F	M	5.7	13.2	BD
9	F	F	5.3	-	-	11	F	F	5.1	11.8	BD

9	F	F	5.6	-	-	11	F	M		6.5	BD
9	F	F	5.3	-	-	11	F	M	5.7	13.7	BD
9	F	F	5.6	-	-	11	F	M	5.2	10.5	BD
9	F	F	5	-	-	11	F	F	5.1	9	BD
9	F	F	5.6	-	-	11	F	M	5.4	10.7	BD
9	F	F	5.7	-	-	11	F	M	5.6	11.6	BD
9	F	F	5.3	-	-	11	F	M	5.8	11.4	BD
9	F	F	5.5	-	-	11	F	M	5.8	10.7	BD
9	F	F	5.6	-	-	11	F	F	5	10.1	BD
9	F	F	5.4	-	-	11	F	F	5.1	11.3	BD
9	F	F		-	-	11	F	M	5.4	10.8	CD
9	F	F		-	-	11	F	F	5	12.3	DD
9	F	M	4.8	-	-	11	F	F	4	4.5	DD
9	F	M	4.9	-	-	11	F	F	5.1	10.7	DD
9	F	M	5.9	-	-	11	F	F	5.1	9.8	DD
9	F	M	5.1	-	-	11	F	F	5.2	10.3	DD
9	F	M	5.9	-	-	11	F	M	6.3	20.7	X
9	F	M	6.4	-	-	11	F	M		11.6	X
9	F	M	5	-	-	11	L	M	6.5	-	-
9	F	M	5.1	-	-	11	L	M	6.7	-	-
9	F	M	5.8	-	-	11	L	M	7	-	-
9	F	M	6.1	-	-	11	L	M	6.8	-	-
9	F	F	5.1	-	-	11	L	M	6.6	-	-
9	F	F	5.5	-	-	11	L	M	5.5	-	-
9	F	F	5.6	-	-	11	L	M	6.2	-	-
9	F	F	5.4	-	-	11	L	M	5.2	-	-
9	F	F	5.1	-	-	11	L	M	6.1	-	-
9	F	F	5.6	-	-	11	L	M	5.4	-	-
9	F	F	5.3	-	-	11	L	M	5.7	-	-
9	F	F	5.5	-	-	11	L	M	6.6	-	-
9	F	F	5.9	-	-	11	L	M	6.2	-	-
9	F	F	5.6	-	-	11	L	M	6.3	-	-
9	F	F	5.2	-	-	11	L	M	6.1	-	-
9	F	F	5.1	-	-	11	L	M	6.5	-	-
9	F	F	5.2	-	-	11	L	M	5.6	-	-
9	F	F	5.5	-	-	11	L	M	6.3	-	-
9	F	F	5.6	-	-	11	L	M	6.6	-	-
9	F	F	5.6	-	-	11	L	F	5.5	-	-
9	F	F	5.6	-	-	11	L	F	5.6	-	-
9	F	F	5.8	-	-	11	L	F	5.2	-	-
9	F	F	5.5	-	-	11	L	F	4.9	-	-
9	F	F	5.5	-	-	11	L	F	5.2	-	-
9	F	F	5	-	-	11	L	F	5.4	-	-
9	F	F	5.6	-	-	11	L	F	5.3	-	-
9	F	F	5.8	-	-	11	L	F	5.6	-	-
9	F	F	5.6	-	-	11	L	F	5.6	-	-
9	F	F	5.3	-	-	11	L	F	4.7	-	-
9	F	F	5.4	-	-	11	L	F	5.8	-	-
9	F	F	5.6	-	-	11	L	F		-	-
9	F	F	5.4	-	-	11	L	F	5.1	-	-
9	F	F	5.5	-	-	11	L	F	5.7	-	-
9	F	F	5.6	-	-	11	L	F		-	-
9	F	F	5.2	-	-	11	L	F	5.4	-	-
9	F	F	5.9	-	-	11	L	F	5.6	-	-

9	F	M	6.2	-	-	11	L	F	5.5	-	-
9	F	M	6.7	-	-	11	L	F	5.5	-	-
9	F	M	4.4	-	-	11	L	F	4.8	-	-
9	F	M	4.8	-	-	11	L	F	5.3	-	-
9	F	M	6	-	-	11	L	F	5.2	-	-
9	F	M	5.1	-	-	11	L	F	5.5	-	-
9	F	M	5.1	-	-	11	L	F	5.3	-	-
9	F	M	6.7	-	-	11	L	F	5.4	-	-
9	F	M	5.2	-	-	11	L	F	5.5	-	-
9	F	M	5.3	-	-	11	L	F	5.3	-	-
9	F	M	5.9	-	-	11	L	F	5.8	-	-
9	F	M	5.1	-	-	11	L	F	5.7	-	-
9	F	M	5.3	-	-	11	L	F		-	-
9	F	M	5.4	-	-	11	L	F	5.8	-	-
9	F	M	5.1	-	-	11	L	M	7.2	-	-
9	F	M	5.1	-	-	11	L	M	6.6	-	-
9	F	M	5.8	-	-	11	L	M	6.8	-	-
9	F	M	6.1	-	-	11	L	M	5.1	-	-
9	F	M	5.2	-	-	11	L	M	5.3	-	-
9	F	M	5	-	-	11	L	M	6.5	-	-
9	F	M	5	-	-	11	L	M	6.4	-	-
9	F	M	5.2	-	-	11	L	M	6.6	-	-
9	F	M	6.4	-	-	11	L	M	6.7	-	-
9	F	M	5.2	-	-	11	L	M	6.2	-	-
9	F	M	4.9	-	-	11	L	M	6.6	-	-
9	F	M	6.3	-	-	11	L	M	6.7	-	-
9	F	M	6.1	-	-	11	L	M	6.1	-	-
9	F	M	5.1	-	-	11	L	M	6.4	-	-
9	F	F	5.4	-	-	11	L	M	5.3	-	-
9	F	F	5.5	-	-	11	L	M	6.1	-	-
9	F	F	5.7	-	-	11	L	M	6.8	-	-
9	F	F	5.3	-	-	11	L	M	5.4	-	-
9	F	F	5.2	-	-	11	L	M	6.5	-	-
9	F	F	5.7	-	-	11	L	M	6.6	-	-
9	F	F	5.5	-	-	11	L	M	6.2	-	-
9	F	F	5.6	-	-	11	L	M		-	-
9	F	F	5.7	-	-	11	L	M		-	-
9	F	F	5	-	-	11	L	M	6.3	-	-
9	F	F	5.6	-	-	11	L	M	6.7	-	-
9	F	F	5.5	-	-	11	L	M	5.6	-	-
9	F	F	5.4	-	-	11	L	F	5.8	-	-
9	F	F	5	-	-	11	L	F	5.1	-	-
9	F	F	5.9	-	-	11	L	F	5.5	-	-
9	F	F	5.7	-	-	11	L	F	5	-	-
9	F	F	5.1	-	-	11	L	F	5.3	-	-
9	F	F	5.7	-	-	11	L	F	5.1	-	-
9	F	F	4.8	-	-	11	L	F	5.4	-	-
9	F	F	5.3	-	-	11	L	F	5.3	-	-
9	F	F	5.6	-	-	11	L	F	5.4	-	-
9	F	F	5.6	-	-	11	L	F		-	-
9	F	F	5.2	-	-	11	L	F	5.2	-	-
9	F	F	5.2	-	-	11	L	F	5.6	-	-
9	F	F	5.5	-	-	11	L	F	5.5	-	-
9	F	F	5.6	-	-	11	L	F	5.5	-	-

9	F	F	5.2	-	-
9	F	F	5.3	-	-
9	F	F	5.8	-	-
9	F	F	5.6	-	-
9	F	F	5.4	-	-
9	F	F	5.6	-	-
9	F	F	5.1	-	-
9	F	F	5.3	-	-
9	F	F	5.7	-	-
9	F	F	5.5	-	-
9	F	F	5.7	-	-
9	F	F	6.1	-	-
9	F	F	5.4	-	-
9	F	F	5.6	-	-
9	F	F	5.5	-	-
9	F	F	5.4	-	-
9	F	F	5.1	-	-
9	F	F	5.2	-	-
9	F	F	5.4	-	-
9	F	F	5.2	-	-
9	F	F	5.2	-	-
9	F	F	5.8	-	-
9	F	F	5.2	-	-
9	F	F	5.2	-	-
9	F	F	5.5	-	-
9	F	F	5.4	-	-
9	F	F	5.6	-	-
9	F	F	5.7	-	-
9	F	F	5.1	-	-
9	F	F	5.7	-	-
9	F	F	5.5	-	-
9	F	F	5.4	-	-
9	F	F	5.4	-	-
9	F	F	5.6	-	-
9	F	F	5.1	-	-
9	F	F	5.7	-	-
9	F	F	5.7	-	-
9	F	F	5.4	-	-
9	F	F	5.4	-	-
9	F	F	5.1	-	-
9	F	F	5.6	-	-
9	F	F	5.6	-	-
9	F	F	5.5	-	-
9	F	F	5.1	-	-
9	F	F	5.4	10.8	BB
9	F	F	4.9	8.3	BB
9	F	F	5	9.1	BB
9	F	M	5.5	10.6	BD
9	F	F	5.5	13.6	BD
9	F	F	5	10	BD
9	F	M	5.9	15	BD
9	F	F	5.2	10.2	BD
9	F	F	5.4	11.8	BD
9	F	F	4.9	9.4	BD
11	L	F	5.7	-	-
11	L	F	5.7	-	-
11	L	M	6.8	-	-
11	L	M	6.6	-	-
11	L	M	5.2	-	-
11	L	M	6	-	-
11	L	M	5.4	-	-
11	L	M	6.5	-	-
11	L	M	6.7	-	-
11	L	M	6.2	-	-
11	L	M	6	-	-
11	L	M	6.9	-	-
11	L	M	6.1	-	-
11	L	M	6.7	-	-
11	L	M	6.2	-	-
11	L	M	6.7	-	-
11	L	M	6.5	-	-
11	L	M	4.8	-	-
11	L	F	5.8	-	-
11	L	F	5.8	-	-
11	L	F	5.6	-	-
11	L	F	5.5	-	-
11	L	F	5.2	-	-
11	L	F	5.8	-	-
11	L	F	5.6	-	-
11	L	F	5.2	-	-
11	L	F	5.3	-	-
11	L	F	5.5	-	-
11	L	F	5.7	-	-
11	L	F	5.4	-	-
11	L	F	5.7	-	-
11	L	F	5.1	-	-
11	L	F	5.4	-	-
11	L	M		-	-
11	L	M	6.4	-	-
11	L	M	6.2	-	-
11	L	M	4.7	-	-
11	L	M	5.6	-	-
11	L	M	6.4	-	-
11	L	M	4.9	-	-
11	L	M	6	-	-
11	L	M	6.6	-	-
11	L	M	6.1	-	-
11	L	M	6.8	-	-
11	L	M	6.1	-	-
11	L	M	6.6	-	-
11	L	F	5.5	-	-
11	L	F	5.2	-	-
11	L	F	5.4	-	-
11	L	F	5.3	-	-
11	L	F	5.5	-	-
11	L	F	5.3	-	-
11	L	F	5.7	-	-
11	L	F	5.2	-	-

9	F	M	5.8	13.6	BD	11	L	F	5.5	-	-
9	F	M	5.7	17.6	BD	11	L	F	5.3	-	-
9	F	F	5.4	10	BD	11	L	F	5.4	-	-
9	F	F	5.1	11.2	BD	11	L	F	5.1	-	-
9	F	F	4.9	10	BD	11	L	F	5.5	-	-
9	F	M	5.8	14.6	BD	11	L	F	5.6	-	-
9	F	M	5.7	11.6	BD	11	L	F	4.8	-	-
9	F	M	5.6	11.4	BD	11	L	F	5.9	-	-
9	F	F	5.3	8.4	BD	11	L	F	5.5	-	-
9	F	F	5.3	9.4	BD	11	L	F	5.1	-	-
9	F	M	4.6	7.2	CC	11	L	F	5.6	-	-
9	F	M	4.9	8.2	CD	11	L	F	5.1	-	-
9	F	F	5.4	10.4	CD	11	L	F	4.5	-	-
9	F	F	5.1	8.7	CD	11	L	F	5.5	-	-
9	F	M	4.8	8.3	DD	11	L	F	5.5	-	-
9	F	F	5.2	10.5	DD	11	L	F	5.6	-	-
9	F	F	5.1	10.5	DD	11	L	F	5.1	-	-
9	F	M	4.6	6	DD	11	L	F	5.6	-	-
9	F	M	4.6	6.2	DD	11	L	F	5.2	11	BB
9	F	M	4.9	8.5	DD	11	L	F		10.8	BB
9	F	F	5.6	9.3	DD	11	L	M	6.6	18.1	BB
9	F	F	5	9.7	DD	11	L	F	5.3	12.8	BB
9	F	M	5	9.4	DD	11	L	M	6.4	18.7	BB
9	F	M	4.2	4.2	DD	11	L	M	6.2	15.6	BB
9	F	M	4.7	7.4	DD	11	L	F	5.1	10.4	BB
9	F	F	4.7	6.6	DD	11	L	F	5	11.3	BC
9	F	M	4.9	8.4	DD	11	L	F	5.3	11.7	BC
9	F	M	4.3	5.3	DD	11	L	M	6.3	16.1	BD
9	F	M	5.6	10.6	X	11	L	M	6.4	19.3	BD
9	F	M	5.7	11.3	X	11	L	M	5.8	14.6	BD
9	L	M	5	-	-	11	L	F	5.4	13.1	BD
9	L	M	5	-	-	11	L	M		20.1	BD
9	L	M	5.1	-	-	11	L	M	6.1	14.8	BD
9	L	M	6.3	-	-	11	L	M	6.3	18.3	BD
9	L	M	5.3	-	-	11	L	F	5.2	9.9	BD
9	L	M	5.2	-	-	11	L	F	5.2	10.4	BD
9	L	M	5.1	-	-	11	L	F	4.9	11.2	BD
9	L	M	5.3	-	-	11	L	M	6.1	16.7	BD
9	L	F	5.4	-	-	11	L	M	6.4	18.3	BD
9	L	F	5	-	-	11	L	M	6.3	21.6	BD
9	L	F	5.4	-	-	11	L	M	6.1	16.6	BD
9	L	F	5.6	-	-	11	L	F	5.5	12.9	BD
9	L	F	5.2	-	-	11	L	F		10.7	BD
9	L	F	5.5	-	-	11	L	F	5.1	10.9	BD
9	L	F	5.7	-	-	11	L	F	5.3	12.1	BD
9	L	F	5.8	-	-	11	L	M	6.8	21.4	BD
9	L	F	5.7	-	-	11	L	M	6.3	17.1	BD
9	L	F	5.2	-	-	11	L	F	5.7	15.4	BD
9	L	F	5.1	-	-	11	L	F	5.3	10.4	BD
9	L	F	5.6	-	-	11	L	F	5.4	10.8	BD
9	L	F	5.2	-	-	11	L	M	4.8	6.6	CC
9	L	F	5.2	-	-	11	L	F	5	7.9	CD
9	L	F	5.5	-	-	11	L	M	6.2	19.1	CD
9	L	F	5.2	-	-	11	L	F	5.5	13.9	CD

9	L	F	5.4	-	-	11	L	M	4.5	8.4	DD
9	L	F	5.6	-	-	11	L	M	4.4	5.9	DD
9	L	F	5.1	-	-	11	L	M	5.4	9.5	X
9	L	F	5.3	-	-	11	L	F	5.5	11.9	X
9	L	F	5.1	-	-	12	F	M	6.9	-	-
9	L	F	5.4	-	-	12	F	M	7	-	-
9	L	F	5.2	-	-	12	F	M	7.3	-	-
9	L	F	5.6	-	-	12	F	M	5.3	-	-
9	L	F	5.6	-	-	12	F	M	7.4	-	-
9	L	F	5.6	-	-	12	F	M	5.8	-	-
9	L	F	5.6	-	-	12	F	M	6.5	-	-
9	L	F	5.5	-	-	12	F	M	5.5	-	-
9	L	F	5.4	-	-	12	F	M	6.3	-	-
9	L	F	5.2	-	-	12	F	M	6.8	-	-
9	L	F	5.1	-	-	12	F	M	5.3	-	-
9	L	F	5.2	-	-	12	F	M	6.3	-	-
9	L	F	5.3	-	-	12	F	M	6.6	-	-
9	L	F	5.1	-	-	12	F	M	5.6	-	-
9	L	F	5	-	-	12	F	M	5.9	-	-
9	L	F	5.3	-	-	12	F	M	7	-	-
9	L	F	5.3	-	-	12	F	M	5.9	-	-
9	L	F	5.1	-	-	12	F	M	5.2	-	-
9	L	F	5.4	-	-	12	F	M	6.2	16	BB
9	L	F	5.4	-	-	12	F	M	6.5	22.3	BB
9	L	F	5.4	-	-	12	F	M	6.4	16.3	BB
9	L	F	5.4	-	-	12	F	M	6.4	16.3	BB
9	L	F	5.2	-	-	12	F	M	5.9	13.5	BB
9	L	F	5	-	-	12	F	M	7	25.4	BC
9	L	F	5.1	-	-	12	F	M	6.9	22.3	BC
9	L	F	5.4	-	-	12	F	M	5.6	12.6	BD
9	L	F	5.1	-	-	12	F	M	5.6	10	BD
9	L	F	5.6	-	-	12	F	M	6	14.2	BD
9	L	F	5.3	-	-	12	F	M	5.8	14.5	BD
9	L	F	5.3	-	-	12	F	M	5.6	11.8	BD
9	L	F	5.4	-	-	12	F	M	5.8	13.2	BD
9	L	F	5	-	-	12	F	M	6.1	14.8	BD
9	L	F	5.3	-	-	12	F	M	5.2	10	BD
9	L	F	5.7	-	-	12	F	F	4.9	10	BD
9	L	F	5.5	-	-	12	F	F	5.1	7.3	BD
9	L	F	5.5	-	-	12	F	M	5.7	14.5	CC
9	L	F	5.6	-	-	12	F	M	6.2	18.1	DD
9	L	F	5.3	-	-	12	F	M	5.4	12	DD
9	L	F	5.1	-	-	12	F	M	5.9	16.7	DD
9	L	F	4.9	-	-	12	F	F	5.2	9.1	DD
9	L	F	5.3	-	-	12	F	M	3.9	3.5	X
9	L	M	5.3	-	-	12	L	M	6.3	-	-
9	L	M	5	-	-	12	L	M	7.1	-	-
9	L	F	5.5	-	-	12	L	M	6.1	-	-
9	L	F	5.6	-	-	12	L	M	6.7	-	-
9	L	F	5.4	-	-	12	L	M	6	-	-
9	L	F	5.2	-	-	12	L	M	6.5	-	-
9	L	F	5.3	-	-	12	L	M		-	-
9	L	F	5.7	-	-	12	L	M	6.1	-	-
9	L	F	5.9	-	-	12	L	M	7.2	-	-

9	L	F	5.3	-	-	12	L	M	6.9	-	-
9	L	F	5.3	-	-	12	L	M	6.9	-	-
9	L	F		-	-	12	L	M	6.7	-	-
9	L	F	5.3	-	-	12	L	M		-	-
9	L	F	5.4	-	-	12	L	M	7	-	-
9	L	F	5.5	-	-	12	L	M	6.3	-	-
9	L	F	5.2	-	-	12	L	M	6.5	-	-
9	L	F	5.3	-	-	12	L	M	6.5	-	-
9	L	F	5.8	-	-	12	L	M	6.4	-	-
9	L	F	5.2	-	-	12	L	M	7.3	-	-
9	L	F	5.4	-	-	12	L	M	6.7	-	-
9	L	F	5.3	-	-	12	L	M	5.3	-	-
9	L	F	5.4	-	-	12	L	M	7.6	-	-
9	L	F	5.6	-	-	12	L	F	6	-	-
9	L	F	5.6	-	-	12	L	F	5.2	-	-
9	L	F	5.7	-	-	12	L	F	5.5	-	-
9	L	F	5.7	-	-	12	L	F	5.7	-	-
9	L	F	5.8	-	-	12	L	F		-	-
9	L	F	5.6	-	-	12	L	F	4.2	-	-
9	L	F	5.2	-	-	12	L	F	5.3	-	-
9	L	F	5.3	-	-	12	L	F	4.8	-	-
9	L	F	5.5	-	-	12	L	M	6.2	-	-
9	L	F	5.6	-	-	12	L	M	6.1	-	-
9	L	F	5.3	-	-	12	L	M	6.9	-	-
9	L	F	5.7	-	-	12	L	M	7.1	-	-
9	L	F	5.7	-	-	12	L	M	6.9	-	-
9	L	F	5.8	-	-	12	L	M	6.1	-	-
9	L	F	5.4	-	-	12	L	M	6.4	-	-
9	L	F	5.7	-	-	12	L	M	5.8	-	-
9	L	F	5.7	-	-	12	L	M	6.8	-	-
9	L	F	5.5	-	-	12	L	M	6.7	-	-
9	L	F	5.5	-	-	12	L	M	6.8	-	-
9	L	F	5.4	-	-	12	L	M	7.1	-	-
9	L	F	5.6	-	-	12	L	M	6.5	-	-
9	L	F	5.3	-	-	12	L	M	7.4	-	-
9	L	F	5.5	-	-	12	L	M	6	-	-
9	L	F	5.2	-	-	12	L	M	6.8	-	-
9	L	F	5.7	-	-	12	L	M	5.5	-	-
9	L	F	5.3	-	-	12	L	M	4.9	-	-
9	L	F	5.5	-	-	12	L	M	7.3	-	-
9	L	F	5.3	-	-	12	L	M	6.9	-	-
9	L	F	5.6	-	-	12	L	M	6.5	-	-
9	L	F	5.8	-	-	12	L	M	7.2	-	-
9	L	F	5.3	-	-	12	L	M	6.6	-	-
9	L	F	5.4	-	-	12	L	M	7.3	-	-
9	L	F	5.1	-	-	12	L	M	7	-	-
9	L	F	5.6	-	-	12	L	M	6.6	-	-
9	L	F	4.6	-	-	12	L	M	5.3	-	-
9	L	F	5.6	-	-	12	L	M	7.1	-	-
9	L	F	5.6	-	-	12	L	M	5.1	-	-
9	L	F	5.4	-	-	12	L	M	6.9	-	-
9	L	F	5.2	-	-	12	L	F	5.3	-	-
9	L	F	5.6	-	-	12	L	F	5.8	-	-
9	L	F	5.6	-	-	12	L	F	5.2	-	-

9	L	F	5.6	-	-
9	L	F	5.8	-	-
9	L	F	5.3	-	-
9	L	F	5	9.9	BB
9	L	F	5.2	10.7	BB
9	L	F	5.3	9.6	BB
9	L	M	4.7	7.3	BC
9	L	F	5.2	10.2	BD
9	L	F	4.7	7.9	BD
9	L	F	4.9	8.8	BD
9	L	F	5.1	10.5	BD
9	L	F	5	8.6	BD
9	L	F	5.2	10.3	BD
9	L	F	5.4	11.3	BD
9	L	F	5.1	8.7	BD
9	L	M	4.8	8.7	CD
9	L	F	5.1	9.7	DD
9	L	M	4.8	7.3	DD
9	L	M	4.9	7.7	DD
9	L	M	4.8	7.7	DD
9	L	F	5.1	9.4	DD
9	L	M	5.2	8.4	DD
9	L	M	4.8	7.6	DD
9	L	M	5	8.7	DD
9	L	M	4.9	7	DD
9	L	F	5	9	DD
9	L	F	4.6	9.1	DD
9	L	M	4.5	5.9	DD
9	L	M	4.5	6.6	DD
9	L	M		7.1	DD
9	L	M	4.8	6.7	DD
9	L	M	5.1	8.8	DD
9	L	F	5.1	10.1	DD
9	L	M	4.7	10.4	X
9	L	M	4.8	8.3	X
9	L	M	4.4	5.5	X
9	L	M	4.6	6.4	X
9	L	M	4.6	6.2	X
9	L	F	5.1	10.7	X
9	L	F	4.7	10.2	X
9	L	F	5.2	10.7	X
9	L	F	5.1	9.9	X
10	F	M	6.3	-	-
10	F	M	6.9	-	-
10	F	M	7	-	-
10	F	M	5.7	-	-
10	F	M	4.7	-	-
10	F	M	5.9	-	-
10	F	M	4.6	-	-
10	F	M	6.2	-	-
10	F	M	6.1	-	-
10	F	M	6.7	-	-
10	F	M	6.3	-	-
10	F	M	7.1	-	-
12	L	F	5.5	-	-
12	L	F	5.7	-	-
12	L	F	5.2	-	-
12	L	F	5.3	-	-
12	L	F	5.3	-	-
12	L	F	5.6	-	-
12	L	M	6.8	-	-
12	L	M	6.5	-	-
12	L	M	6.3	-	-
12	L	M	6.6	-	-
12	L	M	6.7	-	-
12	L	M	6.2	-	-
12	L	M	6.6	-	-
12	L	M	6.7	-	-
12	L	M	4.7	-	-
12	L	M	6.9	-	-
12	L	M	7	-	-
12	L	M	5	-	-
12	L	M	7.4	-	-
12	L	M	7	-	-
12	L	M		-	-
12	L	M	5.5	-	-
12	L	M	6.6	-	-
12	L	M	5.7	-	-
12	L	M	6.4	-	-
12	L	M	6.6	-	-
12	L	M	5.6	-	-
12	L	M	6.5	-	-
12	L	M	6.5	-	-
12	L	M	6.1	-	-
12	L	M	6.2	-	-
12	L	M	4.6	-	-
12	L	M	6.7	-	-
12	L	M	6.8	-	-
12	L	M	6.8	-	-
12	L	M		-	-
12	L	M	6.8	-	-
12	L	F	6	-	-
12	L	F	5.4	-	-
12	L	F	5.5	-	-
12	L	F	5.3	-	-
12	L	F	5.6	-	-
12	L	F	5.4	-	-
12	L	F	5.7	-	-
12	L	F	5.8	-	-
12	L	F	6.2	-	-
12	L	F	5.2	-	-
12	L	F	5.3	-	-
12	L	F	5.9	-	-
12	L	F	5.1	-	-
12	L	F	5.6	-	-
12	L	F	5	-	-
12	L	F	6.1	-	-
12	L	F	5.7	-	-

10	F	M	4.9	-	-	12	L	F	5.5	-	-
10	F	M	7	-	-	12	L	M	5.5	-	-
10	F	M	5.3	-	-	12	L	M	6.7	-	-
10	F	M	5.7	-	-	12	L	M	6.5	-	-
10	F	M	6.2	-	-	12	L	M	6.8	-	-
10	F	M	5.7	-	-	12	L	M	6.3	-	-
10	F	M	5.8	-	-	12	L	M	6.4	-	-
10	F	M	6.1	-	-	12	L	M	6.8	-	-
10	F	M	4.7	-	-	12	L	M	6.8	-	-
10	F	M	6.7	-	-	12	L	M	5.1	-	-
10	F	M	6.2	-	-	12	L	M	6.6	-	-
10	F	M	6.5	-	-	12	L	M	6.9	-	-
10	F	M	6.6	-	-	12	L	M	5	-	-
10	F	M	5.3	-	-	12	L	M	6	-	-
10	F	M	6.2	-	-	12	L	M	6.4	-	-
10	F	M	7	-	-	12	L	M	6.6	-	-
10	F	M	5.9	-	-	12	L	F	5.3	-	-
10	F	M	6.6	-	-	12	L	F	5.4	-	-
10	F	M	4.8	-	-	12	L	F	5.5	-	-
10	F	M	5.2	-	-	12	L	F	5.5	-	-
10	F	F	5.5	-	-	12	L	F	5.5	-	-
10	F	F	5.6	-	-	12	L	F	5.6	-	-
10	F	F		-	-	12	L	F	5.2	-	-
10	F	F	4.7	-	-	12	L	F	5.3	-	-
10	F	F	5.4	-	-	12	L	F	5.4	-	-
10	F	F	5.4	-	-	12	L	F	5.9	-	-
10	F	F	5.5	-	-	12	L	F	5.2	-	-
10	F	F	5.7	-	-	12	L	F	5.4	-	-
10	F	F	5.4	-	-	12	L	F	5.8	-	-
10	F	F	5.4	-	-	12	L	F	5.8	-	-
10	F	F	5.4	-	-	12	L	F	5.8	-	-
10	F	F	4.7	-	-	12	L	F	5.3	-	-
10	F	F	5.1	-	-	12	L	F	5.9	-	-
10	F	F	5.6	-	-	12	L	F	5.4	-	-
10	F	F	5.1	-	-	12	L	F	5.5	-	-
10	F	F	5.3	-	-	12	L	F	5.1	10.6	AD
10	F	F	5.7	-	-	12	L	M	6	17.5	BB
10	F	F	5.2	-	-	12	L	M	6.4	19.4	BB
10	F	F	5.4	-	-	12	L	M	6.3	16.6	BB
10	F	F	5.6	-	-	12	L	M	6.3	20.8	BB
10	F	F	4.8	-	-	12	L	M	6.3	17.4	BB
10	F	F	5.1	-	-	12	L	F	5.1	9.1	BB
10	F	F	5.4	-	-	12	L	F	5.5	12.2	BB
10	F	F	4.9	-	-	12	L	M	6.3	19.7	BB
10	F	F	5	-	-	12	L	M	6.4	19.6	BB
10	F	F	5.4	-	-	12	L	F	5.6	14.9	BB
10	F	M	5.7	-	-	12	L	M	6	19	BB
10	F	M	6.6	-	-	12	L	F	5	8.8	BB
10	F	M	6.6	-	-	12	L	M	6.6	19.1	BB
10	F	M	5.5	-	-	12	L	M	6.6	19.8	BB
10	F	M	6.2	-	-	12	L	M	6.6	17.8	BB
10	F	M	6.3	-	-	12	L	F	5.2	9.2	BC
10	F	M	5	-	-	12	L	M	7	22	BC
10	F	M	5	-	-	12	L	M	6.5	21.5	BC

10	F	M	5.9	-	-
10	F	M	4.9	-	-
10	F	M	6.1	-	-
10	F	M	5.2	-	-
10	F	M		-	-
10	F	M	6.8	-	-
10	F	M	5.2	-	-
10	F	M	5.7	-	-
10	F	M	6.6	-	-
10	F	M	6.3	-	-
10	F	M	6.3	-	-
10	F	M	6.5	-	-
10	F	M	5.8	-	-
10	F	M	6.5	-	-
10	F	M	6.5	-	-
10	F	M	6.3	-	-
10	F	M	6.4	-	-
10	F	M	6.4	-	-
10	F	M	5.8	-	-
10	F	M	7	-	-
10	F	M	6.1	-	-
10	F	M	6.2	-	-
10	F	M	6.3	-	-
10	F	M	5.7	-	-
10	F	M	6.6	-	-
10	F	M	6.7	-	-
10	F	M	6	-	-
10	F	M	5.6	-	-
10	F	M	5.4	-	-
10	F	M	6.7	-	-
10	F	M	6.8	-	-
10	F	M	6.9	-	-
10	F	M	6.5	-	-
10	F	M	6.1	-	-
10	F	F	5.4	-	-
10	F	F	4.9	-	-
10	F	F	5.2	-	-
10	F	F	5.3	-	-
10	F	F	5.8	-	-
10	F	F	5.1	-	-
10	F	F	5.2	-	-
10	F	F	5.5	-	-
10	F	F	5.3	-	-
10	F	F	4.6	-	-
10	F	F	5.1	-	-
10	F	F	5.2	-	-
10	F	F	5.5	-	-
10	F	F	5.6	-	-
10	F	F	5.6	-	-
10	F	F	5.6	-	-
10	F	F	5.4	-	-
10	F	F	5.4	-	-
10	F	F	5.6	-	-
10	F	M		-	-
12	L	M	6.4	19.5	BD
12	L	M	6.2	18.4	BD
12	L	F	5.1	12.1	BD
12	L	F	5.1	9.3	BD
12	L	M	5.9	17.8	BD
12	L	M	6.5	19.5	BD
12	L	F	5.3	10.8	BD
12	L	M	5.5	17.1	BD
12	L	M	5.9	16.6	BD
12	L	F	5.8	11.6	BD
12	L	F	5.4	11.8	BD
12	L	F	5.1	13.4	BD
12	L	F	4.9	8.4	CD
12	L	F	4.9	10.7	CD
12	L	F	5.3	12.5	DD
12	L	F	5.4	12.5	DD
12	L	F	4.9	7.7	DD
12	L	M	5	7.5	DD
12	L	F	5.4	11.9	DD
12	L	F	5.4	10.7	DD
12	L	F	5.5	13.2	DD
13	F	M	6	-	-
13	F	M	6.4	-	-
13	F	M	6	-	-
13	F	M	6.3	22.4	BB
13	F	M	6.1	18.9	BB
13	F	M	6.5	21.3	BB
13	F	M	6	18.3	BB
13	F	M	6.8	20.3	BB
13	F	M	6.3	23.1	BB
13	F	M	5.4	10.9	BB
13	F	M	6	18.8	BD
13	F	M	5.8	11.8	BD
13	F	M	6.1	17	BD
13	F	M	5.6	15.5	BD
13	F	M	6.3	20.4	CD
13	F	M		11.9	X
13	F	M	5.7	12.3	X
13	L	M	6.8	-	-
13	L	M	6.5	-	-
13	L	M	6.5	-	-
13	L	M	7.3	-	-
13	L	M	7.1	-	-
13	L	M	6.8	-	-
13	L	M	6.4	-	-
13	L	F	5.1	-	-
13	L	F	5.3	-	-
13	L	F	5.6	-	-
13	L	F	5.6	-	-
13	L	M	6.9	-	-
13	L	M	6.6	-	-
13	L	M	5.4	-	-
13	L	M		-	-
13	L	M	5.8	-	-

10	F	M	6.2	-	-
10	F	M	6.3	-	-
10	F	M	6.3	-	-
10	F	M	5.7	-	-
10	F	M	5.7	-	-
10	F	M	5.1	-	-
10	F	M	5	-	-
10	F	M	5.5	-	-
10	F	M	5.9	-	-
10	F	M	4.8	-	-
10	F	M	5.4	-	-
10	F	M	6.8	-	-
10	F	M	5.1	-	-
10	F	M	5.4	-	-
10	F	M	6.1	-	-
10	F	M		-	-
10	F	M		-	-
10	F	M	5.4	-	-
10	F	M	5.4	-	-
10	F	M	6	-	-
10	F	M	5.9	-	-
10	F	M	5.6	-	-
10	F	F	5	-	-
10	F	F	5.2	-	-
10	F	F	4.7	-	-
10	F	F	5.2	-	-
10	F	F	5.3	-	-
10	F	F	5	-	-
10	F	F	5.9	-	-
10	F	F	5.9	-	-
10	F	F	5.5	-	-
10	F	F	5.8	-	-
10	F	F	5.4	-	-
10	F	F	5.3	-	-
10	F	F	5.9	-	-
10	F	F	5.3	-	-
10	F	F	5.3	-	-
10	F	F	5.4	-	-
10	F	F	5.4	-	-
10	F	F	5.7	-	-
10	F	F	5.4	-	-
10	F	F	5.3	-	-
10	F	F	5.2	-	-
10	F	M	6.2	-	-
10	F	M	5.7	-	-
10	F	M	7.1	-	-
10	F	M	6	-	-
10	F	M	6.1	-	-
10	F	M	5.7	-	-
10	F	M	6.2	-	-
10	F	M	5.8	-	-
10	F	M		-	-
10	F	M	6.1	-	-
10	F	M	6.3	-	-
13	L	M	7.2	-	-
13	L	M	7.2	-	-
13	L	M	6.8	-	-
13	L	M	4.8	-	-
13	L	M	7.5	-	-
13	L	M	7.6	-	-
13	L	M	6.6	-	-
13	L	M	6.1	-	-
13	L	M	6.5	-	-
13	L	M	7.2	-	-
13	L	F		-	-
13	L	F	4.8	-	-
13	L	F	5.2	-	-
13	L	F	5.3	-	-
13	L	F	5.3	-	-
13	L	F	5.6	-	-
13	L	M	7.5	-	-
13	L	M	7.5	-	-
13	L	M	6.8	-	-
13	L	M	6.6	-	-
13	L	M	6.3	-	-
13	L	M	5.1	-	-
13	L	M	7.2	-	-
13	L	M	6.1	-	-
13	L	M	7.2	-	-
13	L	M	7.1	-	-
13	L	M	7.6	-	-
13	L	M	6.5	-	-
13	L	M	6.2	-	-
13	L	M	6.7	-	-
13	L	M	7.1	-	-
13	L	M	6.8	-	-
13	L	M	7.7	-	-
13	L	M	7.1	-	-
13	L	M	6.6	-	-
13	L	M	6.8	-	-
13	L	M	7.2	-	-
13	L	M	7	-	-
13	L	M	4.8	-	-
13	L	M	6.7	-	-
13	L	M	7.2	-	-
13	L	M	6.5	-	-
13	L	M	6.7	-	-
13	L	M	7.3	-	-
13	L	M	5	-	-
13	L	M	7	-	-
13	L	M	5.8	-	-
13	L	M	6.4	-	-
13	L	M	7.3	-	-
13	L	M	7.1	-	-
13	L	M	7	-	-
13	L	M	6.9	-	-
13	L	M	7.3	-	-
13	L	M	6.9	-	-

10	F	M	5.9	-	-	13	L	M	6.1	-	-
10	F	M	5.4	-	-	13	L	M	6.2	-	-
10	F	M	5.9	-	-	13	L	M	6.9	-	-
10	F	M	5.6	-	-	13	L	M	7.1	-	-
10	F	M	6.2	-	-	13	L	M	6.6	-	-
10	F	M	6.9	-	-	13	L	M	7.5	-	-
10	F	M		-	-	13	L	M	4.6	-	-
10	F	M	5.8	-	-	13	L	M	6.8	-	-
10	F	M	5.6	-	-	13	L	M	6.8	-	-
10	F	M	6.5	-	-	13	L	M	7.4	-	-
10	F	M	6.2	-	-	13	L	M	7.1	-	-
10	F	M	6.6	-	-	13	L	M	6.3	-	-
10	F	M	5.7	-	-	13	L	M	5	-	-
10	F	M	5.8	-	-	13	L	M	7.2	-	-
10	F	M	5.8	-	-	13	L	F	5.3	-	-
10	F	M	6.3	-	-	13	L	F	4.6	-	-
10	F	M	3.9	-	-	13	L	F	5.6	-	-
10	F	M	5.7	-	-	13	L	M	7.1	24	BB
10	F	M	5.9	-	-	13	L	M	6.6	22.4	BB
10	F	M	6.6	-	-	13	L	M	6.5	20.2	BB
10	F	M	5.9	-	-	13	L	M	6.9	27.9	BB
10	F	M	4.8	-	-	13	L	M	6.6	29.3	BB
10	F	M	5.3	-	-	13	L	M	7.1	29.1	BB
10	F	M	5.8	-	-	13	L	M	6.8	22	BB
10	F	M	6.2	-	-	13	L	F	5	9.1	BB
10	F	M	4.8	-	-	13	L	F	5.3	9.1	BB
10	F	M	6.2	-	-	13	L	M	7.1	27.1	BB
10	F	M	6.8	-	-	13	L	M	7.2	25.1	BB
10	F	M	5.1	-	-	13	L	M	6.7	20.9	BB
10	F	M	6.5	-	-	13	L	F	5.2	11.2	BB
10	F	F	5.4	-	-	13	L	F	5.3	10.8	BB
10	F	F	5.6	-	-	13	L	M	6.4	21.8	BB
10	F	F	5.3	-	-	13	L	M	5.8	11.9	BB
10	F	F	5.4	-	-	13	L	M	6.6	21.7	BB
10	F	F	5.6	-	-	13	L	F	5.2	11.4	BB
10	F	F		-	-	13	L	F	4.7	6.7	BB
10	F	F	5.5	-	-	13	L	M	6.6	22.2	BC
10	F	F	5.2	-	-	13	L	F	4.9	8.8	BD
10	F	F	5.6	-	-	13	L	F	5.2	11.7	BD
10	F	F	5.3	-	-	13	L	F	5.1	11.6	BD
10	F	F	4.5	-	-	13	L	M	6.9	25.4	BD
10	F	F	5.3	-	-	13	L	M	6.3	20.3	BD
10	F	F	5.3	-	-	13	L	F	4.6	9.7	BD
10	F	F	5.1	-	-	13	L	F	5.2	9.8	CD
10	F	F	5.4	-	-	13	L	F	4.6	11.1	DD
10	F	F	5.4	-	-	13	L	F	5.1	8	DD
10	F	F	5.2	-	-	13	L	F	5.3	11	DD
10	F	F	4.3	-	-	13	L	F	5	9.9	DD
10	F	F	5.3	-	-	13	L	F	4.7	8.2	DD
10	F	F	5.4	-	-	13	L	F	5	8.9	DD
10	F	F	5.1	-	-	13	L	M	6.5	23.3	X
10	F	F	5.6	-	-	13	L	F	5.2	12.9	X
10	F	F	4.8	-	-	13	L	F	5	12.7	X
10	F	F	5.3	-	-	13	L	M	6.9	26.9	X

10	F	F	5.7	-	-
10	F	M	6.3	16.8	BB
10	F	F	5.2	9.4	BB
10	F	F	5.9	15.2	BB
10	F	F	4.8	10	BB
10	F	M	6.2	15.7	BB
10	F	M	6.7	18.8	BB
10	F	F	5.1	9.9	BB
10	F	F	5.4	11.2	BB
10	F	M	5.8	14.2	BC
10	F	M	5.6	12.8	BD
10	F	M	5.3	10.4	BD
10	F	M	5.5	11.6	BD
10	F	F	5.3	9.9	BD
10	F	F	4.9	8.9	BD
10	F	F	4.8	8.8	BD
10	F	M	5.9	16	BD
10	F	M	5.9	15.7	BD
10	F	M	5.9	15.8	BD
10	F	M	5.4	10	BD
10	F	M	6	14.7	BD
10	F	M	6.1	14.8	BD
10	F	F	5.3	10.3	BD
10	F	F	5.2	10.1	BD
10	F	M	5.8	14.5	BD
10	F	M	5.8	14.3	BD
10	F	M	5.9	14.4	BD
10	F	F	5.1	9	BD
10	F	F	5.1	8.6	BD
10	F	F	4.8	8.8	CD
10	F	F	5.1	10.7	CD
10	F	F	5	9.7	DD
10	F	M	4.8	9.8	DD
10	F	M	4.7	6.7	DD
10	F	F	4.8	6.9	DD
10	F	F	5	8.4	DD
10	F	F	4.9	9.8	DD
10	F	M	4.9	8.8	X
10	F	M	5.8	14	X
10	F	F	5.2	11.5	X
10	F	F	4.5	4.6	X
10	L	M	6.2	-	-
10	L	M		-	-
10	L	M	6.5	-	-
10	L	M	6.2	-	-
10	L	M	5.2	-	-
10	L	M	6.4	-	-
10	L	M	6.2	-	-
10	L	M	6.5	-	-
10	L	M		-	-
10	L	M	6.8	-	-
10	L	M	5.8	-	-
10	L	M		-	-
10	L	M	5.7	-	-
13	L	M	7	29.6	X
13	L	M	6.8	24.3	X
14	F	M	6.1	16.9	BB
14	F	M	6.1	15.3	BB
14	F	F		3.2	DD
14	F	M	6.2	15.5	X
14	F	M	4.6	8.2	X
14	L	M	7.1	-	-
14	L	M	6.4	-	-
14	L	M	6.1	-	-
14	L	M	6.9	-	-
14	L	M	6.4	-	-
14	L	M	7.3	-	-
14	L	M	6.7	-	-
14	L	M	6.5	-	-
14	L	M	6.9	-	-
14	L	M	7.1	-	-
14	L	M	7.3	-	-
14	L	M	7.5	-	-
14	L	M	6.9	-	-
14	L	M	5.3	-	-
14	L	M	7.3	-	-
14	L	M	7.4	-	-
14	L	M	6.6	-	-
14	L	M	6.3	-	-
14	L	M	6.7	-	-
14	L	M	7	-	-
14	L	M	6.3	-	-
14	L	M	6.6	-	-
14	L	M	7.1	-	-
14	L	M	7.4	-	-
14	L	M	7.1	-	-
14	L	M	7.4	-	-
14	L	M	7.2	-	-
14	L	M	6.6	-	-
14	L	M	6.8	-	-
14	L	M	6.8	-	-
14	L	M	5.9	-	-
14	L	M	7.1	-	-
14	L	M	6.5	-	-
14	L	M	6.5	-	-
14	L	M	6.1	-	-
14	L	M	7.3	-	-
14	L	M	6.6	-	-
14	L	M	6.2	-	-
14	L	M	6.6	-	-
14	L	M	6.8	-	-
14	L	M	7.5	-	-
14	L	M	7.1	-	-
14	L	M	6.7	-	-
14	L	M	6.7	-	-
14	L	M		-	-
14	L	M	6.4	-	-
14	L	M	6.8	-	-

10	L	M	6.3	-	-
10	L	F	5.5	-	-
10	L	F		-	-
10	L	F	5.2	-	-
10	L	F	5.1	-	-
10	L	F	5.2	-	-
10	L	F	5.4	-	-
10	L	F	5.7	-	-
10	L	F	5.9	-	-
10	L	F	5.2	-	-
10	L	F	5.7	-	-
10	L	F	5.8	-	-
10	L	F	5.5	-	-
10	L	F	5.5	-	-
10	L	F	5.6	-	-
10	L	F	5.3	-	-
10	L	F	5.8	-	-
10	L	F	5.6	-	-
10	L	F	5.8	-	-
10	L	F	5.5	-	-
10	L	F		-	-
10	L	F	5.6	-	-
10	L	F		-	-
10	L	F	5.2	-	-
10	L	F	5.6	-	-
10	L	F	5.3	-	-
10	L	F	5.5	-	-
10	L	F		-	-
10	L	F		-	-
10	L	F		-	-
10	L	F	5.6	-	-
10	L	F	5.6	-	-
10	L	F	5.6	-	-
10	L	F		-	-
10	L	F	5.4	-	-
10	L	F	5.8	-	-
10	L	F	5.6	-	-
10	L	F	5.2	-	-
10	L	F	5.4	-	-
10	L	F	5.4	-	-
10	L	F	5.2	-	-
10	L	F	5.1	-	-
10	L	F	5.5	-	-
10	L	F	5.6	-	-
10	L	M	5.4	-	-
10	L	M	5.8	-	-
10	L	M	6.3	-	-
10	L	M	5.1	-	-
10	L	M	6.1	-	-
10	L	M	6.3	-	-
10	L	M	5.6	-	-
10	L	M	6.1	-	-
10	L	M	6.5	-	-
10	L	F	5.3	-	-
14	L	M	6.8	-	-
14	L	M	6.3	-	-
14	L	M	7	-	-
14	L	M	6.7	-	-
14	L	M	6.8	-	-
14	L	M	6.4	-	-
14	L	M	6.5	-	-
14	L	M		-	-
14	L	M	7.3	-	-
14	L	M	6.4	-	-
14	L	M	4.5	-	-
14	L	M	7	-	-
14	L	M	7.1	-	-
14	L	M	6.9	-	-
14	L	M	7.1	-	-
14	L	M	7.2	-	-
14	L	M	7.7	-	-
14	L	M	5.7	-	-
14	L	M	6.2	-	-
14	L	M	6.5	-	-
14	L	M	6	-	-
14	L	M	6.9	-	-
14	L	M	6.2	-	-
14	L	M	6.5	-	-
14	L	M	7	-	-
14	L	M		-	-
14	L	M	6.4	-	-
14	L	M	6.8	-	-
14	L	M	4.6	-	-
14	L	M	7.3	-	-
14	L	M	6.1	-	-
14	L	M	7.2	-	-
14	L	M	7.4	-	-
14	L	M	6.1	-	-
14	L	M	7.3	-	-
14	L	M	6.4	-	-
14	L	M	6.7	-	-
14	L	M	6.9	-	-
14	L	M	6.5	-	-
14	L	M	6.7	24.1	BB
14	L	M	6.6	27	BB
14	L	M	6.9	26.7	BB
14	L	M	7	29.2	BB
14	L	M	6.4	22	BB
14	L	M	6.6	24.2	BB
14	L	M	6.8	24.4	BB
14	L	M	7.2	28.7	BB
14	L	M	7	30.9	BB
14	L	M	6.3	21.1	BB
14	L	M	6.9	22.7	BC
14	L	M	7.4	31.8	BC
14	L	M	7.1	26.9	BC
14	L	F	4.9	9.6	BD
14	L	F	4.8	10.2	BD

10	L	F	5.5	-	-	14	L	F	5.6	10.4	BD
10	L	F	5.6	-	-	14	L	F	4.6	6.5	BD
10	L	F	5.3	-	-	14	L	F	4.7	11.4	BD
10	L	F	5.4	-	-	14	L	M	6.5	23.4	BD
10	L	F	5.6	-	-	14	L	M	6.2	18.2	BD
10	L	F	5.5	-	-	14	L	M	5.3	12	BD
10	L	F	5.6	-	-	14	L	F	4.9	10.8	BD
10	L	F	5.7	-	-	14	L	M	6.7	28.2	CC
10	L	F	5.6	-	-	14	L	F	4.8	10.3	DD
10	L	F	5.6	-	-	14	L	F	4.9	9.7	DD
10	L	F	5.7	-	-	14	L	F	4.9	8.7	DD
10	L	F	5.4	-	-	14	L	M	6.2	17.5	X
10	L	F	5.5	-	-	14	L	M	6.4	20.6	X
10	L	F	5.5	-	-	14	L	M	7.2	26.1	X
10	L	F		-	-	14	L	F	5.4	11.6	X
10	L	F	5.4	-	-	15	F	M	7.1	29.6	BB
10	L	F	5.5	-	-	15	L	M	7	-	-
10	L	F	5.5	-	-	15	L	M	7.5	-	-
10	L	F	5.5	-	-	15	L	M	6.2	-	-
10	L	F	5.5	-	-	15	L	M	6.5	-	-
10	L	F	5.4	-	-	15	L	M	6.8	-	-
10	L	F	5.4	-	-	15	L	M	7.4	-	-
10	L	F	5.5	-	-	15	L	M	7.2	-	-
10	L	F	5.4	-	-	15	L	M	7	-	-
10	L	F	5.6	-	-	15	L	M	6.3	-	-
10	L	F	5.7	-	-	15	L	M	7.4	-	-
10	L	F	5.2	-	-	15	L	M	7.5	-	-
10	L	F	5.6	-	-	15	L	M	6.9	-	-
10	L	F	5.9	-	-	15	L	M	6.2	-	-
10	L	F	5.3	-	-	15	L	M	6.6	-	-
10	L	F	5.1	-	-	15	L	M	7.1	-	-
10	L	M	5.3	-	-	15	L	M	7	-	-
10	L	M	5.3	-	-	15	L	M	7.2	-	-
10	L	M	5.5	-	-	15	L	M	6.6	-	-
10	L	M		-	-	15	L	M	7.9	-	-
10	L	M	5.2	-	-	15	L	M	6.8	-	-
10	L	M	5.5	-	-	15	L	M	6.5	-	-
10	L	M	6.2	-	-	15	L	M	7.4	-	-
10	L	M	5.1	-	-	15	L	M	7.1	-	-
10	L	M	4.7	-	-	15	L	M	6.8	-	-
10	L	M	5	-	-	15	L	M	7.1	-	-
10	L	F	5.5	-	-	15	L	M	7.1	-	-
10	L	F	5.6	-	-	15	L	M	7.3	-	-
10	L	F	5.9	-	-	15	L	M	6.6	-	-
10	L	F	5.8	-	-	15	L	M	6.9	-	-
10	L	F	5.8	-	-	15	L	M	7.8	-	-
10	L	F	5.7	-	-	15	L	M	7.2	26	BB
10	L	F	5.3	-	-	15	L	M	6.3	21.6	BB
10	L	F	5.6	-	-	15	L	M	7	25.4	BB
10	L	F	5.9	-	-	15	L	M	6.3	19.9	BB
10	L	F	5	-	-	15	L	M	6.8	23.9	BB
10	L	F	5.2	-	-	15	L	M	6.9	22.1	BB
10	L	F	5.4	-	-	15	L	M	7.2	31.9	BB
10	L	F	5.2	-	-	15	L	M	7.1	32.3	BB

10	L	F	5.5	-	-	15	L	F	5.2	12.2	BB
10	L	F	5.7	-	-	15	L	M	5.7	13.8	BB
10	L	F	5	-	-	15	L	M	6.7	18.9	BB
10	L	F	5.4	-	-	15	L	M		19.6	BB
10	L	F	5.6	-	-	15	L	M		25.9	BB
10	L	F	5.2	-	-	15	L	M	6.9	24.8	BB
10	L	F	5.6	-	-	15	L	M	6.2	17.2	BB
10	L	F	5.9	-	-	15	L	M	7	27.8	BB
10	L	F	5.2	-	-	15	L	M	6.2	17.7	BB
10	L	F	5.3	-	-	15	L	M		24.7	BC
10	L	F	5.4	-	-	15	L	M	6.7	24.7	BC
10	L	F	5.4	-	-	15	L	M	5.6	14.8	BD
10	L	F	5.5	-	-	15	L	F	5.1	9.5	BD
10	L	F	5.7	-	-	15	L	M	6.1	17.2	DD
10	L	F	5.4	-	-	15	L	F	4.6	10.2	DD
10	L	F	5.6	-	-	16	L	M	7.6	34.5	BB
10	L	F	5.6	-	-	16	L	M	6.5	19.5	BB
10	L	F	5.1	-	-	16	L	M	6.9	23.4	BB
10	L	F	5.5	-	-	16	L	M	6.3	20.5	BB
10	L	F	5.9	-	-	16	L	F	5	12.6	BB
10	L	F		-	-	16	L	M	7.1	29.3	BB
10	L	F	5.4	-	-	16	L	M	7.2	28	BB
10	L	F	5.5	-	-	16	L	M	5.8	16.5	BB
10	L	M	4.9	-	-	16	L	M	7.2	31.6	BB
10	L	M	5.1	-	-	16	L	M	7.1	27.8	BB
10	L	M	6.8	-	-	16	L	M	7.1	21.7	BB
10	L	M	5	-	-	16	L	M	6.9	27	BC
10	L	M	5.2	-	-	16	L	M	6.9	29.2	X
10	L	F	5.4	-	-	16	L	M	6.9	24.2	X

Part II – Mating and oviposition trials

Columns are (left to right) male culture alga (***Laminaria*** / ***Fucus***), male treatment (L = exposure to *Laminaria*, F = exposure to *Fucus*), female culture alga (***Laminaria*** / ***Fucus***), female treatment (L = exposure to *Laminaria*, F = exposure to *Fucus*), oviposition status of female (Y = oviposited, N = not oviposited), male harassment (0 = no mount observed, 1 = mount observed), time preceding mount (seconds), struggle duration (seconds), result of mount (0 = no copulation, 1 = copulation), copulation duration (seconds), male wing length (mm), female wing length (mm), male weight (mg) and female weight (mg).

F	C	F	L	Y	1	74	94	1	63	5.5	5.1	9.3	6.8
F	C	F	F	N	1	113	7	1	103	4.8	4.9	6.7	7.2
F	C	F	C	N	1	25	2	0		5.4	5.4	8.5	9.7
F	C	F	L	Y	1	261	3	1	275	4.8	5.2	6.2	9.8

F	C	F	F	N	0					4.9	5.5	6.7	9.0
F	C	F	C	N	1	256	130	0			5.3	11.9	10.8
F	C	F	L	Y	1	235	7	0		5.7	5.3	10.8	9.6
F	C	F	F	Y	1	52	6	1	192	5.9	5.4	13.3	9.5
F	C	F	C	N	0					6.3	5.2	15.2	8.3
F	C	F	L	N	0					6.0	5.3	13.1	14.2
F	C	F	F	Y	0					5.8	5.4	12.0	7.7
F	C	F	C	N	1	559	2	1	309	5.3	5.2	8.9	10.1
F	C	F	C	N	1	33	25	1	596	5.1	5.0	8.2	9.1
F	C	F	F	Y	1	83	8	1	151	6.1	5.2	14.2	9.0
F	C	F	C	N	0					6.4	5.4	14.8	11.2
F	C	F	L	Y	1	542	8	1	79	6.4	5.3	15.0	6.8
F	C	F	F	N	0					6.5	5.2	14.5	7.1
F	C	F	C	N	1	45	3	0		5.9	5.2	10.1	7.9
F	C	F	F	Y	1	17	6	0		5.6	5.0	10.0	6.4
F	C	F	C	N	1	16	7	1	61	5.6	5.2	11.3	10.1
F	C	F	L	N	1	293	2	0		5.8	5.2		
F	C	F	F	Y	0					6.5	4.7		
F	C	L	L	N	1	176	1	1	2243	6.3	5.5	14.2	9.0
F	C	L	F	Y	0					6.1	5.1	13.1	8.3
F	C	L	C	N	0					5.8	5.0	11.1	6.9
F	C	L	L	Y	1	38	6	1	862	4.8	5.3	6.5	9.2
F	C	L	F	Y	1	36	5	0		6.3	5.3	13.4	9.5
F	C	L	C	N	1	62	3	1	36	5.6	5.2	9.6	8.9
F	C	L	L	Y	1	51	12	1	154	6.6	5.1	16.2	8.5
F	C	L	L	Y	1	32	3	0		5.8	5.8	10.8	8.5
F	C	L	C	N	1	153	7	0		5.6	5.3	10.6	8.2
F	C	L	L	N	1	40	9	1	103	5.7	5.3	10.9	9.9
F	C	L	C	N	0					6.2	5.2	13.5	9.1
F	C	L	L	N	0					4.9	5.0	6.8	9.4
F	C	L	F	Y	0					5.8	5.2	9.8	8.2
F	C	L	C	N	1	233	5	0		5.4	4.4	4.9	9.6
F	C	L	L	N	1	106	4	1	67	5.4	5.0	10.2	8.5
F	C	L	F	Y	1	179	4	0		6.6	5.1	15.2	8.4
F	C	L	C	N	1	224	25	0		5.4	5.3	9.7	9.6
F	C	L	F	Y	1	224	5	0		4.3	5.1	5.4	8.0
F	C	L	C	N	0					5.9	5.3	10.2	10.3
F	C	L	C	N	0					5.2	5.4	9.0	11.0
F	C	L	L	Y	1	23	3	1	166	5.8	5.3		
F	C	L	F	Y	1	21	17	1	33	4.5	5.4		
F	C	L	C	N	1	47	2	0		3.9	5.3		
F	F	F	F	N	1	264	6	1	625	5.9	5.3	10.9	9.2
F	F	F	C	N	1	23	7	0		4.9	5.0	7.1	8.8
F	F	F	L	Y	1	27	24	1	275	6.6	5.4	16.1	10.4
F	F	F	F	Y	1	17	14	1	66	6.7	5.1	15.5	8.5
F	F	F	C	N	1	86	34	1	65	6.7	5.3	15.3	8.4
F	F	F	L	Y	1	372	177	0			5.4	12.1	8.4
F	F	F	F	Y	1	566	7	1	48	6.1	4.6	13.0	5.2
F	F	F	C	N	1	20	35	1	1774	6.0	5.6	12.8	12.9
F	F	F	C	N	1	7	9	1	376	5.8	5.3	12.0	9.4
F	F	F	L	Y	1	50	8	1	516	5.6	5.1	9.8	8.5
F	F	F	F	Y	1	232	3	1	75	5.6	5.1	8.8	7.8
F	F	F	C	N	1	273	101	1	796	5.7	5.4	11.1	10.4
F	F	F	L	N	1	112	7	1	228	4.8	5.3	5.8	9.0

F	F	F	F	Y	1	5	11	0		5.9	5.4	10.8	8.4
F	F	F	C	Y	0					5.9	5.5	12.6	8.1
F	F	F	L	Y	1	143	4	0		6.5	5.3	13.9	8.8
F	F	F	F	N	1	22	3	1	238	5.6	5.2	11.0	8.0
F	F	F	L	Y	1	43	39	1	311	5.3	4.9		
F	F	F	F	Y	1	218	4	0		5.8	4.9		
F	F	F	C	N	1	61	5	1	608	5.8	5.3		
F	F	L	L	Y	1	89	2	1	917	6.4	5.0	14.1	8.2
F	F	L	F	N	1	20	17	1	231	5.8	5.4	11.0	10.1
F	F	L	C	N	1	78	4	1	465	5.6		9.9	10.6
F	F	L	L	Y	1	23	48	1	1693	5.2	4.9	6.7	7.4
F	F	L	F	Y	0					6.1	5.1	14.5	8.9
F	F	L	C	N	0					5.0	5.2	6.4	9.3
F	F	L	L	N	1	18	33	1	352	5.8	5.5	8.9	10.1
F	F	L	F	Y	1	52	5	1	442	5.8	5.7	12.3	11.5
F	F	L	C	N	1	176	7	1	146	6.4	5.2	17.0	8.9
F	F	L	L	Y	1	19	27	1	457	5.6	5.2	9.5	7.3
F	F	L	L	Y	1	7	10	1	89	5.1	5.0	6.1	7.7
F	F	L	C	N	0					6.2	5.2	13.1	9.0
F	F	L	F	N	1	21	2	0		4.9	5.5	6.0	10.5
F	F	L	C	N	1	512	5	0		5.4	5.4	8.4	11.1
F	F	L	L	N	1	181	2	1	275	6.7	5.0	16.9	8.7
F	F	L	F	Y	1	36	5	1	137	4.3	4.9	4.2	8.1
F	F	L	C	N	0					6.3	5.2	13.5	8.5
F	F	L	F	Y	1	80	275	1	77	6.5	5.3	14.6	8.3
F	F	L	C	N	1	8	30	1	523	5.8	5.4	10.5	9.4
F	F	L	C	N	1	194	4	0		6.5	5.3	14.4	8.8
F	F	L	C	N	1	17	26	1	280	5.7	5.4	9.9	8.8
F	F	L	L	N	0					5.9	5.2		
F	F	L	F	Y	1	14	4	1	102	4.7	5.3		
F	F	L	C	N	1	66	10	1	1925	5.8	5.2		
F	L	F	L	Y	1	132	13	0		6.0	4.9	11.6	8.2
F	L	F	F	Y	1	63	3	1	248	5.9	5.3	10.5	10.3
F	L	F	C	N	1	22	43	0		6.3	5.2	13.4	9.9
F	L	F	L	N	1	54	32	0		4.1	5.3	5.3	8.8
F	L	F	F	Y	0					6.0	5.4	11.3	10.5
F	L	F	C	N	1	263	32	1	715	5.7	5.2	9.3	9.6
F	L	F	L	N	1	28	38	1	181	5.0	5.1	6.9	7.8
F	L	F	F	N	0					5.8	5.0	11.9	8.1
F	L	F	C	N	1	36	18	1	245	6.3	5.5	14.3	8.7
F	L	F	L	Y	1	116	1	0		6.5	5.3	15.9	8.2
F	L	F	F	Y	1	170	11	1	84	5.3	5.2	6.4	6.1
F	L	F	C	N	1	243	20	1	376	5.1	5.0	7.0	8.3
F	L	F	L	Y	1	35	8	1	615	4.9	5.1	6.9	8.8
F	L	F	F	Y	1	148	8	1	33	6.8	5.4	17.0	10.1
F	L	F	C	N	0					6.1	5.2	12.4	9.5
F	L	F	L	N	1	8	5	0		5.3	4.7	7.0	5.4
F	L	F	F	N	1	263	55	0		5.7	5.4	9.4	9.3
F	L	F	C	N	0					6.0	5.1	13.0	9.7
F	L	F	F	N	1	85	1	0		4.9	4.9	6.8	7.5
F	L	F	C	N	1	27	2	0		5.6	5.1	9.2	9.1
F	L	F	L	Y	1	248	20	0		6.7	5.4		
F	L	F	F	N	1	31	1	0		6.5	5.3		
F	L	F	C	N	1	104	27	0		5.8	5.5		

F	L	F	C	N	0					6.8	5.2		
F	L	L	L	Y	1	5	4	0		4.8	5.2	7.0	10.2
F	L	L	F	Y	1	30	3	1	268	5.4	5.4	8.5	8.7
F	L	L	C	N	1	135	14	1	329	4.7	4.9	5.8	7.9
F	L	L	L	Y	1	75	38	1	2730	4.9	5.3	6.6	10.8
F	L	L	F	N	0					6.3	5.4	15.4	10.6
F	L	L	C	N	1	88	8	1	348	6.0	5.2	11.7	9.4
F	L	L	L	Y	1	28	3	1	1390	5.9	5.1	10.5	9.1
F	L	L	F	N	1	278	3	0		5.7	5.2	10.1	6.7
F	L	L	C	N	1	23	65	0		4.9	5.6	6.9	10.3
F	L	L	L	Y	0						4.8	9.6	6.3
F	L	L	F	Y	1	29	102	0		6.2	5.0	11.5	10
F	L	L	C	N	1	44	4	0		5.6	5.6	8.9	10.6
F	L	L	L	Y	1	25	5	1	137	5.0	5.1	6.5	6.8
F	L	L	F	Y	1	58	7	1	810	6.2	4.9	11.8	7.7
F	L	L	C	N	0					6.1	5.6	12.3	10.9
F	L	L	L	N	1	19	15	0		5.3	5.5	8.3	9.3
F	L	L	F	Y	1	23	97	1	814	5.9	5.4	10.2	10.3
F	L	L	C	N	1	57	3	0		6.2	5.3	14.4	9.6
F	L	L	L	Y	1	40	8	1	103	5.1	5.0		
F	L	L	F	Y	1	10	7	1	86	5.0	4.9		
F	L	L	C	N	1	238	88	1	303	6.5	5.2		
F	L	L	F	Y	1	34	3	1	527	4.8	5.4		
F	L	L	C	N	1	55	143	1	81	6.0	5.3		
L	C	F	L	Y	0					6.8	5.2	19.2	8.3
L	C	F	F	Y	0					7.6	5.3	26.8	7.8
L	C	F	C	N	0					6.5	5.2	17.9	8
L	C	F	L	Y	1	166	6	1	105	6.9	5.6	21	11.4
L	C	F	F	Y	1	28	11	1	32	6.6	5.2	17.8	6.4
L	C	F	C	N	0					7.1	5.2	23.1	8.8
L	C	F	L	N	1	59	435	0		6.4	5.2	13.5	9.6
L	C	F	F	Y	0					7.1	5.3	25.1	10.4
L	C	F	C	N	1	190	4	1	401	4.7	5.2	5.4	8.4
L	C	F	L	Y	1	42	4	1	77	6.4	5.3	15.1	8.2
L	C	F	F	N	0					5.4	5.5	8.5	8.5
L	C	F	C	N	0						5.4	6.3	9.5
L	C	F	L	Y	0					6.7	5.3	19.4	8.4
L	C	F	F	N	0					6.5	4.9	16.3	6.4
L	C	F	C	N	0					6.2	5.1	15.6	8.8
L	C	F	L	N	0					6.6	5.1	17.1	8.0
L	C	F	F	N	1	239	6	0		6.9	5.1	22.0	8.3
L	C	F	C	N	0					6.5	5.0	18.8	5.8
L	C	F	L	Y	1	319	50	0		6.9	5.1	22.4	6.7
L	C	F	F	N	0					6.5	5.3	16.2	8.5
L	C	F	C	N	1	60	1	0		5.7	4.9	12.3	6.7
L	C	F	L	Y	1	19	9	0		6.7	5.4		
L	C	F	F	Y	1	35	4	1	51	6.1	5.5		
L	C	F	C	N	1	32	13	1	316	6.9	5.2		
L	C	F	L	Y	1	31	5	0		6.5	4.9		
L	C	F	C	N	1	136	4	0		6.3	5.1		
L	C	F	C	N	1	31	8	0		6.5	5.2		
L	C	L	L	Y	1	155	3	0		6.6	4.9	14.0	7.8
L	C	L	F	Y	0					6.6	5.2	19.5	8.5
L	C	L	C	N	1	9	3	1	1282	6.0	5.1	11.6	9.2

L	C	L	F	Y	1	46	1	0		5.9	5.4	12.2	11.2
L	C	L	C	N	0					6.2	5.0	14.6	8.9
L	C	L	L	N	1	574	47	0		6.2	5.4	14.3	10.0
L	C	L	F	Y	1	230	4	1	48	6.5	5.4	17.8	8.9
L	C	L	C	N	1	236	7	1	84	6.0	4.9	13.3	6.6
L	C	L	L	N	0					5.0	5.3	7.9	10.2
L	C	L	L	Y	1	206	9	1	67	6.4	5.4	15.8	9.4
L	C	L	C	N	0					6.8	5.1	17.1	8.6
L	C	L	L	Y	1	227	5	0		6.6	5.4	16.8	10.2
L	C	L	F	Y	1	189	5	1	183	5.1	5.3	7.0	9.4
L	C	L	C	N	1	268	3	0		4.3	5.0	5.8	7.7
L	C	L	L	Y	1	180	7	0		6.5	5.1	15.7	7.4
L	C	L	F	Y	1	212	8	0		4.7	5.3	6.1	8.2
L	C	L	C	N	1	423	2	0		6.0	4.9	13.4	8.0
L	C	L	L	N	1	33	52	1	56	5.4	5.4	9.9	10.3
L	C	L	F	N	0					7.1	4.9	22.0	5.5
L	C	L	C	N	1	36	8	0		5.9	5.0	12.4	8.5
L	C	L	L	Y	1	135	22	1	61	6.9	5.6		
L	C	L	F	Y	1	8	7	1	315	6.6	4.9		
L	C	L	C	N	1	21	5	1	195	6.2	5.5		
L	C	L	F	Y	1	109	5	0		6.6	5.3		
L	F	F	L	N	1	11	7	1	608	5.7		8.7	9.9
L	F	F	F	Y	0					6.9	5.2	18.9	7.5
L	F	F	C	N	0					6.9	4.9	19.4	8.6
L	F	F	L	N	1	158	36	1	200	7.2	5.6	22.8	11.0
L	F	F	F	Y	1	25	6	0		6.9	5.2	19.7	7.6
L	F	F	C	N	1	66	31	1	110	5.6	5.4	11.6	9.0
L	F	F	L	N	1	244	31	0		6.9	5.0	22.0	8.3
L	F	F	F	Y	1	20	3	1	41	6.5	5.0	16.3	7.7
L	F	F	C	N	1	46	10	1	439	5.6	5.2	9.7	8.7
L	F	F	L	N	1	34	151	1	1130	4.8	5.0	5.6	7.5
L	F	F	F	Y	1	139	26	1	45	6.3	5.3	14.2	8.1
L	F	F	C	N	1	11	3	1	491	6.1	5.2	14.2	8.8
L	F	F	L	Y	0					6.1	5.1	14.1	8.6
L	F	F	F	N	1	59	49	1	2278	4.8	5.5	6.6	10.4
L	F	F	C	N	1	41	15	0		4.9	5.0	8.2	9.5
L	F	F	L	Y	1	307	3	0		7.2	5.5	23.0	8.0
L	F	F	F	N	1	262	5	1	336	6.1	5.1	13.0	7.8
L	F	F	C	N	1	140	11	1	479	6.3	5.0	14.7	7.6
L	F	F	L	Y	1	71	12	0		7.0	5.1	19.7	6.9
L	F	F	F	Y	1	23	16	0		5.4	4.9	7.8	6.5
L	F	F	C	N	1	296	10	1	252	6.2	5.3	14.0	9.0
L	F	F	L	Y	1	21	12	1	112	6.2	5.4		
L	F	F	F	N	0					6.3	4.7		
L	F	F	C	N	1	335	8	1	427	6.0	5.4		
L	F	F	C	N	1	8	5	1	157	6.2	5.4		
L	F	F	C	N	0					7.1	5.0		
L	F	L	L	Y	1	74	14	0		7.0	5.1	17.3	6.8
L	F	L	F	Y	1	592	6	1	66	6.2	5.4	15.0	8.2
L	F	L	C	N	1	101	8	1	1169	5.9	5.2	12.1	9.0
L	F	L	L	Y	0					7.0	4.9	19.2	8.5
L	F	L	F	N	1	132	2	0		6.7	5.2	17.4	10.5
L	F	L	C	N	1	34	56	0		5.7	5.3	10.7	9.1
L	F	L	L	Y	1	32	25	1	153	5.5	5.9	13.2	9.7

L	F	L	F	Y	1	155	2	1	85	6.8	4.8	18.8	9.8
L	F	L	C	N	1	22	5	1	1078	4.8	5.2	8.0	10.0
L	F	L	L	Y	1	24	3	1	64	6.4	5.1	15.5	8.9
L	F	L	C	N	0					6.6	5.1	18.1	9.8
L	F	L	C	N	1	369	11	1	793	6.0	5.4	10.7	10.3
L	F	L	L	Y	1	28	2	0		6.4	4.9	16.5	5.8
L	F	L	F	Y	1	54	40	1	668	5.1	5.0	6.8	7.2
L	F	L	C	N	1	549	7	0		6.2	5.4	14.1	10.5
L	F	L	L	N	1	218	157	1	76	7.0	5.1	19.2	8.0
L	F	L	F	Y	1	16	5	0		6.9	4.8	18.6	6.1
L	F	L	C	N	1	463	16	1	333	6.2	5.0	13.9	8.3
L	F	L	L	Y	1	11	12	1	379	5.2	5.1	9.0	7.1
L	F	L	F	Y	1	58	1379	0		6.2	5.3	15.3	9.5
L	F	L	C	N	0					6.3	5.4	14.0	8.4
L	F	L	C	N	1	53	10	1	96	6.0	5.0	13.0	7.9
L	F	L	C	N	1	25	18	1	796	6.5	5.3	15.1	9.8
L	F	L	L	Y	1	82	3	1	55	6.1	5.1		
L	F	L	F	N	0					7.4	5.3		
L	F	L	C	N	1	17	26	1	67	6.3	5.6		
L	F	L	F	Y	1	14	9	1	327	6.6	5.2		
L	F	L	C	N	1	25	31	1	2858	4.9	5.0		
L	L	F	L	Y	0					7.0	5.0	21.3	5.8
L	L	F	F	N	1	14	7	0		6.2	5.5	15.5	10.8
L	L	F	C	N	1	27	15	0		6.8	4.9	19.0	5.9
L	L	F	L	N	1	154	2	0		6.3	5.2	16.5	9.6
L	L	F	F	Y	1	25	19	0		6.5	5.0	17.0	6.7
L	L	F	C	N	0					7.2	5.3	24.3	10.2
L	L	F	L	N	1	87	1	0		7.0	4.5	18.7	5.7
L	L	F	F	Y	1	269	8	1	95	6.4	5.4	13.7	9.4
L	L	F	C	N	0					5.9	5.5	13.0	10.8
L	L	F	L	N	1	63	48	0		6.4	5.2	14.2	7.9
L	L	F	F	Y	1	79	12	1	62	6.1	5.1	11.3	9.6
L	L	F	C	N	1	543	4	0		6.6	5.0	16.7	7.5
L	L	F	L	N	1	106	39	1	661	6.4	5.1	16.2	9.5
L	L	F	F	N	0					7.1	5.1	21.6	7.4
L	L	F	C	N	0					6.8	5.1	18.4	8.1
L	L	F	L	Y	1	392	11	1	69	6.3	5.5	15.3	8.3
L	L	F	F	N	1	117	2	0		7.0	5.3	22.9	8.0
L	L	F	C	N	1	42	20	1	1143	5.8	5.3	11.6	9.7
L	L	F	L	Y	1	199	10	1	46	5.9	5.2		
L	L	F	F	N	1	56	7	0		7.1	5.5		
L	L	F	C	N	1	259	8	1	282	6.5	5.3		
L	L	F	L	Y	1	257	22	0		6.7	5.5		
L	L	L	L	Y	1	23	7	1	516	6.3	5.3	17.4	9.1
L	L	L	F	Y	0					6.4	5.0	17.7	7.2
L	L	L	C	N	1	17	10	1	119	5.9	5.1	11.9	10.3
L	L	L	L	N	0					6.4	5.1	15.4	9.3
L	L	L	F	Y	1	24	6	1	63	6.0	5.0	10.9	7.6
L	L	L	C	N	1	72	87	1	143	6.6	5.2	16.4	12.0
L	L	L	L	Y	1	28	3	1	618	6.7	5.6	18.4	11.3
L	L	L	F	N	1	394	7	1	116	6.2	5.4	14.3	9.0
L	L	L	C	N	1	43	4	1	477	5.8	5.1	12.4	10.4
L	L	L	L	Y	0					6.5	5.2	17.1	7.3
L	L	L	F	Y	1	363	82	1	42	6.8	5.4	18.2	8.3

L	L	L	C	N	1	21	10	1	96		5.3	13.5	9.7
L	L	L	L	Y	1	268	11	1	174	6.2	5.5	17.4	8.8
L	L	L	F	Y	1	14	15	1	376	5.9		11.2	8.5
L	L	L	C	N	0					5.1	5.1	8.2	8.4
L	L	L	L	Y	1	97	9	1	50	6.4	5.2	15.8	8.2
L	L	L	F	N	1	7	5	1	349	5.2	5.5	7.2	8.3
L	L	L	C	N	1	28	32	0		6.8	5.5	19.0	11.0
L	L	L	C	N	0					5.8	5.2	10.8	8.5
L	L	L	L	N	1	31	3	1	292	5.9	5.4		
L	L	L	F	Y	0					6.8	4.9		
L	L	L	C	N	1	6	7	0		6.2	4.9		
L	L	L	L	Y	1	136	14	0		6.6	5.7		
L	L	L	F	Y	1	93	6	1	98	6.6	4.9		
L	L	L	C	N	1	55	20	0		4.9	5.5		
		F	L	Y							5.3		9.0
		F	C	N							5.2		8.2
		F	C	N							5.3		
		L	L	Y							5.2		7.4

Appendix 5 – Chapter 7

Dataset A

Columns are (from left to right) male culture alga (***Laminaria*** / ***Fucus***), female culture alga (***Laminaria*** / ***Fucus***), mating success (0 = no copulation, 1 = copulation), time preceding mount (seconds ($\log_{10}$ transformed), struggle duration (seconds (reciprocal cube root transformed)) and male wing length (mm).

F	F	0	1.929	-1.000	4.9
L	F	0	1.778	-1.000	5.7
L	L	0	1.663	-1.000	5.9
F	L	1	2.246	-1.000	6.3
F	F	0	2.064	-1.000	6.5
F	F	0	1.491	-1.000	6.5
L	F	0	1.940	-1.000	7.0
F	L	0	1.672	-0.707	3.9
F	L	0	1.322	-0.707	4.9
F	F	1	2.747	-0.707	5.3
F	F	0	1.398	-0.707	5.4
F	F	0	1.431	-0.707	5.6
F	F	0	2.467	-0.707	5.8
L	L	0	2.626	-0.707	6.0
L	F	0	2.188	-0.707	6.3
L	L	0	1.447	-0.707	6.4
F	L	1	1.949	-0.707	6.4
F	L	1	2.258	-0.707	6.7
L	L	0	2.121	-0.707	6.7
L	L	1	2.190	-0.707	6.8
L	F	0	2.068	-0.707	7.0
L	L	0	2.428	-0.577	4.3
F	F	1	2.417	-0.577	4.8
F	L	1	1.531	-0.577	4.8
F	L	1	1.477	-0.577	5.4
F	F	1	2.365	-0.577	5.6
F	L	1	1.792	-0.577	5.6
F	F	1	1.342	-0.577	5.6
F	L	0	2.444	-0.577	5.7
F	L	1	1.362	-0.577	5.8
F	L	0	1.505	-0.577	5.8
F	L	1	1.447	-0.577	5.9
F	F	0	1.653	-0.577	5.9
F	F	1	1.799	-0.577	5.9
L	L	1	1.491	-0.577	5.9
L	L	1	0.954	-0.577	6.0
L	L	1	1.914	-0.577	6.1
L	F	1	1.041	-0.577	6.1
F	L	0	1.756	-0.577	6.2
L	L	1	1.380	-0.577	6.4
F	F	1	1.544	-0.354	4.9
F	L	1	1.602	-0.354	5.1
F	F	1	1.699	-0.354	5.6
L	L	0	1.556	-0.354	5.9
L	L	1	2.004	-0.354	5.9
F	L	1	1.944	-0.354	6.0
L	F	1	2.525	-0.354	6.0
F	F	1	1.919	-0.354	6.1
F	F	1	2.734	-0.354	6.4
L	F	1	2.430	-0.354	6.4
L	F	0	1.491	-0.354	6.5
L	F	1	2.413	-0.354	6.5
F	F	1	2.170	-0.354	6.8
F	L	1	1.602	-0.333	5.7
F	F	1	0.845	-0.333	5.8
L	L	1	1.987	-0.333	6.4
L	L	1	2.314	-0.333	6.4
L	L	1	1.146	-0.333	6.6
L	F	0	1.279	-0.333	6.7
F	L	1	0.845	-0.316	5.1
L	F	1	1.663	-0.316	5.6
F	L	1	1.820	-0.316	5.8
L	L	1	1.230	-0.316	5.9
L	F	1	2.299	-0.316	5.9
L	L	1	1.724	-0.316	6.0
L	F	1	2.471	-0.316	6.2
F	F	1	2.230	-0.302	5.3
F	F	0	0.699	-0.302	5.9
L	L	1	2.567	-0.302	6.0
L	L	1	2.428	-0.302	6.2
L	F	1	2.146	-0.302	6.3
L	F	1	2.593	-0.302	6.3
L	F	1	1.447	-0.302	6.6
L	L	1	1.041	-0.289	5.2
L	F	1	1.898	-0.289	6.1
L	F	1	1.322	-0.289	6.2
F	L	1	1.708	-0.289	6.6
L	F	0	1.851	-0.289	7.0
F	F	0	2.121	-0.277	6.0
L	F	1	1.505	-0.277	6.9

L	F	1	1.301	-0.577	6.5
L	L	0	2.190	-0.577	6.6
L	L	1	1.447	-0.577	6.7
L	F	0	2.487	-0.577	7.2
L	F	1	2.279	-0.500	4.7
F	L	1	1.146	-0.500	4.7
F	L	0	0.699	-0.500	4.8
F	L	1	2.025	-0.500	5.4
F	L	1	1.892	-0.500	5.6
F	L	0	1.643	-0.500	5.6
F	F	0	2.338	-0.500	5.8
L	L	1	1.633	-0.500	5.8
L	F	1	1.544	-0.500	6.1
L	F	0	2.134	-0.500	6.3
L	F	1	1.623	-0.500	6.4
F	L	0	2.288	-0.500	6.5
F	F	0	2.155	-0.500	6.5
L	L	1	2.362	-0.500	6.5
L	F	0	2.735	-0.500	6.6
F	L	0	2.253	-0.500	6.6
F	L	1	1.556	-0.447	4.3
F	L	0	2.350	-0.447	4.3
L	L	1	1.342	-0.447	4.8
F	L	1	1.398	-0.447	5.0
L	L	1	2.276	-0.447	5.1
L	L	1	0.845	-0.447	5.2
F	F	0	0.903	-0.447	5.3
F	L	0	2.367	-0.447	5.4
F	L	0	2.709	-0.447	5.4
F	F	1	1.785	-0.447	5.8
F	L	1	1.716	-0.447	5.8
L	F	1	2.418	-0.447	6.1
L	F	1	0.903	-0.447	6.2
L	L	1	1.322	-0.447	6.2
F	L	0	1.556	-0.447	6.3
L	F	0	1.491	-0.447	6.5
L	L	0	2.037	-0.447	6.6
L	L	0	2.356	-0.447	6.6
L	L	0	1.204	-0.447	6.9
F	L	1	1.580	-0.408	4.8
F	F	0	1.230	-0.408	5.6
F	F	1	2.422	-0.408	5.9
F	F	1	1.716	-0.408	5.9
L	L	1	1.380	-0.408	6.0
L	L	1	2.772	-0.408	6.2
L	L	1	1.968	-0.408	6.6
L	F	0	2.378	-0.408	6.9
L	F	0	1.398	-0.408	6.9
L	F	1	2.220	-0.408	6.9
F	F	1	2.053	-0.378	4.8
F	F	1	2.049	-0.378	4.8
F	F	0	1.362	-0.378	4.9
F	L	1	1.000	-0.378	5.0
F	F	1	1.204	-0.378	5.6
F	L	1	2.130	-0.267	4.7
L	L	0	2.134	-0.267	6.6
F	F	1	1.230	-0.267	6.7
L	L	0	1.869	-0.267	7.0
L	F	0	1.613	-0.258	4.9
F	L	0	1.279	-0.258	5.3
L	L	1	1.146	-0.258	5.9
L	F	0	1.431	-0.258	6.8
L	F	0	1.362	-0.250	5.4
L	L	1	2.666	-0.250	6.2
F	L	1	1.322	-0.243	4.5
F	L	1	1.301	-0.243	5.8
F	F	1	1.556	-0.236	6.3
L	L	1	1.398	-0.236	6.5
L	F	0	1.398	-0.229	6.5
L	L	0	1.740	-0.224	4.9
F	F	1	2.386	-0.224	5.1
L	F	1	1.623	-0.224	5.8
F	F	0	2.394	-0.224	6.7
L	F	0	2.410	-0.213	6.7
L	L	1	2.130	-0.213	6.9
F	F	1	1.431	-0.204	6.6
F	F	1	1.519	-0.200	5.1
F	L	0	2.350	-0.200	5.4
L	L	1	1.505	-0.200	5.5
F	L	1	1.230	-0.196	5.7
L	F	1	2.143	-0.196	6.3
L	L	1	1.230	-0.196	6.3
F	L	1	1.279	-0.192	5.6
F	F	0	2.017	-0.192	5.8
F	L	1	0.903	-0.183	5.8
L	L	1	1.398	-0.180	4.9
L	F	1	1.820	-0.180	5.6
L	F	0	2.387	-0.180	6.9
F	F	0	1.732	-0.177	4.1
F	F	1	2.420	-0.177	5.7
L	L	0	1.447	-0.177	6.8
F	L	1	1.255	-0.174	5.8
F	F	1	1.934	-0.171	6.7
F	F	1	1.301	-0.169	6.0
L	F	1	2.199	-0.167	7.2
F	L	1	1.875	-0.162	4.9
F	F	1	1.447	-0.162	5.0
F	F	1	1.633	-0.160	5.3
L	F	1	2.025	-0.160	6.4
L	L	1	1.732	-0.158	5.1
F	F	0	1.342	-0.152	6.3
L	L	0	2.759	-0.146	6.2
F	L	1	1.362	-0.144	5.2
L	F	0	1.799	-0.144	6.4
L	F	1	1.771	-0.143	4.8
L	F	0	2.504	-0.141	6.9
L	L	1	1.519	-0.139	5.4
F	F	0	2.420	-0.135	5.7

F	L	0	2.185	-0.378	5.6
L	F	1	1.041	-0.378	5.7
F	F	0	2.371	-0.378	5.7
L	L	1	2.373	-0.378	6.0
F	F	1	2.753	-0.378	6.1
F	L	1	1.763	-0.378	6.2
L	L	0	0.778	-0.378	6.2
L	L	1	2.595	-0.378	6.2
L	L	0	2.740	-0.378	6.2
L	F	0	1.146	-0.378	6.2
L	L	1	1.362	-0.378	6.3
F	L	1	2.246	-0.378	6.4
L	L	0	2.255	-0.378	6.5
L	L	1	0.903	-0.378	6.6
L	F	0	1.748	-0.378	7.1
L	L	0	2.326	-0.354	4.7
L	L	0	1.531	-0.134	5.7
F	L	0	1.362	-0.124	4.9
L	L	1	2.560	-0.110	6.8
L	L	1	1.857	-0.107	6.6
F	L	1	2.377	-0.107	6.5
F	F	1	1.869	-0.103	5.5
F	L	1	1.362	-0.102	5.9
F	F	1	2.436	-0.100	5.7
F	L	0	1.462	-0.099	6.2
F	L	1	1.740	-0.084	6.0
L	F	1	1.531	-0.081	4.8
L	L	1	2.338	-0.080	7.0
F	L	1	1.903	-0.060	6.5
L	F	0	1.771	-0.048	6.4
L	L	0	1.763	-0.027	6.2

Dataset B

Columns are (from left to right) mating success (0 = no copulation, 1 = copulation), time preceding mount (seconds ($\log_{10}$ transformed)), struggle duration (seconds (reciprocal square root transformed)) and male wing length (mm (($\log_{10}$ transformed)).

1	0.301	-1.000	0.690
1	0.477	-1.000	0.690
1	0.699	-1.000	0.653
0	1.079	-1.000	0.681
0	1.176	-1.000	0.724
1	1.362	-1.000	0.699
0	1.491	-1.000	0.813
0	1.505	-1.000	0.643
1	1.602	-1.000	0.602
1	1.602	-1.000	0.663
0	1.663	-1.000	0.771
0	1.778	-1.000	0.756
0	1.929	-1.000	0.690
0	1.940	-1.000	0.845
0	1.973	-1.000	0.663
0	1.996	-1.000	0.699
0	2.064	-1.000	0.813
1	2.246	-1.000	0.799
1	2.528	-1.000	0.732
0	2.728	-1.000	0.771
0	2.770	-1.000	0.623
1	0.000	-0.707	0.778
1	0.602	-0.707	0.716
1	0.903	-0.707	0.591
0	1.633	-0.378	0.699
0	1.663	-0.378	0.672
0	1.663	-0.378	0.690
1	1.681	-0.378	0.785
1	1.716	-0.378	0.740
1	1.716	-0.378	0.845
1	1.740	-0.378	0.672
0	1.748	-0.378	0.851
1	1.763	-0.378	0.708
1	1.763	-0.378	0.763
1	1.763	-0.378	0.792
1	1.826	-0.378	0.672
1	1.886	-0.378	0.623
1	1.886	-0.378	0.681
1	1.987	-0.378	0.716
1	2.033	-0.378	0.672
1	2.049	-0.378	0.681
0	2.053	-0.378	0.633
1	2.053	-0.378	0.681
1	2.064	-0.378	0.672
0	2.068	-0.378	0.663
1	2.140	-0.378	0.643
0	2.185	-0.378	0.748
1	2.230	-0.378	0.724

1	0.903	-0.707	0.613
1	0.903	-0.707	0.633
0	0.903	-0.707	0.643
1	0.903	-0.707	0.716
0	0.954	-0.707	0.623
0	1.000	-0.707	0.663
0	1.114	-0.707	0.623
0	1.114	-0.707	0.699
1	1.146	-0.707	0.732
1	1.176	-0.707	0.591
1	1.176	-0.707	0.653
1	1.230	-0.707	0.556
1	1.255	-0.707	0.653
0	1.255	-0.707	0.732
0	1.255	-0.707	0.740
1	1.279	-0.707	0.716
1	1.279	-0.707	0.716
1	1.301	-0.707	0.653
0	1.301	-0.707	0.663
0	1.322	-0.707	0.690
1	1.342	-0.707	0.653
1	1.342	-0.707	0.663
1	1.362	-0.707	0.556
0	1.380	-0.707	0.681
1	1.398	-0.707	0.653
0	1.398	-0.707	0.724
0	1.398	-0.707	0.732
1	1.415	-0.707	0.643
0	1.415	-0.707	0.690
0	1.415	-0.707	0.708
0	1.431	-0.707	0.748
1	1.431	-0.707	0.799
1	1.447	-0.707	0.708
1	1.447	-0.707	0.708
0	1.447	-0.707	0.806
0	1.462	-0.707	0.591
1	1.491	-0.707	0.699
0	1.491	-0.707	0.724
1	1.505	-0.707	0.663
1	1.505	-0.707	0.681
0	1.505	-0.707	0.699
0	1.519	-0.707	0.672
0	1.544	-0.707	0.653
0	1.556	-0.707	0.556
0	1.556	-0.707	0.681
0	1.580	-0.707	0.681
1	1.591	-0.707	0.633
1	1.591	-0.707	0.732
1	1.602	-0.707	0.681
0	1.633	-0.707	0.681
1	1.633	-0.707	0.681
1	2.236	-0.378	0.699
1	2.243	-0.378	0.748
1	2.246	-0.378	0.806
0	2.255	-0.378	0.813
1	2.274	-0.378	0.778
0	2.371	-0.378	0.756
1	2.373	-0.378	0.778
0	2.473	-0.378	0.716
1	2.529	-0.378	0.716
0	2.565	-0.378	0.763
1	2.595	-0.378	0.792
0	2.740	-0.378	0.792
1	2.753	-0.378	0.785
1	0.602	-0.354	0.568
1	0.699	-0.354	0.653
1	0.699	-0.354	0.763
1	0.845	-0.354	0.602
1	0.903	-0.354	0.699
1	0.903	-0.354	0.748
1	0.954	-0.354	0.591
0	1.000	-0.354	0.724
1	1.000	-0.354	0.732
1	1.000	-0.354	0.763
1	1.041	-0.354	0.653
1	1.041	-0.354	0.663
0	1.041	-0.354	0.716
1	1.079	-0.354	0.690
1	1.114	-0.354	0.898
1	1.146	-0.354	0.763
1	1.176	-0.354	0.748
1	1.230	-0.354	0.663
1	1.301	-0.354	0.602
0	1.322	-0.354	0.716
1	1.342	-0.354	0.643
1	1.342	-0.354	0.690
0	1.362	-0.354	0.716
1	1.362	-0.354	0.724
1	1.431	-0.354	0.699
1	1.462	-0.354	0.681
0	1.477	-0.354	0.623
0	1.491	-0.354	0.813
1	1.505	-0.354	0.708
1	1.519	-0.354	0.653
0	1.519	-0.354	0.699
1	1.519	-0.354	0.708
1	1.519	-0.354	0.724
1	1.544	-0.354	0.690
1	1.556	-0.354	0.699
0	1.556	-0.354	0.771
1	1.602	-0.354	0.708
1	1.613	-0.354	0.681

1	1.633	-0.707	0.732
1	1.653	-0.707	0.623
0	1.653	-0.707	0.708
0	1.672	-0.707	0.591
1	1.672	-0.707	0.763
0	1.681	-0.707	0.716
0	1.681	-0.707	0.785
1	1.699	-0.707	0.653
0	1.716	-0.707	0.681
0	1.724	-0.707	0.672
1	1.724	-0.707	0.833
1	1.732	-0.707	0.613
1	1.748	-0.707	0.839
0	1.756	-0.707	0.708
0	1.763	-0.707	0.591
0	1.763	-0.707	0.740
1	1.771	-0.707	0.591
0	1.771	-0.707	0.681
1	1.833	-0.707	0.653
1	1.839	-0.707	0.699
0	1.839	-0.707	0.732
1	1.851	-0.707	0.708
1	1.863	-0.707	0.708
1	1.881	-0.707	0.785
1	1.886	-0.707	0.591
1	1.940	-0.707	0.653
1	1.940	-0.707	0.681
1	1.949	-0.707	0.806
0	1.959	-0.707	0.613
0	1.973	-0.707	0.568
1	2.000	-0.707	0.602
0	2.009	-0.707	0.591
1	2.017	-0.707	0.690
0	2.057	-0.707	0.681
0	2.068	-0.707	0.845
0	2.107	-0.707	0.771
1	2.117	-0.707	0.690
0	2.121	-0.707	0.826
0	2.155	-0.707	0.623
1	2.155	-0.707	0.716
0	2.188	-0.707	0.799
1	2.190	-0.707	0.833
1	2.199	-0.707	0.613
1	2.210	-0.707	0.663
0	2.215	-0.707	0.708
0	2.250	-0.707	0.653
1	2.258	-0.707	0.826
0	2.294	-0.707	0.792
1	2.318	-0.707	0.633
1	2.344	-0.707	0.690
0	2.391	-0.707	0.716
1	1.633	-0.354	0.663
1	1.643	-0.354	0.672
1	1.663	-0.354	0.732
1	1.699	-0.354	0.672
1	1.699	-0.354	0.748
1	1.792	-0.354	0.732
1	1.820	-0.354	0.681
0	1.826	-0.354	0.591
0	1.875	-0.354	0.672
1	1.919	-0.354	0.785
1	1.944	-0.354	0.778
0	1.982	-0.354	0.653
1	1.996	-0.354	0.724
1	2.004	-0.354	0.771
0	2.137	-0.354	0.663
1	2.149	-0.354	0.708
1	2.170	-0.354	0.833
1	2.188	-0.354	0.708
1	2.260	-0.354	0.792
1	2.307	-0.354	0.672
0	2.326	-0.354	0.672
1	2.413	-0.354	0.813
1	2.430	-0.354	0.806
1	2.473	-0.354	0.633
1	2.525	-0.354	0.748
1	2.525	-0.354	0.778
1	2.624	-0.354	0.919
1	2.673	-0.354	0.778
1	2.734	-0.354	0.806
0	0.845	-0.333	0.672
1	0.845	-0.333	0.763
0	0.903	-0.333	0.643
1	1.146	-0.333	0.672
1	1.146	-0.333	0.820
1	1.204	-0.333	0.602
1	1.204	-0.333	0.690
1	1.230	-0.333	0.732
0	1.279	-0.333	0.826
1	1.322	-0.333	0.643
1	1.322	-0.333	0.732
1	1.415	-0.333	0.663
1	1.447	-0.333	0.690
1	1.519	-0.333	0.708
1	1.531	-0.333	0.663
1	1.580	-0.333	0.708
1	1.591	-0.333	0.716
1	1.602	-0.333	0.756
0	1.613	-0.333	0.544
1	1.623	-0.333	0.699
1	1.672	-0.333	0.653
1	1.740	-0.333	0.724

1	2.407	-0.707	0.623
0	2.467	-0.707	0.763
0	2.486	-0.707	0.580
1	2.508	-0.707	0.653
1	2.540	-0.707	0.663
0	2.547	-0.707	0.716
0	2.626	-0.707	0.778
0	2.646	-0.707	0.613
1	2.736	-0.707	0.806
1	2.747	-0.707	0.724
1	2.760	-0.707	0.653
1	0.477	-0.577	0.716
1	0.699	-0.577	0.881
1	0.778	-0.577	0.613
0	0.778	-0.577	0.748
1	0.845	-0.577	0.690
1	0.903	-0.577	0.653
1	0.903	-0.577	0.690
1	0.903	-0.577	0.732
0	0.954	-0.577	0.643
1	0.954	-0.577	0.699
1	0.954	-0.577	0.724
1	0.954	-0.577	0.771
1	0.954	-0.577	0.778
1	1.000	-0.577	0.643
1	1.000	-0.577	0.653
0	1.000	-0.577	0.663
1	1.041	-0.577	0.633
1	1.041	-0.577	0.643
1	1.041	-0.577	0.663
1	1.041	-0.577	0.681
1	1.041	-0.577	0.699
1	1.041	-0.577	0.763
1	1.041	-0.577	0.785
0	1.079	-0.577	0.580
0	1.079	-0.577	0.591
1	1.079	-0.577	0.613
1	1.079	-0.577	0.623
1	1.114	-0.577	0.602
1	1.114	-0.577	0.672
0	1.114	-0.577	0.681
1	1.146	-0.577	0.591
1	1.176	-0.577	0.672
1	1.176	-0.577	0.672
1	1.176	-0.577	0.708
1	1.204	-0.577	0.699
1	1.230	-0.577	0.716
1	1.255	-0.577	0.690
0	1.279	-0.577	0.716
0	1.301	-0.577	0.690
1	1.301	-0.577	0.699

1	1.748	-0.333	0.724
1	1.892	-0.333	0.643
1	1.987	-0.333	0.806
1	2.017	-0.333	0.681
1	2.104	-0.333	0.756
1	2.117	-0.333	0.672
1	2.314	-0.333	0.806
1	2.430	-0.333	0.724
1	2.494	-0.333	0.799
0	2.766	-0.333	0.623
1	0.602	-0.316	0.690
1	0.778	-0.316	0.763
1	0.845	-0.316	0.708
1	0.903	-0.316	0.690
1	0.954	-0.316	0.724
1	1.079	-0.316	0.672
1	1.079	-0.316	0.681
1	1.114	-0.316	0.580
1	1.176	-0.316	0.724
1	1.204	-0.316	0.602
1	1.230	-0.316	0.771
1	1.255	-0.316	0.580
1	1.279	-0.316	0.756
1	1.301	-0.316	0.633
1	1.301	-0.316	0.748
0	1.380	-0.316	0.748
1	1.398	-0.316	0.748
1	1.447	-0.316	0.724
1	1.477	-0.316	0.681
1	1.531	-0.316	0.799
1	1.591	-0.316	0.653
1	1.663	-0.316	0.748
1	1.690	-0.316	0.799
1	1.699	-0.316	0.623
1	1.708	-0.316	0.690
1	1.724	-0.316	0.778
1	1.785	-0.316	0.732
1	1.820	-0.316	0.763
0	1.908	-0.316	0.672
1	1.924	-0.316	0.732
1	2.000	-0.316	0.716
0	2.072	-0.316	0.763
0	2.111	-0.316	0.724
0	2.114	-0.316	0.716
1	2.233	-0.316	0.724
1	2.236	-0.316	0.643
1	2.299	-0.316	0.771
1	2.342	-0.316	0.623
1	2.461	-0.316	0.613
1	2.471	-0.316	0.792
1	2.568	-0.316	0.681

1	1.301	-0.577	0.732
1	1.301	-0.577	0.813
1	1.301	-0.577	0.826
0	1.322	-0.577	0.716
1	1.342	-0.577	0.708
1	1.342	-0.577	0.748
1	1.342	-0.577	0.756
1	1.362	-0.577	0.732
1	1.362	-0.577	0.763
1	1.362	-0.577	0.771
1	1.380	-0.577	0.633
1	1.380	-0.577	0.663
1	1.380	-0.577	0.672
1	1.380	-0.577	0.806
1	1.398	-0.577	0.699
1	1.415	-0.577	0.690
0	1.415	-0.577	0.699
0	1.431	-0.577	0.602
0	1.447	-0.577	0.613
1	1.447	-0.577	0.663
1	1.447	-0.577	0.748
1	1.447	-0.577	0.771
1	1.447	-0.577	0.771
1	1.447	-0.577	0.826
1	1.447	-0.577	0.833
1	1.462	-0.577	0.602
1	1.477	-0.577	0.732
1	1.491	-0.577	0.672
1	1.491	-0.577	0.681
1	1.491	-0.577	0.771
0	1.505	-0.577	0.544
0	1.505	-0.577	0.623
1	1.505	-0.577	0.643
0	1.505	-0.577	0.763
1	1.519	-0.577	0.653
1	1.519	-0.577	0.724
1	1.531	-0.577	0.681
1	1.544	-0.577	0.663
1	1.544	-0.577	0.690
1	1.556	-0.577	0.623
1	1.556	-0.577	0.785
1	1.568	-0.577	0.653
1	1.580	-0.577	0.681
1	1.591	-0.577	0.681
1	1.602	-0.577	0.724
1	1.602	-0.577	0.724
1	1.602	-0.577	0.732
1	1.643	-0.577	0.663
0	1.653	-0.577	0.681
0	1.653	-0.577	0.771
1	1.690	-0.577	0.708
1	2.571	-0.316	0.653
0	2.685	-0.316	0.771
1	0.699	-0.302	0.732
0	0.699	-0.302	0.771
1	1.041	-0.302	0.740
0	1.146	-0.302	0.681
1	1.146	-0.302	0.778
1	1.230	-0.302	0.724
0	1.255	-0.302	0.699
1	1.301	-0.302	0.672
1	1.431	-0.302	0.681
1	1.447	-0.302	0.653
1	1.447	-0.302	0.820
1	1.491	-0.302	0.699
1	1.568	-0.302	0.672
1	1.699	-0.302	0.653
1	1.756	-0.302	0.602
0	1.839	-0.302	0.580
1	1.944	-0.302	0.623
1	2.013	-0.302	0.716
1	2.076	-0.302	0.732
1	2.146	-0.302	0.799
1	2.212	-0.302	0.820
1	2.230	-0.302	0.724
1	2.236	-0.302	0.724
1	2.297	-0.302	0.740
1	2.396	-0.302	0.602
1	2.428	-0.302	0.792
1	2.567	-0.302	0.778
1	2.593	-0.302	0.799
1	0.000	-0.289	0.663
1	0.602	-0.289	0.699
1	0.845	-0.289	0.763
1	1.000	-0.289	0.732
1	1.041	-0.289	0.716
1	1.079	-0.289	0.740
1	1.114	-0.289	0.699
1	1.114	-0.289	0.778
1	1.176	-0.289	0.580
1	1.230	-0.289	0.613
1	1.279	-0.289	0.544
1	1.301	-0.289	0.748
1	1.322	-0.289	0.756
1	1.322	-0.289	0.792
1	1.362	-0.289	0.690
1	1.398	-0.289	0.708
1	1.415	-0.289	0.690
0	1.544	-0.289	0.681
0	1.591	-0.289	0.724
1	1.663	-0.289	0.681
1	1.708	-0.289	0.820

1	1.699	-0.577	0.672
1	1.699	-0.577	0.785
1	1.716	-0.577	0.708
1	1.716	-0.577	0.771
1	1.724	-0.577	0.602
1	1.732	-0.577	0.643
0	1.740	-0.577	0.672
1	1.740	-0.577	0.716
1	1.740	-0.577	0.756
0	1.756	-0.577	0.792
0	1.778	-0.577	0.653
0	1.785	-0.577	0.708
1	1.785	-0.577	0.771
1	1.792	-0.577	0.748
1	1.792	-0.577	0.748
1	1.799	-0.577	0.771
1	1.857	-0.577	0.699
1	1.857	-0.577	0.699
0	1.881	-0.577	0.724
0	1.886	-0.577	0.863
0	1.903	-0.577	0.756
1	1.914	-0.577	0.681
1	1.914	-0.577	0.785
0	1.940	-0.577	0.633
1	1.954	-0.577	0.716
1	1.959	-0.577	0.716
1	1.959	-0.577	0.740
1	1.978	-0.577	0.602
0	1.978	-0.577	0.756
1	2.004	-0.577	0.699
0	2.029	-0.577	0.708
1	2.057	-0.577	0.799
1	2.072	-0.577	0.708
0	2.076	-0.577	0.681
0	2.083	-0.577	0.653
1	2.083	-0.577	0.699
0	2.140	-0.577	0.763
1	2.170	-0.577	0.633
1	2.179	-0.577	0.602
0	2.190	-0.577	0.820
1	2.210	-0.577	0.826
1	2.223	-0.577	0.716
1	2.250	-0.577	0.690
1	2.365	-0.577	0.748
0	2.417	-0.577	0.602
1	2.417	-0.577	0.681
0	2.428	-0.577	0.633
0	2.431	-0.577	0.716
1	2.439	-0.577	0.681
0	2.444	-0.577	0.756
0	2.487	-0.577	0.857
1	1.716	-0.289	0.580
0	1.716	-0.289	0.716
1	1.724	-0.289	0.653
0	1.799	-0.289	0.663
1	1.833	-0.289	0.869
0	1.851	-0.289	0.845
0	1.869	-0.289	0.602
1	1.898	-0.289	0.785
1	1.954	-0.289	0.690
1	2.068	-0.289	0.623
1	2.083	-0.289	0.672
1	2.143	-0.289	0.591
0	2.747	-0.289	0.748
1	0.903	-0.277	0.613
0	0.954	-0.277	0.613
1	1.041	-0.277	0.716
1	1.079	-0.277	0.771
1	1.204	-0.277	0.591
1	1.301	-0.277	0.716
1	1.322	-0.277	0.748
0	1.447	-0.277	0.898
1	1.462	-0.277	0.771
1	1.505	-0.277	0.839
1	1.568	-0.277	0.633
1	1.699	-0.277	0.708
1	1.716	-0.277	0.699
1	2.000	-0.277	0.690
0	2.004	-0.277	0.633
1	2.009	-0.277	0.690
0	2.121	-0.277	0.778
0	2.230	-0.277	0.740
1	2.710	-0.277	0.690
0	2.769	-0.277	0.672
1	0.845	-0.267	0.732
1	0.903	-0.267	0.653
1	0.954	-0.267	0.681
1	1.041	-0.267	0.863
0	1.204	-0.267	0.732
1	1.230	-0.267	0.826
0	1.362	-0.267	0.716
1	1.544	-0.267	0.663
1	1.544	-0.267	0.740
1	1.643	-0.267	0.602
1	1.643	-0.267	0.623
1	1.663	-0.267	0.690
1	1.708	-0.267	0.724
0	1.820	-0.267	0.690
0	1.869	-0.267	0.845
0	1.898	-0.267	0.681
0	1.991	-0.267	0.699
1	2.130	-0.267	0.672

0	2.512	-0.577	0.690
0	2.515	-0.577	0.591
1	2.591	-0.577	0.716
0	2.617	-0.577	0.690
0	2.644	-0.577	0.763
0	2.682	-0.577	0.633
1	2.711	-0.577	0.716
1	2.733	-0.577	0.748
0	0.699	-0.500	0.681
1	0.845	-0.500	0.623
1	0.845	-0.500	0.672
1	0.903	-0.500	0.623
1	0.903	-0.500	0.633
1	0.903	-0.500	0.681
1	0.954	-0.500	0.681
1	0.954	-0.500	0.778
0	1.000	-0.500	0.653
1	1.000	-0.500	0.681
1	1.000	-0.500	0.690
0	1.000	-0.500	0.716
1	1.041	-0.500	0.633
1	1.041	-0.500	0.681
1	1.041	-0.500	0.732
1	1.079	-0.500	0.708
0	1.079	-0.500	0.851
1	1.114	-0.500	0.633
1	1.114	-0.500	0.708
1	1.146	-0.500	0.672
1	1.146	-0.500	0.716
1	1.146	-0.500	0.826
0	1.176	-0.500	0.690
1	1.176	-0.500	0.708
1	1.230	-0.500	0.643
1	1.230	-0.500	0.672
1	1.230	-0.500	0.681
0	1.230	-0.500	0.708
1	1.230	-0.500	0.732
1	1.255	-0.500	0.681
1	1.255	-0.500	0.716
1	1.279	-0.500	0.653
1	1.279	-0.500	0.690
1	1.279	-0.500	0.699
1	1.279	-0.500	0.724
1	1.279	-0.500	0.771
1	1.301	-0.500	0.643
1	1.301	-0.500	0.806
0	1.322	-0.500	0.623
1	1.342	-0.500	0.653
1	1.342	-0.500	0.672
1	1.342	-0.500	0.724
1	1.342	-0.500	0.748
0	2.134	-0.267	0.820
1	2.185	-0.267	0.875
0	2.228	-0.267	0.633
0	2.708	-0.267	0.568
1	0.602	-0.258	0.653
0	0.778	-0.258	0.740
1	0.903	-0.258	0.643
1	1.041	-0.258	0.681
1	1.146	-0.258	0.771
0	1.279	-0.258	0.724
1	1.362	-0.258	0.708
1	1.415	-0.258	0.690
0	1.431	-0.258	0.833
0	1.447	-0.258	0.568
1	1.462	-0.258	0.681
0	1.613	-0.258	0.690
1	1.778	-0.258	0.732
1	1.892	-0.258	0.875
0	2.384	-0.258	0.653
0	2.567	-0.258	0.740
1	2.746	-0.258	0.778
1	1.041	-0.250	0.663
0	1.041	-0.250	0.724
0	1.362	-0.250	0.732
1	1.477	-0.250	0.732
1	1.531	-0.250	0.643
1	1.633	-0.250	0.740
1	1.748	-0.250	0.732
1	1.799	-0.250	0.633
1	1.929	-0.250	0.740
0	1.940	-0.250	0.708
0	2.083	-0.250	0.681
1	2.666	-0.250	0.792
0	0.699	-0.243	0.672
1	1.041	-0.243	0.653
0	1.146	-0.243	0.591
1	1.176	-0.243	0.663
1	1.301	-0.243	0.740
1	1.301	-0.243	0.763
1	1.322	-0.243	0.653
1	2.520	-0.243	0.643
1	1.342	-0.236	0.544
1	1.398	-0.236	0.690
1	1.398	-0.236	0.771
1	1.398	-0.236	0.813
1	1.415	-0.236	0.845
1	1.556	-0.236	0.799
1	1.681	-0.236	0.672
1	1.740	-0.236	0.580
1	1.919	-0.236	0.740
1	2.702	-0.236	0.778

1	1.362	-0.500	0.663
1	1.362	-0.500	0.724
1	1.362	-0.500	0.857
1	1.380	-0.500	0.591
1	1.380	-0.500	0.623
0	1.380	-0.500	0.681
1	1.380	-0.500	0.708
1	1.415	-0.500	0.756
1	1.447	-0.500	0.623
1	1.447	-0.500	0.633
1	1.462	-0.500	0.602
1	1.477	-0.500	0.690
0	1.491	-0.500	0.623
0	1.531	-0.500	0.724
1	1.544	-0.500	0.785
1	1.556	-0.500	0.672
1	1.568	-0.500	0.681
1	1.580	-0.500	0.699
1	1.602	-0.500	0.602
1	1.602	-0.500	0.690
1	1.602	-0.500	0.740
1	1.623	-0.500	0.643
1	1.623	-0.500	0.806
1	1.633	-0.500	0.681
1	1.633	-0.500	0.763
1	1.643	-0.500	0.716
0	1.643	-0.500	0.748
1	1.708	-0.500	0.716
1	1.716	-0.500	0.732
1	1.732	-0.500	0.708
0	1.748	-0.500	0.672
1	1.756	-0.500	0.663
1	1.756	-0.500	0.690
0	1.763	-0.500	0.591
1	1.792	-0.500	0.708
1	1.792	-0.500	0.748
0	1.851	-0.500	0.663
1	1.881	-0.500	0.763
1	1.886	-0.500	0.643
1	1.892	-0.500	0.748
1	1.908	-0.500	0.681
1	1.934	-0.500	0.724
1	1.944	-0.500	0.672
0	1.954	-0.500	0.623
1	1.964	-0.500	0.613
1	1.964	-0.500	0.708
1	2.025	-0.500	0.732
1	2.049	-0.500	0.690
0	2.134	-0.500	0.799
0	2.137	-0.500	0.633
0	2.155	-0.500	0.813
0	0.301	-0.229	0.643
1	0.903	-0.229	0.643
0	1.230	-0.229	0.690
1	1.398	-0.229	0.623
0	1.398	-0.229	0.813
1	1.477	-0.229	0.708
0	1.544	-0.229	0.623
0	2.281	-0.229	0.886
0	2.299	-0.229	0.623
0	2.697	-0.229	0.544
1	1.114	-0.224	0.602
0	1.146	-0.224	0.623
1	1.301	-0.224	0.672
1	1.342	-0.224	0.724
1	1.362	-0.224	0.681
1	1.505	-0.224	0.663
1	1.623	-0.224	0.763
0	1.740	-0.224	0.690
1	2.009	-0.224	0.716
1	2.386	-0.224	0.708
0	2.394	-0.224	0.826
1	2.497	-0.224	0.792
1	0.845	-0.218	0.833
1	0.903	-0.218	0.663
1	1.000	-0.218	0.778
1	1.322	-0.218	0.740
1	1.322	-0.218	0.740
1	1.602	-0.218	0.633
0	1.724	-0.218	0.778
1	1.756	-0.218	0.653
0	1.919	-0.218	0.690
1	1.146	-0.213	0.681
0	1.176	-0.213	0.591
1	1.204	-0.213	0.672
1	1.380	-0.213	0.748
1	1.505	-0.213	0.708
0	1.580	-0.213	0.672
1	1.851	-0.213	0.623
0	1.954	-0.213	0.724
1	2.130	-0.213	0.839
0	2.410	-0.213	0.826
0	2.601	-0.213	0.643
1	0.954	-0.209	0.633
1	1.342	-0.209	0.820
0	1.380	-0.209	0.623
1	1.519	-0.209	0.724
1	1.898	-0.209	0.602
0	2.161	-0.209	0.708
0	2.483	-0.209	0.785
1	1.079	-0.204	0.653
0	1.114	-0.204	0.591

1	2.167	-0.500	0.716
1	2.207	-0.500	0.663
1	2.217	-0.500	0.681
0	2.253	-0.500	0.820
1	2.260	-0.500	0.672
1	2.279	-0.500	0.672
0	2.288	-0.500	0.813
1	2.307	-0.500	0.724
1	2.310	-0.500	0.778
0	2.338	-0.500	0.763
1	2.362	-0.500	0.813
1	2.410	-0.500	0.663
0	2.498	-0.500	0.875
1	2.528	-0.500	0.732
0	2.555	-0.500	0.724
0	2.622	-0.500	0.716
1	2.701	-0.500	0.556
0	2.703	-0.500	0.778
0	2.735	-0.500	0.820
0	2.744	-0.500	0.653
1	0.477	-0.447	0.653
1	0.477	-0.447	0.740
1	0.845	-0.447	0.716
1	0.903	-0.447	0.613
0	0.903	-0.447	0.724
1	0.903	-0.447	0.792
1	0.954	-0.447	0.663
1	0.954	-0.447	0.681
0	1.041	-0.447	0.724
1	1.079	-0.447	0.623
1	1.079	-0.447	0.681
1	1.079	-0.447	0.724
1	1.114	-0.447	0.633
1	1.114	-0.447	0.663
1	1.146	-0.447	0.681
1	1.146	-0.447	0.699
1	1.146	-0.447	0.732
1	1.176	-0.447	0.623
1	1.176	-0.447	0.623
0	1.176	-0.447	0.708
1	1.204	-0.447	0.653
0	1.204	-0.447	0.839
1	1.204	-0.447	0.892
0	1.230	-0.447	0.653
1	1.230	-0.447	0.681
1	1.230	-0.447	0.708
1	1.255	-0.447	0.716
1	1.255	-0.447	0.740
1	1.279	-0.447	0.633
1	1.279	-0.447	0.690
1	1.301	-0.447	0.633
0	1.255	-0.204	0.633
0	1.342	-0.204	0.699
1	1.431	-0.204	0.756
1	1.431	-0.204	0.820
1	1.556	-0.204	0.672
0	1.591	-0.204	0.740
1	1.643	-0.204	0.724
1	1.699	-0.204	0.653
1	1.041	-0.200	0.681
0	1.462	-0.200	0.681
1	1.505	-0.200	0.623
1	1.505	-0.200	0.740
1	1.519	-0.200	0.708
1	1.653	-0.200	0.633
1	2.130	-0.200	0.799
0	2.350	-0.200	0.732
0	0.301	-0.196	0.833
0	0.778	-0.196	0.785
1	0.903	-0.196	0.653
1	1.230	-0.196	0.672
1	1.230	-0.196	0.756
1	1.230	-0.196	0.799
1	1.301	-0.196	0.716
1	1.415	-0.196	0.672
1	1.613	-0.196	0.663
1	2.143	-0.196	0.799
1	2.508	-0.196	0.833
1	1.230	-0.192	0.690
0	1.230	-0.192	0.857
1	1.279	-0.192	0.748
1	1.362	-0.192	0.602
1	1.431	-0.192	0.699
0	2.017	-0.192	0.763
1	2.290	-0.192	0.740
1	2.749	-0.192	0.613
1	1.146	-0.189	0.681
0	2.134	-0.189	0.690
1	2.167	-0.189	0.690
1	2.609	-0.189	0.699
0	1.000	-0.186	0.591
1	1.114	-0.186	0.748
1	0.903	-0.183	0.763
1	0.954	-0.183	0.663
1	1.748	-0.183	0.708
0	1.826	-0.183	0.708
1	2.422	-0.183	0.708
1	1.362	-0.180	0.771
1	1.398	-0.180	0.690
0	1.415	-0.180	0.633
0	1.477	-0.180	0.724
1	1.771	-0.180	0.708

1	1.301	-0.447	0.663
1	1.301	-0.447	0.663
1	1.322	-0.447	0.623
1	1.322	-0.447	0.633
0	1.322	-0.447	0.690
1	1.322	-0.447	0.792
1	1.342	-0.447	0.681
1	1.342	-0.447	0.699
1	1.342	-0.447	0.732
1	1.362	-0.447	0.690
1	1.380	-0.447	0.672
1	1.380	-0.447	0.708
1	1.398	-0.447	0.653
1	1.398	-0.447	0.690
1	1.398	-0.447	0.699
1	1.398	-0.447	0.740
1	1.415	-0.447	0.602
1	1.415	-0.447	0.681
1	1.431	-0.447	0.591
1	1.462	-0.447	0.672
1	1.462	-0.447	0.681
1	1.477	-0.447	0.690
0	1.491	-0.447	0.813
1	1.505	-0.447	0.643
1	1.519	-0.447	0.681
1	1.519	-0.447	0.724
0	1.531	-0.447	0.602
1	1.531	-0.447	0.672
1	1.556	-0.447	0.633
0	1.556	-0.447	0.799
0	1.568	-0.447	0.633
1	1.580	-0.447	0.663
0	1.591	-0.447	0.663
1	1.613	-0.447	0.672
1	1.613	-0.447	0.732
1	1.623	-0.447	0.839
0	1.663	-0.447	0.716
1	1.672	-0.447	0.672
1	1.690	-0.447	0.653
1	1.690	-0.447	0.672
1	1.699	-0.447	0.643
0	1.699	-0.447	0.663
0	1.699	-0.447	0.716
1	1.699	-0.447	0.716
1	1.708	-0.447	0.653
1	1.716	-0.447	0.763
0	1.724	-0.447	0.699
0	1.763	-0.447	0.672
1	1.763	-0.447	0.681
1	1.778	-0.447	0.690
1	1.785	-0.447	0.763
1	1.820	-0.180	0.748
0	2.013	-0.180	0.643
0	2.387	-0.180	0.839
1	2.516	-0.180	0.672
0	0.699	-0.177	0.806
0	1.447	-0.177	0.833
0	1.732	-0.177	0.613
1	2.420	-0.177	0.756
1	1.255	-0.174	0.763
1	1.602	-0.174	0.623
1	2.678	-0.174	0.699
1	0.301	-0.171	0.732
0	0.903	-0.171	0.663
1	1.690	-0.171	0.643
1	1.934	-0.171	0.826
1	1.301	-0.169	0.724
1	1.301	-0.169	0.778
1	1.580	-0.169	0.681
1	1.914	-0.169	0.724
0	2.496	-0.169	0.681
0	1.041	-0.167	0.799
1	1.114	-0.167	0.690
1	1.886	-0.167	0.663
0	2.000	-0.167	0.690
1	2.199	-0.167	0.857
1	1.000	-0.164	0.771
1	2.134	-0.164	0.763
0	1.431	-0.162	0.792
1	1.447	-0.162	0.699
1	1.875	-0.162	0.690
1	1.987	-0.162	0.771
0	1.991	-0.162	0.568
0	2.766	-0.162	0.633
1	1.633	-0.160	0.724
1	2.025	-0.160	0.806
1	2.107	-0.160	0.724
1	1.732	-0.158	0.708
0	1.322	-0.156	0.613
1	2.086	-0.156	0.785
0	2.565	-0.154	0.851
0	1.079	-0.152	0.851
0	1.342	-0.152	0.799
1	1.633	-0.152	0.580
1	1.633	-0.152	0.672
0	1.908	-0.152	0.690
0	2.318	-0.152	0.613
1	1.580	-0.151	0.623
0	2.290	-0.151	0.613
1	1.146	-0.149	0.602
1	2.000	-0.149	0.756
1	2.262	-0.149	0.732

0	1.833	-0.447	0.792
1	1.851	-0.447	0.643
0	1.851	-0.447	0.672
0	1.869	-0.447	0.568
1	1.869	-0.447	0.716
1	1.924	-0.447	0.724
1	1.940	-0.447	0.716
1	1.949	-0.447	0.771
0	2.013	-0.447	0.690
1	2.021	-0.447	0.708
0	2.037	-0.447	0.820
1	2.167	-0.447	0.681
1	2.170	-0.447	0.748
1	2.188	-0.447	0.716
0	2.217	-0.447	0.613
1	2.255	-0.447	0.672
1	2.255	-0.447	0.681
1	2.276	-0.447	0.708
0	2.297	-0.447	0.681
1	2.303	-0.447	0.663
1	2.338	-0.447	0.690
0	2.350	-0.447	0.633
1	2.356	-0.447	0.591
0	2.356	-0.447	0.820
0	2.367	-0.447	0.732
1	2.371	-0.447	0.672
0	2.400	-0.447	0.623
1	2.410	-0.447	0.672
1	2.417	-0.447	0.699
1	2.418	-0.447	0.785
1	2.423	-0.447	0.799
1	2.467	-0.447	0.748
0	2.496	-0.447	0.672
1	2.542	-0.447	0.792
0	2.548	-0.447	0.672
0	2.563	-0.447	0.613
1	2.575	-0.447	0.724
0	2.660	-0.447	0.544
1	2.692	-0.447	0.690
0	2.709	-0.447	0.732
1	0.000	-0.408	0.681
1	0.778	-0.408	0.699
1	0.845	-0.408	0.716
1	0.903	-0.408	0.653
1	0.903	-0.408	0.732
0	0.954	-0.408	0.663
1	0.954	-0.408	0.681
1	1.000	-0.408	0.663
1	1.000	-0.408	0.681
1	1.041	-0.408	0.643
1	1.079	-0.408	0.724
1	1.613	-0.147	0.740
1	2.223	-0.147	0.568
1	0.301	-0.146	0.681
0	0.954	-0.146	0.740
0	2.759	-0.146	0.792
1	1.041	-0.144	0.732
1	1.362	-0.144	0.716
1	1.763	-0.144	0.740
0	1.799	-0.144	0.806
1	1.079	-0.143	0.643
1	1.230	-0.143	0.708
1	1.519	-0.143	0.602
1	1.771	-0.143	0.681
1	1.914	-0.143	0.708
0	2.029	-0.143	0.699
1	1.362	-0.141	0.633
0	2.504	-0.141	0.839
1	1.176	-0.140	0.613
1	2.225	-0.140	0.623
1	1.519	-0.139	0.732
1	2.480	-0.139	0.681
1	1.362	-0.137	0.633
1	2.093	-0.137	0.699
1	2.167	-0.136	0.756
1	2.255	-0.136	0.690
1	1.362	-0.135	0.663
0	1.732	-0.135	0.643
0	2.420	-0.135	0.756
1	1.531	-0.134	0.748
0	1.531	-0.134	0.756
1	2.100	-0.132	0.778
1	1.204	-0.131	0.740
1	1.568	-0.130	0.663
0	1.491	-0.129	0.826
1	1.851	-0.129	0.663
1	2.121	-0.129	0.699
1	1.079	-0.127	0.643
1	1.653	-0.127	0.613
0	0.954	-0.126	0.633
1	1.176	-0.126	0.724
1	1.176	-0.125	0.740
1	1.301	-0.125	0.672
1	1.301	-0.125	0.724
1	1.580	-0.125	0.672
1	1.892	-0.125	0.663
0	1.362	-0.124	0.690
1	0.699	-0.123	0.672
1	1.633	-0.123	0.699
1	1.833	-0.122	0.690
0	2.382	-0.121	0.613
0	1.041	-0.120	0.633

1	1.114	-0.408	0.613
1	1.114	-0.408	0.763
1	1.146	-0.408	0.591
0	1.176	-0.408	0.613
0	1.176	-0.408	0.681
1	1.230	-0.408	0.672
1	1.230	-0.408	0.699
0	1.230	-0.408	0.732
0	1.230	-0.408	0.748
1	1.255	-0.408	0.623
1	1.255	-0.408	0.716
1	1.279	-0.408	0.613
1	1.279	-0.408	0.653
1	1.301	-0.408	0.633
1	1.301	-0.408	0.681
1	1.322	-0.408	0.623
1	1.380	-0.408	0.653
1	1.380	-0.408	0.778
1	1.398	-0.408	0.785
0	1.398	-0.408	0.839
0	1.415	-0.408	0.756
1	1.431	-0.408	0.699
1	1.462	-0.408	0.672
1	1.477	-0.408	0.716
1	1.477	-0.408	0.748
1	1.519	-0.408	0.716
1	1.568	-0.408	0.653
1	1.568	-0.408	0.716
1	1.580	-0.408	0.681
1	1.580	-0.408	0.699
1	1.580	-0.408	0.771
1	1.591	-0.408	0.643
1	1.602	-0.408	0.813
1	1.613	-0.408	0.839
1	1.633	-0.408	0.690
1	1.653	-0.408	0.690
1	1.663	-0.408	0.681
0	1.672	-0.408	0.643
1	1.690	-0.408	0.602
0	1.690	-0.408	0.663
1	1.690	-0.408	0.708
0	1.699	-0.408	0.602
1	1.716	-0.408	0.771
1	1.732	-0.408	0.643
1	1.732	-0.408	0.724
1	1.740	-0.408	0.613
1	1.748	-0.408	0.672
1	1.771	-0.408	0.681
1	1.792	-0.408	0.643
1	1.813	-0.408	0.633
0	1.833	-0.408	0.672
0	2.130	-0.120	0.699
1	2.305	-0.120	0.681
0	1.230	-0.120	0.623
1	1.792	-0.119	0.690
1	1.301	-0.117	0.653
0	2.004	-0.117	0.681
1	1.681	-0.116	0.699
1	0.954	-0.115	0.690
1	1.079	-0.115	0.740
0	1.114	-0.115	0.613
0	2.068	-0.115	0.699
0	2.167	-0.113	0.623
0	1.602	-0.113	0.663
0	0.699	-0.112	0.623
1	1.415	-0.112	0.771
1	1.477	-0.112	0.792
0	2.053	-0.112	0.708
0	1.398	-0.110	0.778
1	2.560	-0.110	0.833
0	1.633	-0.110	0.699
1	1.380	-0.108	0.748
1	2.079	-0.108	0.778
1	1.857	-0.107	0.820
1	2.377	-0.107	0.813
1	2.446	-0.107	0.708
1	2.017	-0.106	0.785
1	1.903	-0.105	0.716
1	1.531	-0.105	0.690
0	1.778	-0.105	0.672
0	2.632	-0.105	0.643
0	0.903	-0.104	0.623
1	1.462	-0.104	0.740
1	1.204	-0.103	0.724
0	1.462	-0.103	0.724
1	1.869	-0.103	0.740
1	1.875	-0.102	0.732
0	2.679	-0.102	0.839
1	1.362	-0.102	0.771
1	2.727	-0.102	0.633
0	1.602	-0.101	0.681
1	1.230	-0.100	0.732
1	2.436	-0.100	0.756
1	1.114	-0.099	0.724
1	1.146	-0.099	0.681
0	1.462	-0.099	0.792
1	1.724	-0.096	0.708
1	2.086	-0.096	0.740
0	1.863	-0.095	0.778
1	2.563	-0.095	0.708
0	1.903	-0.094	0.613
1	1.633	-0.094	0.699

0	1.869	-0.408	0.623
1	1.875	-0.408	0.771
0	1.881	-0.408	0.672
1	1.886	-0.408	0.763
1	1.908	-0.408	0.756
1	1.949	-0.408	0.699
1	1.959	-0.408	0.690
1	1.968	-0.408	0.820
1	2.033	-0.408	0.568
1	2.045	-0.408	0.681
1	2.076	-0.408	0.708
1	2.167	-0.408	0.653
1	2.176	-0.408	0.833
0	2.193	-0.408	0.643
1	2.220	-0.408	0.839
1	2.233	-0.408	0.623
1	2.303	-0.408	0.763
0	2.310	-0.408	0.708
0	2.378	-0.408	0.839
1	2.418	-0.408	0.653
1	2.422	-0.408	0.771
0	2.431	-0.408	0.716
0	2.505	-0.408	0.763
0	2.551	-0.408	0.740
0	2.610	-0.408	0.672
0	2.719	-0.408	0.531
1	2.750	-0.408	0.820
1	2.772	-0.408	0.792
0	0.778	-0.378	0.792
0	0.845	-0.378	0.568
1	0.845	-0.378	0.681
0	0.845	-0.378	0.740
1	0.903	-0.378	0.643
1	0.903	-0.378	0.820
1	1.000	-0.378	0.643
1	1.000	-0.378	0.699
1	1.041	-0.378	0.756
1	1.079	-0.378	0.740
1	1.114	-0.378	0.633
1	1.114	-0.378	0.633
1	1.114	-0.378	0.663
1	1.114	-0.378	0.748
1	1.146	-0.378	0.681
0	1.146	-0.378	0.792
1	1.176	-0.378	0.613
1	1.176	-0.378	0.690
1	1.204	-0.378	0.623
1	1.204	-0.378	0.724
1	1.204	-0.378	0.748
0	1.255	-0.378	0.732
1	1.255	-0.378	0.813
0	1.447	-0.094	0.663
1	2.029	-0.094	0.623
0	2.438	-0.093	0.740
0	1.041	-0.092	0.724
0	1.146	-0.092	0.708
1	1.708	-0.091	0.663
0	2.021	-0.091	0.602
0	2.483	-0.091	0.724
1	1.146	-0.090	0.845
1	0.778	-0.090	0.653
0	2.107	-0.090	0.690
0	1.690	-0.089	0.690
1	1.568	-0.089	0.663
1	1.708	-0.088	0.708
0	1.881	-0.087	0.857
1	1.886	-0.087	0.653
0	1.740	-0.087	0.699
1	1.681	-0.084	0.613
1	1.740	-0.084	0.778
1	1.531	-0.081	0.681
0	1.792	-0.081	0.663
1	1.663	-0.081	0.724
0	2.537	-0.081	0.643
1	1.568	-0.080	0.690
1	2.338	-0.080	0.845
1	1.447	-0.078	0.681
0	2.639	-0.078	0.690
0	2.255	-0.077	0.633
1	1.623	-0.076	0.724
0	1.230	-0.073	0.690
1	2.452	-0.073	0.748
1	1.342	-0.073	0.556
1	1.204	-0.071	0.643
1	1.799	-0.071	0.708
1	1.613	-0.071	0.699
1	2.459	-0.071	0.716
1	1.146	-0.068	0.732
1	1.462	-0.067	0.748
0	1.322	-0.066	0.716
0	0.778	-0.066	0.643
1	1.398	-0.065	0.643
1	0.903	-0.063	0.623
1	2.513	-0.063	0.699
1	1.663	-0.062	0.663
1	1.903	-0.060	0.813
1	1.699	-0.060	0.653
1	0.845	-0.060	0.708
1	2.418	-0.059	0.653
1	1.869	-0.059	0.613
1	1.919	-0.058	0.785
1	1.556	-0.056	0.633

0	1.301	-0.378	0.708
1	1.322	-0.378	0.613
1	1.322	-0.378	0.633
0	1.322	-0.378	0.681
1	1.322	-0.378	0.724
1	1.322	-0.378	0.756
0	1.362	-0.378	0.690
1	1.362	-0.378	0.699
1	1.362	-0.378	0.708
1	1.362	-0.378	0.799
1	1.380	-0.378	0.623
1	1.415	-0.378	0.681
0	1.415	-0.378	0.699
1	1.415	-0.378	0.771
1	1.431	-0.378	0.643
1	1.431	-0.378	0.681
1	1.431	-0.378	0.699
1	1.462	-0.378	0.699
1	1.462	-0.378	0.748
0	1.477	-0.378	0.724
1	1.491	-0.378	0.690
0	1.519	-0.378	0.556
1	1.519	-0.378	0.740
1	1.531	-0.378	0.672
1	1.531	-0.378	0.716
1	1.544	-0.378	0.653
1	1.556	-0.378	0.623
1	1.556	-0.378	0.813
0	1.602	-0.378	0.591
1	1.602	-0.378	0.653
0	2.425	-0.055	0.690
1	1.973	-0.055	0.756
1	1.000	-0.054	0.740
1	0.699	-0.053	0.778
1	1.146	-0.053	0.568
0	1.924	-0.052	0.623
1	0.301	-0.051	0.748
0	2.332	-0.051	0.556
1	0.477	-0.050	0.756
1	1.230	-0.050	0.716
1	2.049	-0.050	0.740
1	1.869	-0.049	0.672
0	1.771	-0.048	0.806
1	1.898	-0.048	0.763
0	1.279	-0.047	0.681
0	2.149	-0.047	0.732
1	1.255	-0.046	0.813
0	2.064	-0.045	0.826
0	1.845	-0.045	0.699
1	1.079	-0.044	0.756
1	1.230	-0.044	0.708
1	1.342	-0.043	0.699
0	2.723	-0.042	0.724
0	1.431	-0.042	0.672
0	1.415	-0.042	0.602
0	1.255	-0.041	0.740
0	1.415	-0.034	0.681
0	1.763	-0.027	0.792
1	1.204	-0.027	0.672

Appendix 6 – Chapter 8

Columns are (left to right) sex (**m**ale/**f**emale), alga exposed to (***L**aminaria* / ***F**ucus*), longevity (days), wing length (mm) and number of flies of the opposite sex present.

M	L	18	5.6	0	M	F	14	6.2	3
M	L	1	5.7	0	M	F	14	5.8	3
M	L	21	6.8	0	M	F	9	5.1	3
M	L	6	4.4	0	M	F	16	6.1	3
M	L	24	6.5	0	M	F	27	7.1	3
M	L	6	7.0	0	M	F	9	5.2	3
M	L	1	6.0	0	M	F	28	6.0	3
M	L	26	6.5	0	M	F	15	6.9	3
M	L	6	5.4	0	M	F	8	5.5	3
M	L	15	6.2	0	M	F	2	6.2	3
M	L	2	6.5	0	M	F	14	6.3	3
M	L	42	5.0	0	M	F	13	6.6	3
M	L	14	5.8	0	M	F	19	6.8	3
M	L	19	5.7	0	M	F	15	6.7	3
M	L	12	6.1	0	M	F	14	6.4	3
M	L	13	4.1	0	M	F	5	5.8	3
M	L	13	6.2	0	M	F	12	7.1	3
M	L	2	6.1	0	M	F	15	6.7	3
M	L	33	4.9	0	M	F	12	6.2	3
M	L	39	4.8	0	M	F	28	6.4	3
M	L	15	6.9	0	M	F	13	5.5	3
M	L	5	6.7	0	M	F	22	6.5	3
M	L	24	5.1	0	M	F	18	6.1	3
M	L	14	5.2	0	M	F	13	6.4	3
M	L	15	4.9	0	M	F	13	7.0	3
M	L	31	5.6	0	M	F	13	6.8	3
M	L	18	4.8	0	M	F	13	6.1	3
M	L	23	5.3	0	M	F	14	4.6	3
M	L	15	6.0	0	M	F	13	5.8	3
M	L	44	5.9	0	M	F	14	6.1	3
M	L	16	5.8	0	F	F	15	6.3	1
M	L	13	5.4	0	F	F	18	5.6	1
M	L	9	5.8	0	F	F	12	6.0	1
M	L	38	5.0	0	F	F	27	4.9	1
M	L	3	6.2	0	F	F	3	5.1	1
M	L	13	6.1	0	F	F	18	5.7	1
M	L	16	6.7	0	F	F	19	5.4	1
M	L	9	4.9	0	F	F	20	5.6	1
M	L	6	4.6	0	F	F	25	5.9	1
M	L	36	3.9	0	F	F	13	4.9	1
M	L	23	5.9	0	F	F	18	5.1	1
M	L	30	5.2	0	F	F	15	5.3	1
M	L	17	6.6	0	F	F	12	5.6	1
M	L	35	4.5	0	F	F	15	5.2	1
M	L	38	4.9	0	F	F	24	5.0	1

M	L	11	5.5	0
M	L	34	4.3	0
M	L	20	5.3	0
M	L	16	6.7	0
M	F	31	6.8	0
M	F	25	5.8	0
M	F	8	5.7	0
M	F	58	4.1	0
M	F	38	5.7	0
M	F	1	5.1	0
M	F	27	6.2	0
M	F	1	6.1	0
M	F	41	4.4	0
M	F	25	6.7	0
M	F	27	6.1	0
M	F	45	4.5	0
M	F	29	5.5	0
M	F	3	6.3	0
M	F	2	6.2	0
M	F	15	6.5	0
M	F	21	6.9	0
M	F	2	5.3	0
M	F	29	5.1	0
M	F	16	7.0	0
M	F	12	6.2	0
M	F	1	5.8	0
M	F	15	5.6	0
M	F	3	6.0	0
M	F	57	4.6	0
M	F	10	4.7	0
M	F	62	6.1	0
M	F	1	5.9	0
M	F	22	6.1	0
M	F	15	4.7	0
M	F	31	6.1	0
M	F	43	6.0	0
M	F	17	4.6	0
M	F	37	6.3	0
M	F	10	4.3	0
M	F	49	5.4	0
M	F	7	6.1	0
M	F	45	5.0	0
M	F	1	5.5	0
M	F	16	6.1	0
M	F	37	4.7	0
M	F	21	5.9	0
M	F	7	5.8	0
M	F	51	5.2	0
M	F	26	5.1	0
M	F	9	6.0	0
M	F	59	4.8	0
M	F	8	5.7	0
M	F	16	5.5	0
F	L	46	5.6	0
F	F	29	5.4	1
F	F	15	5.1	1
F	F	24	5.8	1
F	F	15	4.8	1
F	F	14	5.2	1
F	F	10	5.2	1
F	F	2	4.4	1
F	F	10	5.4	1
F	F	1	5.5	1
F	F	5	5.5	1
F	F	1	6.1	1
F	F	18	4.1	1
F	F	10	5.5	1
F	F	19	5.1	1
F	F	19	5.7	1
F	F	17	5.3	1
F	F	14	5.2	1
F	F	26	6.0	1
F	F	2	5.5	3
F	F	2	4.2	3
F	F	2	5.5	3
F	F	4	5.8	3
F	F	16	5.6	3
F	F	9	5.5	3
F	F	15	5.4	3
F	F	21	5.1	3
F	F	13	5.0	3
F	F	10	5.3	3
F	F	13	5.5	3
F	F	11	5.3	3
F	F	11	5.1	3
F	F	14	5.9	3
F	F	12	4.9	3
F	F	2	5.0	3
F	F	13	5.3	3
F	F	13	5.5	3
F	F	4	5.8	3
F	F	11	5.6	3
F	F	17	4.7	3
F	F	15	4.9	3
F	F	9	5.6	3
F	F	12	5.9	3
F	F	13	6.1	3
F	F	12	5.7	3
F	F	12	5.0	3
F	F	14	5.1	3
F	F	16	5.2	3
F	F	11	5.7	3
F	F	1	5.5	3
F	F	19	5.8	3
F	F	14	5.1	3
F	F	15	5.2	3
M	L	7	6.2	1
M	L	13	6.3	1

F	L	42	5.0	0
F	L	3	5.3	0
F	L	17	5.2	0
F	L	1	5.1	0
F	L	16	5.2	0
F	L	23	6.1	0
F	L	33	5.4	0
F	L	16	4.4	0
F	L	24	5.9	0
F	L	31	5.8	0
F	L	38	5.1	0
F	L	2	5.4	0
F	L	38	4.7	0
F	L	51	5.2	0
F	L	9	4.7	0
F	L	57	4.7	0
F	L	38	4.8	0
F	L	34	5.6	0
F	L	30	5.0	0
F	L	1	5.5	0
F	L	17	4.9	0
F	L	7	5.0	0
F	L	27	4.1	0
F	L	48	4.9	0
F	L	6	4.2	0
F	L	1	5.3	0
F	L	51	4.6	0
F	L	2	4.2	0
F	L	16	4.8	0
F	L	42	5.1	0
F	L	2	5.2	0
F	L	31	5.1	0
F	L	33	4.6	0
F	L	4	5.2	0
F	L	1	4.6	0
F	L	13	4.8	0
F	L	12	4.9	0
F	L	22	4.9	0
F	L	37	5.7	0
F	L	23	3.9	0
F	L	37	5.3	0
F	L	10	5.1	0
F	L	17	4.7	0
F	L	28	5.0	0
F	L	38	5.8	0
F	L	18	4.8	0
F	L	2	4.7	0
F	L	46	4.8	0
F	L	16	5.4	0
F	F	15	5.5	0
F	F	53	4.6	0
F	F	60	5.1	0
F	F	38	5.2	0
F	F	2	3.6	0

M	L	6	6.5	1
M	L	4	6.1	1
M	L	6	6.4	1
M	L	6	6.5	1
M	L	5	5.1	1
M	L	6	6.5	1
M	L	5	6.2	1
M	L	1	6.3	1
M	L	12	6.6	1
M	L	6	6.8	1
M	L	7	5.8	1
M	L	0	6.7	1
M	L	11	6.2	1
M	L	6	5.5	1
M	L	3	6.6	1
M	L	5	6.1	1
M	L	7	6.2	1
M	L	3	6.0	1
M	L	10	5.3	1
M	L	9	5.8	1
M	L	5	5.9	1
M	L	5	5.4	1
M	L	10	6.2	1
M	L	8	5.5	1
M	L	7	6.0	1
M	L	12	5.3	1
M	L	6	7.1	1
M	L	6	6.1	1
M	L	7	5.1	1
M	L	6	5.6	1
M	L	3	5.8	1
M	L	3	5.5	1
M	L	3	7.0	1
M	L	6	6.5	3
M	L	11	5.6	3
M	L	10	5.3	3
M	L	9	5.2	3
M	L	7	6.1	3
M	L	3	6.3	3
M	L	3	6.4	3
M	L	2	6.3	3
M	L	10	5.3	3
M	L	2	5.2	3
M	L	5	5.4	3
M	L	5	7.0	3
M	L	1	5.2	3
M	L	3	5.0	3
M	L	7	5.2	3
M	L	6	5.4	3
M	L	7	6.3	3
M	L	10	5.8	3
M	L	6	6.2	3
M	L	6	6.8	3
M	L	6	7.1	3

F	F	21	5.2	0
F	F	32	5.3	0
F	F	28	5.2	0
F	F	50	5.1	0
F	F	44	5.5	0
F	F	48	5.6	0
F	F	42	5.2	0
F	F	40	5.2	0
F	F	2	5.2	0
F	F	62	5.5	0
F	F	18	4.8	0
F	F	21	5.3	0
F	F	49	5.4	0
F	F	63	4.9	0
F	F	38	4.8	0
F	F	32	5.1	0
F	F	28	5.5	0
F	F	72	4.5	0
F	F	20	5.8	0
F	F	42	5.9	0
F	F	38	4.9	0
F	F	11	4.8	0
F	F	28	5.3	0
F	F	56	5.6	0
F	F	1	5.4	0
F	F	36	5.5	0
F	F	16	5.3	0
F	F	58	4.5	0
F	F	28	4.7	0
F	F	12	4.8	0
F	F	29	4.4	0
F	F	44	5.1	0
F	F	20	5.3	0
F	F	58	4.8	0
F	F	2	4.8	0
F	F	60	5.5	0
F	F	19	5.3	0
F	F	2	5.3	0
F	F	29	5.2	0
F	F	55	5.5	0
F	F	57	5.1	0
F	F	2	4.6	0
F	F	17	5.4	0
F	F	47	5.3	0
M	F	18	5.9	1
M	F	6	5.2	1
M	F	20	6.1	1
M	F	15	6.3	1
M	F	11	7.2	1
M	F	14	6.2	1
M	F	17	5.9	1
M	F	0	5.2	1
M	F	17	6.5	1
M	F	17	6.6	1
M	L	2	6.7	3
M	L	6	6.6	3
M	L	1	6.2	3
M	L	5	4.8	3
M	L	5	5.7	3
M	L	4	6.1	3
M	L	7	6.7	3
M	L	2	5.3	3
M	L	8	4.7	3
M	L	6	4.5	3
M	L	3	6.2	3
M	L	8	4.8	3
M	L	5	6.2	3
M	L	7	5.7	3
F	L	5	5.4	1
F	L	5	4.7	1
F	L	6	5.1	1
F	L	6	5.6	1
F	L	3	6.3	1
F	L	4	5.2	1
F	L	6	5.4	1
F	L	2	5.8	1
F	L	6	5.3	1
F	L	3	6.3	1
F	L	6	6.0	1
F	L	5	4.9	1
F	L	2	4.8	1
F	L	12	4.8	1
F	L	14	5.7	1
F	L	12	5.4	1
F	L	13	5.2	1
F	L	7	5.1	1
F	L	7	5.6	1
F	L	12	6.1	1
F	L	1	4.6	1
F	L	6	5.2	1
F	L	6	5.1	1
F	L	4	4.9	1
F	L	6	5.6	1
F	L	6	5.6	1
F	L	6	4.9	1
F	L	2	5.4	1
F	L	7	5.8	1
F	L	3	4.4	1
F	L	3	5.7	1
F	L	3	5.2	1
F	L	6	4.5	1
F	L	0	5.1	1
F	L	5	5.2	1
F	L	4	5.5	3
F	L	3	5.2	3
F	L	2	4.6	3
F	L	11	5.2	3
F	L	9	5.1	3

M	F	17	4.9	1
M	F	2	6.6	1
M	F	19	5.2	1
M	F	21	6.4	1
M	F	15	5.1	1
M	F	12	5.2	1
M	F	11	5.9	1
M	F	18	4.5	1
M	F	27	5.8	1
M	F	8	6.1	1
M	F	29	6.6	1
M	F	14	6.2	1
M	F	15	5.2	1
M	F	7	5.8	1
M	F	16	5.7	1
M	F	12	5.6	1
M	F	22	5.3	1
M	F	12	6.3	1
M	F	29	5.9	1
M	F	16	6.5	1
M	F	15	6.4	1
M	F	12	6.0	1
M	F	18	4.8	1
M	F	16	6.6	1
M	F	1	6.3	1
M	F	8	6.0	3
M	F	13	6.6	3
M	F	20	5.3	3
M	F	13	6.1	3
M	F	17	4.8	3
F	L	14	5.6	3
F	L	9	4.9	3
F	L	3	4.8	3
F	L	4	5.3	3
F	L	2	5.0	3
F	L	3	5.1	3
F	L	10	4.8	3
F	L	13	4.3	3
F	L	3	5.2	3
F	L	7	4.6	3
F	L	12	4.5	3
F	L	3	5.5	3
F	L	6	6.0	3
F	L	13	4.9	3
F	L	6	6.1	3
F	L	7	5.4	3
F	L	11	5.6	3
F	L	1	4.7	3
F	L	5	5.4	3
F	L	10	4.9	3
F	L	3	5.5	3
F	L	5	4.5	3
F	L	1	5.4	3
F	L	1	5.1	3
F	L	5	5.3	3
F	L	12	4.8	3
F	L	4	5.1	3
F	L	12	5.2	3
F	L	5	4.8	3

Appendix 7 – Chapter 9

Stable isotope ratios of coelopids. Columns are (left to right) species of coelopid (*C.* ***f****rigida* / *C.* ***p****ilipes*), source (F = laboratory *Fucus* culture, L = laboratory *Laminaria* culture, M = laboratory mixed algal culture, W = wild type), sex (**m**ale / **f**emale), weight of sample (mg), δ^{13}C (‰) and δ^{15}N (‰).

F	F	M	0.677	-13.75	9.35
F	F	M	0.988	-12.43	10.05
F	F	M	0.474	-13.93	10.42
F	F	M	0.594	-13.54	10.74
F	F	M	0.806	-13.88	10.50
F	F	M	0.833	-13.63	10.25
F	F	M	0.689	-13.84	10.88
F	F	M	0.550	-13.68	9.92
F	F	M	0.790	-13.53	11.03
F	F	M	0.614	-14.15	10.13
F	F	F	0.545	-13.54	10.85
F	F	F	0.539	-13.10	10.00
F	F	F	0.803	-12.86	9.76
F	F	F	0.654	-13.73	9.73
F	F	F	0.770	-14.43	10.55
F	F	F	0.520	-12.86	10.62
F	F	F	0.446	-13.13	11.22
F	F	F	0.558	-14.00	10.12
F	F	F	0.550	-13.89	9.48
F	F	F	0.702	-13.68	10.27
F	L	M	0.523	-12.96	9.04
F	L	M	0.924	-12.42	10.84
F	L	M	0.802	-13.24	9.26
F	L	M	0.892	-12.80	9.18
F	L	M	0.688	-12.57	9.54
F	L	M	0.973	-13.26	9.54
F	L	M	0.752	-12.50	8.79
F	L	M	0.634	-12.88	9.98
F	L	M	0.539	-13.13	9.16
F	L	M	0.760	-12.41	10.83
F	L	F	0.522	-12.52	10.26
F	L	F	0.582	-12.77	9.47
F	L	F	0.520	-12.98	9.77
F	L	F	0.767	-12.36	10.61
F	L	F	0.588	-12.20	10.76
F	L	F	0.598	-12.54	10.87
F	L	F	0.648	-12.94	9.25
F	L	F	0.622	-12.58	10.42
F	L	F	0.637	-12.27	10.64
F	L	F	0.549	-12.54	10.95
P	F	M	0.674	-13.61	10.06
P	F	M	0.734	-12.78	10.25
P	W	F	0.416	-16.84	8.96
P	W	F	0.806	-16.23	9.62
P	W	F	0.519	-17.53	7.18
P	W	F	0.581	-17.57	6.91
P	W	F	0.649	-16.81	8.71
F	W	M	0.684	-16.26	11.67
F	W	M	0.664	-16.59	9.63
F	W	M	0.541	-18.29	8.81
F	W	M	0.713	-18.55	10.55
F	W	M	0.867	-17.36	10.40
F	W	M	0.431	-16.40	9.36
F	W	M	0.523	-16.12	8.99
F	W	M	0.776	-16.25	9.72
F	W	M	0.308	-18.17	10.17
F	W	M	0.599	-17.88	8.31
F	W	F	0.995	-19.03	10.38
F	W	F	0.768	-18.41	9.58
F	W	F	0.475	-17.33	7.63
F	W	F	0.514	-19.31	8.65
F	W	F	0.510	-19.10	9.10
F	W	F	0.347	-18.86	8.43
F	W	F	0.661	-16.63	11.17
F	W	F	0.446	-16.78	8.77
F	W	F	0.347	-20.09	7.74
F	W	F	0.573	-18.79	8.01
P	W	M	0.595	-16.00	9.78
P	W	M	0.441	-16.81	9.46
P	W	M	0.773	-15.72	11.33
P	W	M	0.503	-16.26	9.41
P	W	M	0.623	-18.62	8.21
P	W	M	0.881	-16.90	10.31
P	W	M	0.574	-17.02	8.36
P	W	M	0.503	-17.77	9.08
P	W	M	0.898	-17.38	7.97
P	W	M	0.834	-16.25	9.55
P	W	F	0.479	-16.79	8.99
P	W	F	0.592	-17.19	8.37
P	W	F	0.638	-16.19	9.31
P	W	F	0.495	-17.21	8.52
P	W	F	0.333	-16.72	10.17
P	W	F	0.518	-16.29	10.82
P	W	F	0.612	-16.38	9.81

P	F	M	0.775	-12.74	10.53
P	F	M	0.730	-12.82	10.16
P	F	M	0.835	-12.82	10.44
P	F	M	0.782	-12.71	10.43
P	F	M	0.791	-12.75	10.63
P	F	M	0.707	-12.81	10.92
P	F	M	0.690	-13.01	9.90
P	F	M	0.701	-12.48	10.42
P	F	F	0.636	-14.04	9.92
P	F	F	0.603	-13.18	9.84
P	F	F	0.673	-13.43	9.66
P	F	F	0.613	-13.53	9.83
P	F	F	0.738	-13.18	10.44
P	F	F	0.738	-13.14	10.57
P	F	F	0.629	-13.35	9.55
P	F	F	0.683	-13.35	10.15
P	F	F	0.716	-13.59	9.52
P	F	F	0.804	-13.12	9.65
P	L	M	0.736	-12.60	11.13
P	L	M	0.787	-12.24	11.05
P	L	M	0.970	-12.47	11.59
P	L	M	0.698	-12.66	10.87
P	L	M	0.653	-12.38	10.84
P	L	M	0.507	-12.26	11.28
P	L	M	0.606	-12.61	10.84
P	L	M	0.666	-11.85	11.82
P	L	M	0.581	-12.10	11.28
P	L	M	0.575	-12.28	11.51
P	L	F	0.692	-12.47	10.79
P	L	F	0.633	-12.46	10.45
P	L	F	0.732	-12.42	10.84
P	L	F	0.783	-12.43	11.30
P	L	F	0.683	-12.65	11.32
P	L	F	0.567	-12.57	11.11
P	L	F	0.540	-12.44	11.50
P	L	F	0.538	-12.63	10.70
P	L	F	0.692	-12.09	11.18
P	L	F	0.552	-12.20	11.62
F	W	M	0.707	-18.36	9.14
F	W	M	0.655	-18.15	10.06
F	W	M	0.657	-16.69	10.45
F	W	M	0.882	-16.55	10.54
F	W	M	0.643	-17.91	8.87
F	W	M	0.619	-18.74	11.59
F	W	M	0.593	-16.44	10.16
F	W	M	0.537	-17.70	11.10
F	W	M	0.921	-16.18	10.64
F	W	M	0.470	-18.23	8.96
F	W	F	0.515	-18.83	9.70
F	W	F	0.605	-17.55	11.45
F	W	F	0.563	-16.96	9.75
F	W	F	0.714	-19.59	10.75
F	W	F	0.631	-16.98	11.56
F	W	F	0.612	-17.97	11.84
P	W	F	0.374	-16.22	8.93
P	W	F	0.662	-16.20	9.53
P	W	F	0.458	-16.76	8.59
F	W	M	0.351	-18.89	11.07
F	W	M	0.447	-18.55	9.78
F	W	M	0.378	-17.14	9.63
F	W	M	0.447	-16.76	
F	W	M	0.500	-18.75	9.38
F	W	M	1.054	-15.59	11.41
F	W	F	0.460	-18.28	8.75
F	W	F	0.558	-19.00	11.15
F	W	F	0.532	-18.04	8.49
F	W	F	0.487	-19.17	9.27
P	W	M	0.366	-17.21	8.98
P	W	M	0.399	-16.19	8.64
P	W	M	0.705	-17.26	7.92
P	W	M	0.730	-17.36	8.94
P	W	M	0.454	-17.36	7.41
P	W	M	0.843	-16.50	9.62
P	W	M		-16.54	7.57
P	W	M	0.469	-16.14	9.08
P	W	M	0.759	-16.47	8.48
P	W	M	0.660	-16.41	8.71
P	W	F	0.460	-16.71	8.93
P	W	F	0.610	-17.31	8.31
P	W	F	0.413	-16.64	8.61
P	W	F	0.507	-16.67	9.93
P	W	F	0.579	-16.40	8.61
P	W	F	0.511	-16.89	7.87
P	W	F	0.685	-16.42	10.58
P	W	F	0.690	-16.62	9.33
P	W	F	0.470	-17.01	8.64
P	W	F	0.629	-16.54	8.60
F	W	F	0.417	-17.01	10.12
F	M	M	0.794	-12.30	10.07
F	M	M	0.857	-12.68	10.61
F	M	M	0.887	-13.41	9.95
F	M	M	0.908	-12.27	10.48
F	M	M	0.937	-12.36	9.92
F	M	F	0.609	-12.74	10.92
F	M	F	0.298	-14.06	10.71
F	M	F	0.672	-12.66	10.61
F	M	F	0.687	-12.76	10.47
F	M	M	0.991	-12.25	10.66
F	M	M	0.885	-12.20	10.97
F	M	M	0.504	-12.20	11.82
F	M	M	0.948	-12.54	10.60
F	M	M	0.908	-12.47	10.38
F	M	M	0.782	-12.61	9.85
F	M	M	0.494	-13.05	9.45
F	M	M	0.937	-12.60	10.48
F	M	M	0.871	-12.16	10.39
F	M	M	0.760	-12.48	9.91
F	M	M	0.856	-12.73	9.88

F	W	F	0.421	-17.31	9.00
F	W	F	0.447	-17.40	8.55
F	W	F	0.498	-16.98	8.96
F	W	F	0.561	-18.11	8.39
P	W	M	0.572	-16.59	10.23
P	W	M	0.560	-17.29	9.07
P	W	M	0.688	-18.22	7.81
P	W	M	0.726	-16.92	9.05
P	W	M	0.497	-16.47	9.88
P	W	M	0.700	-16.75	10.45
P	W	M	0.560	-16.64	9.62
P	W	M	0.858	-16.04	10.79
P	W	M	0.892	-16.71	10.23
P	W	M	0.505	-16.35	9.96
P	W	F	0.458	-16.32	9.74
P	W	F	0.790	-17.84	8.14
P	W	F	0.653	-15.72	8.68
P	W	F	0.705	-16.42	9.85
P	W	F	0.510	-16.46	8.32
F	M	M	0.499	-12.73	10.07
F	M	M	0.692	-12.48	9.97
F	M	M	0.927	-12.51	10.23
F	M	M	0.755	-12.46	10.34
F	M	F	0.458	-12.48	11.08
F	M	F	0.412	-12.91	10.18
F	M	F	0.321	-13.12	10.15
F	M	F	0.489	-13.11	10.41
F	M	F	0.604	-12.89	9.54
F	M	F	0.553	-12.88	9.57
F	M	F	0.569	-13.33	9.19
F	M	F	0.544	-12.64	10.62
F	M	F	0.448	-12.69	10.52
F	M	F	0.426	-13.03	10.14
F	M	F	0.245	-12.15	11.24
F	M	F	0.550	-12.60	9.96
F	M	F	0.644	-13.63	8.89
F	M	F	0.376	-12.97	10.20

Stable isotope ratios of marine algae. Columns are (left to right) species of alga (C = *Ceramium virgatum*, F = *Fucus*, L = *Laminaria*, P = *Palmaria palmata*), weight of sample (mg), δ^{13}C (‰) and δ^{15}N (‰).

C	1.446	-29.82	7.03
C	1.322	-29.75	6.83
C	1.167	-27.49	6.59
C	1.190	-28.83	6.20
C	1.074	-29.08	6.58
C	1.006	-30.25	6.15
C	1.208	-26.96	7.04
C	1.361	-28.47	6.40
C	1.292	-28.08	6.64
C	1.433	-28.70	6.69
F	0.988	-13.68	5.80
F	1.081	-14.51	6.11
F	0.884	-14.23	6.95
F	0.980	-13.71	6.43
F	0.880	-14.31	6.50
F	1.042	-14.42	6.57
F	0.942	-14.70	6.46
F	0.946	-15.22	5.35
F	1.077	-15.66	5.92
F	0.898	-15.44	4.90
L	0.830	-16.63	4.49
L	0.994	-16.59	4.75
C	1.020	-31.02	6.40
C	1.124	-27.93	6.59
C	1.258	-29.60	6.59
C	1.065	-28.19	6.60
C	1.387	-26.72	6.74
C	1.202	-27.11	6.86
C	1.361	-30.05	6.74
C	1.471	-29.46	6.91
C	1.141	-29.08	6.40
C	1.466	-29.35	6.50
F	1.009	-15.41	3.98
F	0.841	-16.34	5.31
F	0.860	-14.93	5.69
F	0.858	-16.59	3.24
F	0.868	-16.68	3.67
F	1.016	-16.10	4.79
F	0.823	-13.82	5.72
F	0.801	-13.99	5.63
F	0.878	-12.92	5.38
F	1.098	-14.73	4.84
L	0.881	-14.66	6.84
L	1.077	-16.67	3.86

L	0.797	-13.93	6.65
L	0.963	-14.86	6.46
L	0.963	-14.66	5.07
L	0.854	-17.22	5.35
L	0.922	-15.41	4.69
L	0.788	-16.02	6.23
L	0.874	-15.10	5.32
L	0.903	-15.40	4.33
P	1.063	-20.75	5.31
P	1.074	-21.42	4.56
P	1.559	-20.08	5.80
P	1.224	-20.87	5.33
P	1.271	-20.97	4.93
P	1.054	-20.81	4.41
P	1.418	-20.11	5.84
P	1.046	-19.50	6.00
P	1.230	-20.82	5.10
L	0.843	-16.07	4.91
L	0.833	-15.93	6.02
L	0.854	-14.59	5.87
L	1.089	-13.78	3.98
L	1.030	-15.35	6.19
L	1.088	-14.98	5.49
L	0.802	-15.46	4.02
L	0.950	-14.22	5.10
P	1.138	-20.54	5.53
P	1.490	-20.83	5.36
P	1.445	-21.11	4.66
P	1.168	-20.42	5.37
P	1.357	-19.68	5.99
P	1.071	-20.68	5.14
P	1.043	-20.72	6.05
P	1.005	-20.62	4.28
P	1.009	-19.98	5.68

Appendix 8 – Chapter 10

Columns are (left to right) primer pair (A = *Mse*I – ACAG/*Eco*RI – CAAG, B = *Mse*I – ACAG/*Eco*RI – CAAC, C = *Mse*I – ACAT/*Eco*RI – CAAG ,D = *Mse*I – ACAT/*Eco*RI – CAAC), fragment size (bp), samples 1 through 16 first cycle and samples 1 through 16 second cycle (0 / 1 representing the absence or presence of the fragment respectively).

A	62	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
A	63	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
A	64	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	65	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	66	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	67	0	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0
A	68	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	69	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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A	72	0	0	1	0	0	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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A	157	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A 159	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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A 278	0	0	1	0	1	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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B 60	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
B 61	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	1	0	1	0	1	1	1	1	1	0	1
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B 89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 90	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
B 91	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
B 92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
B 93	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 101	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 102	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 103	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 104	0	1	1	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0
B 105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
B 108	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
B 109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
B 110	0	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	0	1	1	1	1	1	1	0	1
B 111	0	1	1	0	0	1	0	0	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1	1	1	1	1	0	1
B 112	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	1	1	1	0	1	0	1	1	0	1	0
B 113	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0
B 114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
B 115	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 122	0	0	0	0	1	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 124	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
B 129	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 134	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
B 135	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
B 136	0	1	1	0	0	1	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0	1	1	1	1	0	1	1	0	1
B 137	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
B 141	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 143	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 144	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 145	0	0	1	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1
B 146	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	1	0

B 148	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 149	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
B 150	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
B 151	0	0	0	0	0	0	1	1	0	0	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
B 152	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0
B 153	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1
B 154	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	1	0
B 155	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
B 157	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
B 158	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
B 160	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
B 161	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 162	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 163	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 166	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
B 173	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
B 176	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 181	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 183	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 184	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 188	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 190	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
B 192	0	1	0	0	0	0	0	0	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1
B 193	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	1	0	0	0	0	0
B 195	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
B 198	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0
B 199	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1
B 200	0	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	1	0	0	1	0	0	0	0	0
B 201	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 204	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1
B 205	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 208	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 222	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
B 223	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 235	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
B 240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
B 242	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
B 243	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
B 250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
B 251	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	0	1	0	0
B 252	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	1	1
B 253	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0
B 254	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1
B 255	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
B 262	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 272	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
B 274	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
B 275	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
B 283	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B 291	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
B 292	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
B 293	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1
B 294	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0
B 296	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0

B 297	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	1	0	0
B 299	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0		0	0	0	0	1	1	0	0	0	1	0	0
B 300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 302	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	1	0
B 304	0	1	1	0	0	1	0	0	1	1	1	1	1	1	1	1	0	1	1		0	0	0	0	1	1	1	0	1	1	0	1
B 305	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0		0	0	0	1	1	0	0	0	0	1	0	0
B 307	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0		0	0	0	0	1	0	0	0	0	0	0	0
B 308	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	1	0
B 309	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0		0	0	0	0	0	1	0	0	0	1	0	0
B 310	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	1	0	0	0	0	0	0	1	0
B 311	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0		0	0	0	0	0	1	0	0	1	0	0	1
B 312	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1		0	0	0	0	0	0	0	0	0	0	0	0
B 316	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 318	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	0	0
B 322	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0		0	0	0	0	0	1	0	0	0	0	0	0
B 340	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0		0	0	0	0	1	0	0	0	1	1	0	1
B 341	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		0	0	0	1	0	0	0	0	0	0	0	0
B 348	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 357	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 360	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 369	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 398	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		0	0	0	0	0	0	0	0	1	0	0	0
B 418	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 419	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 421	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0		0	0	0	0	0	0	0	1	0	0	0	0
B 422	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 424	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0		0	0	0	0	0	0	0	0	0	0	0	0
B 436	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	1	0	0	0	0	0	0	0	0	0	0
B 448	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	1	0	0	0	0	0	0	0	0	0
B 450	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	1	1		0	0	0	0	1	1	1	1	1	1	0	0
B 451	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		0	0	0	0	0	0	0	0	1	0	1	0
B 453	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
C 61	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 62	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	0	1		0	0	0		1	1	1	1	1	1	1	1	1
C 64	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0		0	0	0		0	0	0	0	0	1	0	0	0
C 65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	1		0	0	0	0	0	0	0	0	0
C 66	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 67	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	0	0	0		0	0	0		0	0	0	0	1	0	1	0	0
C 68	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 69	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 71	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 72	0	1	1	0	0	1	0	0	0	1	1	1	1	0	1	1	0	1		0	0	1		1	0	1	1	0	1	0	1	1
C 73	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 74	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		0	0	1		0	0	0	0	1	0	0	0	0
C 76	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	0	1		0	0	1		1	1	1	1	0	1	1	1	1
C 79	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		1	0	0		0	0	0	0	0	0	0	0	0
C 82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	1		0	0	0	0	0	0	0	0	0
C 83	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1		0	0	0		1	1	1	1	1	1	1	1	1
C 84	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	1	0	0	0	1	0
C 88	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		0	0	0		0	0	0	0	1	0	0	0	0
C 89	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 93	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 95	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 97	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0
C 98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		1	0	0	0	0	0	0	0	0
C 100	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1		0	0	1		1	1	1	1	1	1	1	1	1

C 102	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0
C 105	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
C 111	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 115	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 116	0	1	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	1	0	0	0	0	0	1	1	1	1	1	1	0
C 117	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 121	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 123	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 125	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 134	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 142	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
C 146	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
C 147	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
C 157	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 159	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 169	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 170	0	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0	0	0	0	1	1	1	1	1	1	1	1
C 172	0	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	1	1	1	1	1	1	1	1
C 173	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
C 178	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1
C 187	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 189	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 190	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 193	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 194	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 199	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
C 203	0	0	1	0	0	0	0	0	1	1	1	0	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1
C 212	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 220	0	1	1	0	0	1	0	0	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1
C 244	1	1	1	0	0	0	0	0	0	0	1	0	1	1	1	1	0	1	0	0	0	1	0	0	1	0	1	1	1	1
C 247	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1
C 250	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 251	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	0	0	0	0	0	0
C 252	0	1	1	0	0	1	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1
C 258	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
C 259	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0
C 260	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0
C 261	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1	1	1	1
C 263	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
C 272	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	0	1	0	1	0	0
C 274	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 278	0	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1
C 280	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 322	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 323	0	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1	0	0	0	1	0	1	1	0	1	0	1	1
C 337	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	0	0	1	1	0
C 340	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
C 345	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
C 346	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1
C 373	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
C 376	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 423	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
D 60	0	1	1	0	0	1	0	0	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0

D	61	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	62	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	63	1	0	1	0	1	1	0	1	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1
D	64	1	1	0	1	1	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D	65	1	0	1	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	1	0	1	1	1	1
D	66	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	67	1	0	1	1	1	1	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	68	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
D	69	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	70	1	0	1	1	1	1	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	71	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D	73	1	0	1	0	1	1	0	1	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D	74	0	0	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D	76	1	1	1	0	1	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0
D	77	0	1	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	78	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0
D	79	0	0	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	81	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
D	82	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	83	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D	84	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	85	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
D	86	0	0	1	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	1	1	0	1	0	1	1	0
D	87	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
D	89	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	90	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1
D	91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D	92	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	93	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D	94	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	95	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	96	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0
D	97	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	98	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	99	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	100	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D	101	1	1	0	1	1	1	0	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	102	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	103	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	104	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	105	1	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
D	107	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	108	1	0	0	0	1	0	0	0	0	0	0	1	1	1	1	0	0	1	0	1	1	0	1	0	1	1	0	0	0	0
D	109	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
D	110	0	0	1	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	1	0	1	1	0	0	0	1	1	0
D	111	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0
D	112	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	113	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	114	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	1	1	1	1	1	0	1	1
D	115	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	116	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	117	1	0	0	0	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	118	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	119	1	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	120	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

D 121	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 122	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 124	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 125	1	0	1	0	1	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D 126	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 127	1	0	1	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 128	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	1	0	0	1	1	1	1	1	1	1	1
D 129	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0
D 130	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 131	0	0	1	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D 133	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 134	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 136	0	0	0	1	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 137	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	1	1	1	1	1	1	1	1
D 138	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
D 139	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
D 140	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
D 142	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
D 143	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 145	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 146	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 148	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D 149	1	1	0	0	1	1	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	1	1	1	0	1	1	1	0
D 150	1	0	1	1	1	1	0	0	0	1	1	1	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0
D 151	0	0	0	0	0	0	1	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 152	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	1	1
D 153	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 154	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1
D 155	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 156	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D 157	0	0	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 158	0	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
D 159	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 160	0	0	0	0	1	0	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 161	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 162	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 163	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 164	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 166	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 167	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 169	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 170	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 172	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 173	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 174	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 175	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 176	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 177	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
D 178	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1	1	1	1
D 179	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 180	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 181	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 182	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 183	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 184	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 187	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

D 189	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 190	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 192	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 193	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 194	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 195	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 197	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 198	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D 199	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D 200	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	1	1	1	1	1	1	1	1	1
D 201	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D 202	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 204	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
D 205	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	0	1	1	1	1
D 206	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 207	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 208	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 209	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 210	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 211	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1
D 212	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 213	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 216	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 217	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0
D 218	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 221	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 222	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0
D 223	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 225	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D 228	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
D 230	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 231	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 232	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 233	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0
D 235	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 237	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
D 238	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1	1	0	1	0	0
D 239	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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D 243	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	1	1	1	1	1	1	1	0
D 244	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	1	1	1	1	0	1	1	1	1
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D 247	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 254	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
D 255	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
D 256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0
D 258	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 260	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0
D 261	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 262	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 263	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 265	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0
D 266	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
D 267	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 268	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

D 271	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	1	0	0	0	0
D 275	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 278	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 279	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 280	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 281	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 283	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 284	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 285	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 286	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 288	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 291	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 294	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 297	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 301	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 302	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
D 303	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 304	0	0	0	0	0	1	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 305	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 306	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 308	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 312	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
D 314	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 315	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
D 316	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 317	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
D 320	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
D 321	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 322	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
D 323	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
D 325	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
D 329	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 334	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 341	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 342	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 343	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 344	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 348	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 349	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 350	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 354	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
D 356	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 357	1	0	1	0	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 360	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 366	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 368	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 373	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 377	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
D 379	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
D 381	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 391	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	1	0	0	1	0
D 398	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 407	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D 410	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 411	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

D 412	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 413	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 417	0	1	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 419	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 421	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 422	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 424	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 426	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 428	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 436	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 437	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 439	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 445	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D 456	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0