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Phenotypic plasticity in marine intertidal gastropods

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Phenotypic plasticity in marine intertidal gastropods

By

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A thesis submitted to the University of Plymouth in partial fulfilment for the degree of

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Phenotypic plasticity in marine intertidal gastropods

by
Ahmed Mohammed Al- Mazrouai

Abstract

Phenotypic plasticity, the differential phenotypic expression of the same genotype in response to different environmental conditions, is a paradigm central of the study of evolution and ecology and is at the core of the “nature versus nurture” debate. Here, the marine gastropod *Littorina littorea* was used as a model to further our understanding of the potential role of phenotypic plasticity in intertidal systems. In the first study *L. littorea* was included in an investigation of induced defences across six species of intertidal marine gastropods in the families Littorinidae and Trochidae. Species differed in the magnitude and type of plastic response, which appeared to relate to their susceptibility to crab predation. Chapters three and four revealed that *L. littorea* was able to alter its degree of morphological plasticity depending on temporal variation in predation threat. Snails exposed to predation threat half way through trials appeared to “catch up” snails continuously exposed to predator cues in terms of their shell size, whereas snails experiencing a removal of predation cues showed a significant reduction in growth rate following this switch in predation environment. A further investigation suggested that *Littorina littorea* demonstrated no significant difference in the morphological traits under variable predator threat versus a constant predator threat environment. Finally, the interaction between biotic (predator) and abiotic (temperature) environmental effects revealed that snails maintained at 16 and 20° C demonstrated significant induced defences by growing larger and thicker shells, but there was no significant difference in induced defences between these two temperatures. However, the expression of induced defences was much lower at 24° C with only negative significant response in two of shell traits

between control and predator cue treatments which may indicate that induced defences was inhibited at this temperature treatment. The implications of these results are discussed as is the potential applications of induced defences.

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Authors Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other university award without prior agreement of the Graduate committee.

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Chapter 1

Introduction

PHENOTYPIC PLASTICITY

General Background

The study of phenotypic plasticity, the differential phenotypic expression of genotypes in response to the environment (DeWitt and Scheiner, 2004; Pigliucci, 2005; Pigliucci *et al*, 2006; Fordyce, 2006), has its roots in work by Woltereck at the beginning of the 1900s (Woltereck, 1909). Woltereck investigated the responses of different clones of the freshwater cladocerans, *Daphnia* and *Hyalodaphnia*, to various environmental factors and found that, for some variables (e.g. food quality), clones responded differently in terms of their morphological responses. He used the German term *Reaktionsnorm* to describe this phenomenon and the reaction norm has since become a cornerstone of phenotypic plasticity studies (Woltereck, 1909; Schlichting and Pigliucci, 1998). Despite this early interest in how the expression of the genotype could be influenced by the environment, for the vast part of the last century the interaction of the environmental conditions with the phenotype was considered as 'environmental noise' (Sultan, 2000). It was not until the early or mid 1980s that there was renewed interest in phenotypic plasticity, leading to several reviews of the subject during the 1990s (Sultan, 1992; Via *et al*, 1995; Sultan, 2000; Pigliucci, 2001; Sarkar, 2004; Pigliucci, 2005).

What is now clear is that phenotypic plasticity can include variation in an individual's behaviour, morphology, physiology, growth, life history and demography (Price *et al*, 2003; Miner *et al*, 2005). One of its great strengths as a paradigm, is that it potentially unites all the biological disciplines of genetics, development, ecology and evolution (DeWitt and Scheiner, 2004). At the same time, as it enables an organism to maximise its fitness through an ability to match its phenotype to a range of environmental

conditions, hence its study is highly pertinent to scientists aiming to predict how organisms might respond to changing environmental conditions (DeWitt *et al*, 1998; Kraft *et al*, 2006). Finally, the study of the role of plasticity in evolution is perhaps where this field may make its biggest contribution, in essence, by investigating the “nature versus nurture debate” (Schlichting, 1986; Schlichting and Pigliucci, 1998; Pigliucci, 2001; West-Eberhard, 2003; DeWitt and Scheiner, 2004; Pigliucci, 2005; Brakefield, 2006).

Costs of and Limits to Plasticity

One of the aspects of plasticity that has received considerable attention is its associated costs, plus those factors that limit its expression (DeWitt, 1988; DeWitt *et al*, 1988; Relyea, 2002; Pigliucci, 2005). Costs of plasticity are defined as reduced fitness associated with plastic genotypes, in comparison to fixed genotypes, when producing the same phenotype in a same natural environment (DeWitt *et al*, 1998; Pigliucci, 2005). Ultimately, costs are viewed as being associated with the expression of plasticity and should be traded off against the fitness benefits of plastic responses; if this were not the case, the organism should produce a fixed phenotype irrespective of environmental conditions (Trussell, 2000b; Pigliucci, 2005). Costs of plasticity may be traded off against several other traits such as decreased body mass, reduced growth rate or altered reproduction (Kemp and Bertness, 1984; Trussell, 1996; Trussell, 2000a; Trussell, 2000b; Trussell and Smith, 2000). Five costs of plasticity were proposed by DeWitt *et al* (1998): i) *maintenance costs* as a result of keeping the sensory and regulatory mechanisms for the plasticity to occur; ii) *production costs* as a result of producing the phenotypic response; iii) *information acquisition costs* for assessing the variability of the environment; iv) *developmental instability costs* that

result from phenotypic imprecisions (e.g. mistakes during development, fluctuating asymmetry), thus causing reduced fitness under stabilizing selection; v) *genetic costs* that are caused by interactions among genes through linkage (associated with the link between the genes that promote plasticity with other costly genes leading to lower fitness), pleiotropy (when genes that promote plasticity have negative pleiotrophy effects on other traits) and epistasis (when the genes that promote plasticity alter the expression of other genes leading to indirect effects of other traits) (DeWitt *et al*, 1998; Pigliucci, 2005).

Limits of plasticity are functional restrictions that lead to the failure of an organism to produce the optimal phenotype in any given environment (DeWitt *et al*, 1998; Pigliucci, 2005). Four types of plasticity limits were proposed by DeWitt *et al* (1998): i) *information reliability limits* associated with weak information about the environmental conditions; ii) *time lag limits* resulting from the time delay from the detection of a change in the environmental conditions until the phenotypic responses; iii) *developmental range limits* that prevent the production of extreme phenotypes; iv) *epiphenotypic problems* that result from add-on phenotypes being less effective than the same phenotype produced in early ontogeny (DeWitt, 1998; DeWitt *et al*, 1998).

There has been considerable interest in plasticity constraints (costs and limits) due to their important effect in ecological and evolutionary studies; however, research in this field still in its early stages (DeWitt *et al*, 1998; Pigliucci, 2005). Theoretical models have been produced to demonstrate plasticity constraints, but empirical studies have lagged behind these advances (DeWitt *et al*, 1998; Pigliucci, 2005). In particular there are difficulties in demonstrating costs of plasticity; and some recent studies such as

Van Buskirk's (2000) investigation of costs across 15 species of anuran tadpoles show that costs arise from shifts in allocation of time and energy and not necessarily actual (fitness) costs (DeWitt *et al*, 1998; Pigliucci, 2005). It is also clear that less research has been undertaken on limits which indicates the need for a greater focus on this aspect of plasticity (DeWitt and Scheiner, 2004).

Adaptive Value of Phenotypic Plasticity

The adaptive value of phenotypic plasticity has featured in evolutionary studies, yet formal testing of whether plasticity is adaptive has been very difficult, mainly due to the need to examine responses and fitness in multiple environments (DeWitt and Scheiner, 2004; Pigliucci, 2005). There is little evidence that plasticity is truly adaptive, although some recent examples do help in this regard. The maintenance of eyespots in the butterfly *Bicyclus anynana* in response to predation, for example, made these insects more difficult for predatory birds to recognise them (Lyytinen *et al*, 2003). Similarly, Nilsson *et al* (1995) showed that crucian carp *Carrasius carassius* with predator-induced deeper bodies were taken less by predatory pike in laboratory experiments. Likewise, the plastic response in the thickness of water-storing leaves of the Hawaiian tree *Metrosideros polymorpha* in response to variable moisture availability allow this species to attain a wide ecological distribution (Cordell, *et al*, 1988; Sultan, 2000).

It is likely that plasticity may enhance the potential for species to be invasive, the ability of species to withstand sudden changes in environmental conditions and, ultimately, the patterns of evolutionary diversification (Sultan, 2000). Several authors have suggested that plasticity may in fact play a significant role in the evolutionary process (Appleton and Palmer, 1988; Via *et al*, 1995; McCollum and Buskirk, 1996;

Price *et al*, 2003; Price, 2006). First, plasticity is essential for the survival and continuation of populations under fluctuating environmental conditions and, second, the selection pressures that drive evolution occur within the plasticity context (Price, 2006). Although the development of phenotypic plasticity is advantageous for plastic organisms, it is essential to explore how plasticity leads to adaptation and whether plasticity is linked to evolution. One approach is to compare plastic responses across a range of species within a described phylogeny. Such an approach was used by Van Buskirk (2002) for tadpoles, demonstrating support for the adaptive plasticity hypothesis, i.e. a positive correlation between variation in predation risk species experienced and their morphological plasticity.

Hence, there is evidence that plasticity has played role in the evolution of some groups of organism. Another key point is whether there are genes for plasticity. However, the genetic basis of phenotypic plasticity has been one of the more controversial issues among evolutionary biologists (Frankino and Raff, 2004). There are two different models that describe the genetic base for plasticity: i) *The regulatory gene model* whereby genes for plasticity of regulate other gene under changes in environmental conditions (Schlichting and Pigluicch, 1998); ii) *The genetic sensitivity model* which proposes that there are no genes for plasticity but that plasticity occurs through differential expression of trait genes depending on the environment (Via, 1993). At present the question concerning which model best applies is still ongoing, although new molecular approaches may help to address this question (Yang *et al*, 2006; Gutteling *et al*, 2007).

INDUCED DEFENCES

General Background

Inducible defences are a common form of plasticity induced by cues associated with predators of predation events (Harvell and Tollrian, 1999; Trussell and Nicklin, 2002). This form of plasticity occurs in a wide range of organisms, including invertebrates, vertebrates and plants. Examples include: chemical defences in marine algae (Toth and Pavia, 2000; Coleman *et al*, 2007), and in cephalaspidean gastropods (Marin *et al*, 1999); formation of defensive spines in marine bryozoans (*Membranipora membranacea* -Iyengar and Harvell, 2002); increased carapace strength in *Daphnia pulex* and *Daphnia cucullata* (Laforsch *et al*, 2004); altered shell thickness and morphology in marine gastropods (*Littorina obtusata*) and barnacles (*Strombus gigas*) (Delgado *et al*, 2002; Trussell and Nicklin, 2002) and changes in body shape of fish (Andersson *et al*, 2006). There are also numerous taxa that exhibit behavioural predator-induced defences such as the diel vertical migration in freshwater zooplankton (e.g. *Daphnia mendotae* - Pangle and Peacor, 2006), reduced activity in amphibians (e.g. the larval wood frog *Rana sylvatica* - Releya, 2002), anti-predator avoidance behaviour in dragonflies (e.g. *Pachydiplax longipennis* - Hopper, 2001), and avoidance behaviour in freshwater snails (e.g. *Physella virgata* - Alexander and Covich, 1991, *Physa gyrina* - Wojdak and Luttbeg, 2005, *Lymnaea stagnalis* - Dalesman *et al*, 2007). Finally, there may also be life-history plasticity that is initiated by the presence of a predation threat, such as increased time to reach maturity in *Daphnia pulex* (Tollrian, 1995) and rapid growth with reduced reproduction in freshwater snails, e.g. *Physella virgata* (Crowl and Covich, 1990) although it could be argued that these are costs of induced defensive traits and not traits that are themselves induced by the presence of a predator.

Ecological Consequences of Induced Defences

Most work on interactions in ecological systems has been concerned with direct interactions such as the consumption of prey by predators (Van Tamelen, 1987; Appleton and Palmer, 1988), but more recently, there has been growing focus on indirect interactions, such as density mediated indirect interactions (DMIIs) and trait mediated indirect interactions (TMIIs) (Lima, 2002; Trussell *et al*, 2002; Trussell *et al*, 2003; Trussell *et al*, 2004; Trussell *et al*, 2006).

DMIIs are forms of species interactions which arise through direct consumption (lethal effects) of prey by predators (Peacor and Werner, 2001; Trussell *et al*, 2004; Ohaba and Nakasuji, 2007). Indirect effects from DMIIs occur as predators consume prey, reducing their density, which in turn indirectly affects populations further down the food chain (Ohaba and Nakasuji, 2007; Peacor and Werner, 2001; Trussell *et al*, 2004; Trussell *et al*, 2006). The result is that DMIIs may have positive or negative effects (Raimondi *et al*, 2000). For example, crabs feeding on intertidal gastropods may reduce their density, and hence the grazing pressure that they exert on algae, resulting in a positive effect of an increase in algal cover. On the other hand, less predation may cause increased gastropods grazing efforts, resulting in a negative effect of a decrease in algal cover.

TMIIs are non-lethal forms of species interaction that occur when plastic phenotypic responses (such as changes to development, morphology, physiology, life history or behaviour) affect other species in the food web (Raimondi *et al*, 2000; Peacor and Werner, 2001; Dill *et al*, 2003; Trussell *et al*, 2003; Werner and Peacor, 2003; Trussell *et al*, 2004; Wojdak and Luttbeg, 2005). For example, TMIIs occur when the

presence of predators, such as crabs, reduce the foraging behaviour of gastropods, releasing algae from grazing pressure (Trussell *et al*, 2003; Wojdak and Luttbeg, 2005). Thus, in this case, gastropods prey are not removed from the environment, but alter their grazing behaviour. Consequently, TMIs may have significant implications for the diversity and dynamics of ecological communities (Peacor and Werner, 2001; Dill *et al*, 2003; Werner and Peacor, 2003) and several papers suggest that the effects of TMIs may be greater than DMIs (Peacor and Werner, 2001; Trussell *et al*, 2002; Trussell *et al*, 2003; Trussell *et al*, 2004; Trussell *et al*, 2006).

Marine Gastropods as Models for Studying Induced Defences

Intertidal rocky shores are typically very heterogeneous environments that are affected by extreme changes in physical and biological characteristics. They are also recognised as hosting high species diversity with a wide diversity of organisms adapted to these environmental conditions (Newell, 1979; Yamada and Boulding, 1996; Johannesson, 2003). The physical factors that intertidal organisms experience include desiccation, extreme temperature ranges, exposure to wave action, tidal variation, varied salinities and oxygen concentrations, plus heterogeneity of resources (Newell, 1979; Bertness, 1999; Johannesson, 2003). Biological factors that have an influence in the intertidal include larval recruitment, predation, competition, and grazing (Newell, 1979; Johannesson, 2003). These physical and biological factors play a major role in the organisms' mobility, size and morphology and, hence, affect the functioning of the intertidal community (Bertness, 1999; Johannesson, 2003). At the same time, these factors influence the evolution of intertidal species and offer a powerful tool for studying evolutionary ecology (Johannesson, 2003; Helmuth *et al*, 2006).

Marine gastropods from intertidal communities have played an important role in the investigation of phenotypic plasticity for several reasons. First, these species occupy a heterogeneous environment that is subject to varied physical and biological stress (Yamada and Boulding, 1996; Bertness, 1999; Johannesson, 2003). Second, when exposed to predator stress, they have displayed a range of plastic traits in morphology, life history and behaviour (Appleton and Palmer, 1988; Trussell, 1997; Trussell, 2000a; Trussell and Smith, 2000; Smith and Ruiz, 2004). Gastropods also have high abundance, with relatively low mobility, and their biology is well known from a suite of ecological studies (Yamada and Boulding, 1996; Bertness, 1999; Trussell, 2000a; Trussell and Smith, 2000; Trussell, 2002; Trussell and Nicklin, 2002; Trussell *et al*, 2002; Johannesson, 2003; Smith and Ruiz, 2004; Trussell *et al*, 2006).

Gastropoda is the largest class within the phylum Mollusca, with more than 60,000 species described worldwide (Gibson *et al*, 2001). Most species have a shell formed of one piece, which is usually spirally coiled. Shells are deposited on the edge of the mantle and consist of minute particles of calcium carbonate intimately associated with organic material. In nature, calcium carbonate comes in two different mineral forms: aragonite and the slightly harder, less dense and less soluble form, calcite (Vermeij, 1993). Gastropod shells are built for protection and their characteristics often reflect a specific type of predation pressure (Ray and Stoner, 1995). For example, thick shells and narrow apertures are used to deter decapod crustaceans, which crush shells and peel them back from the aperture to expose soft tissues. On the other hand, spinose shell sculptures are used to defend against gape-limited predators such as fish and turtles (Ray and Stoner, 1995). Certain morphological features such as spines, which

appear to be protection mechanisms, might not necessarily give adaptive advantage, however. For example, the spines of thorny oysters *Spondylus americanus* function primarily as settlement sites for camouflage-providing epibionts and not as structures directly defending against predators (Feifarek, 1987; Ray and Stoner, 1995).

The co-evolution of gastropods and predatory crabs has been suggested as a mechanism behind the high crush resistance of snail shells and efficient crab claws (Boulding *et al*, 1999). This co-evolutionary relationship varies latitudinally; heavier predation pressure by crabs in tropical latitudes is thought to have led to heavier shells and more elaborate shell armour in tropical gastropods (Bertness, 1981). The effectiveness of the shell as a defensive structure is indicated by the duration of the handling time, i.e. the longer handling times by a predator, the more effective the shell. Direct measures of shell strength also provide a measurement of shell effectiveness and indicate resistance to predation (Preston *et al*, 1996). One suggestion is that evolutionary escalation has occurred whereby predator-prey co-evolution has given rise to the kinds of extreme shell forms we now see in marine gastropods. Evidence from the frequency of shell repair of gastropods within the fossil record does provide some evidence of this potential mechanism with shell damage increasing in of shells from the Pennsylvanian and Triassic periods, through Cretaceous and Miocene eras to recent times (Vermeij *et al*, 1981).

THESIS AIMS

The overall aim of this thesis was to increase our understanding of induced defences in the rocky intertidal in the temperate zone by cross-species comparison of induced defences in marine gastropods. Few cross-species comparisons have been conducted

on intertidal gastropods and so my first objective was to compare responses across several taxa and assess whether responses could be explained by species relatedness or life histories. My second objective was concerned with investigating the flexibility of induced defences in respond to variation in biotic and abiotic environmental variables. My approach was to use a series of laboratory trials to investigate how plastic responses in *Littorina littorea* (Trussell *et al*, 2003; Relyea, 2004; Rundle *et al*, 2004; Bibby *et al*, 2007) were affected by both variation in the timing and degree of predation threat and environmental temperature. Gastropods were used as model group that are known to be amenable to such laboratory work and to exhibit both morphological and behavioural responses to predators (see above). At the same time, gastropods are known to play an important functional role in intertidal ecosystems. Hence, the results of my work should be of general importance for our understanding of rocky intertidal ecology.

The aims of individual chapters were as follows.

Chapter 2

In this first chapter I carried out a cross-species comparison of induced defences for six gastropod species common in the U.K. intertidal. The main aim of this chapter was to examine whether: i) species differed in the type and magnitude of induced defences they exhibited; and ii) whether the expression of induced defences was linked to species relatedness.

Chapter 3

This chapter was the first of three focusing on induced defences in *Littorina littorea*. Plasticity is costly, thus it is important for the organisms to express induced defenses only when appropriate. Hence, the main aim of this chapter was to assess whether *L. littorea* was able to match its environment closely either by reversing plastic responses or increasing its response to better match its environment (i.e. by exhibiting “catch up” plastic responses). By using experimental animals from two size classes I also investigated whether such responses were size dependent.

Chapter 4

In this chapter I maintained the theme of investigating how closely *Littorina littorea* was able to match its responses to environmental conditions by investigating how plastic responses varied in the face of variable predation threats (systematic predator cue versus random predator cue). Hence, the main aim in this chapter was to test the predation risk allocation hypothesis from a different angle, using the expression of morphological induced defences rather than behavioural traits.

Chapter 5

Due to global warming, the effects of rising temperature have received a great deal of attention in the past few years. Temperature plays an important role in the determining species physiology, behaviour and distributions hence, it is likely that induced defences will be influenced by temperature. In this chapter, I examined the effect of varied temperature regimes on the expression of induced defences in *L. littorea*.

Chapter 6

This final chapter presents a general summary of my experimental observations and draws the main conclusions from my work as well as making recommendations for further study.

STUDY SPECIES AND FIELD COLLECTION SITES

In the first part of this thesis I compared six species from two families of marine gastropods: Littorinidae - *Littorina litorea*, *L. fabalis*, and *L. obtusata*; Trochidae - *Osilinus lineata*, *Gibbula cinerarea*, and *G. umbilicalis*. Here, I provide a brief outline of the biology/ecology of these species. These species are abundant on the intertidal rocky shores of Cornwall and Devon in the United Kingdom.

Common Periwinkle *Littorina littorea* (Linnaeus)

The herbivorous common periwinkle *Littorina littorea* (Figure 1.1) has a sharply conical shell with a prominent spire, and is the largest species of intertidal gastropod in the UK, reaching up to 25-30 mm in height on average and with a maximum recorded size of 52.8 mm (Hayward *et al*, 1995a; Reid, 1996; Gibson *et al*, 2001). It inhabits the rocky intertidal between the upper and lower shores with a preference for sheltered shores on rocks. It feeds on a wide range of algae including *Enteromorpha lactuca* and, in addition, is usually associated with algae such as *Fucus vesiculosus*, *Ascophyllum nodosum*, *Fucus serratus*, and *Laminaria* spp. (Dippolito *et al*, 1975; Bakke, 1988; Reid, 1996; Gibson *et al*, 2001; Carlson *et al*, 2006).

Littorina littorea can tolerate a wide range of salinity and temperature (Dippolito *et al*, 1975 see also Chapter 5) and its regional distribution extends from the White Sea in Northern Russia to northern Spain and, subsequently, the UK (Gibson *et al*, 2001). This species is planktotrophic and has relatively long planktonic stages of 4-6 weeks (Kemp and Bertness, 1984; Moran, 1999; Johnson *et al*, 2001; Johannesson, 2003; Hollander *et al*, 2006).



Figure 1.1 Common Periwinkle *Littorina littorea* (Linnaeus). Scale bar = 5 mm.

Flat Periwinkle *Littorina fabalis* (Turton)

It is important to note that *L. fabalis* (Figure 1.2) is a synonym of *L. mariaae* used in most of the references cited in this study (for example in: Raffaelli, 1982; Reimchen, 1982; Watson and Norton, 1987; Reid 1990; Lowell *et al*. 1994; Hayward *et al*.

1995a; and Gibson *et al.* 2001). The name *L. mariae* was used until the recent reclassification (Reid, 1996). *Littorina fabalis* is typically 11-12 mm in length and has various colour morphs such as olive green, brown, yellow, light reticulate and dark reticulate which are thought to reflect the algal habitat individuals experience (Raffaelli, 1982; Hayward *et al.*, 1995a; Johannesson and Ekendahl, 2002). This herbivorous species is mainly found on *Fucus serratus*, from mean low water to the middle shore; it is more resistant to wave action but less resistant to desiccation than *L. obtusata*. It has no larval stage but exhibits direct development, hatching as miniature adults (Paterson *et al.*, 2001; Johannesson and Ekendahl, 2002; Kemppainen *et al.*, 2005) that reach maturity in one year (Reimchen, 1982) and usually reproduces once a year (Johannesson and Ekendahl, 2002).



Figure 1.2 Flat Periwinkle *Littorina fabalis* (Turton). Scale bar = 5 mm.

Flat Periwinkle *Littorina obtusata* (Linnaeus)

The flat periwinkle *Littorina obtusata* (Figure 1.3) occurs in shallow water and is very tolerant of a wide range of exposures. It generally lives on macroalgae, especially *Fucus vesiculosus* and *Ascophyllum nodosum* (Raffaelli, 1982; Watson and Norton, 1987; Reid, 1990; Lowell *et al*, 1994; Ekendahl, 1995; Hayward *et al*, 1995a; Reid 1996; Gibson *et al*, 2001). In some areas, such as New England, USA, it is only found in association with *Ascophyllum nodosum* (Bertness, 1999). It has wide range of colours such as olive-brown, yellow, orange and black, but tends to be brown or yellow as a cryptic adaptation for its brown algal habitat (Raffaelli, 1982; Reid, 1996; Johannesson and Ekendahl, 2002). *Littorina obtusata* reaches lengths of 15-17 mm (Hayward *et al*, 1995a) and is thought to exhibit the most intraspecific morphological variation in the Littorinidae (Reid, 1996). This species has direct development without a larval stage (Paterson *et al*, 2001; Johannesson and Ekendahl, 2002; Kemppainen *et al*, 2005) and reaches maturity in two years (Reimchen, 1982; Johannesson and Ekendahl, 2002). *Littorina obtusata* has a similar appearance to *L. fabalis*. The two species are often confused and before they were split in 1980 were considered a single species (*Littorina littoralis*) (Fretter and Graham, 1980; Reid, 1996). However, *L. obtusata* has distinctive features such as a more oval aperture with the most recent whorl larger than the aperture, while *L. fabalis* has a lower (drop) shaped aperture view and the aperture larger than the body whorl (Hayward *et al*, 1996).



Figure 1.3 Flat Periwinkle *Littorina obtusata* (Linnaeus). Scale bar = 5 mm..

Thick Top Shell *Osilinus lineata* (da Costa)

Osilinus lineata (Figure 1.4) has a thick, heavy, conical shell with a spire height up to 25 mm and a maximum width of 30 mm (*Osilinus lineata* = *Monodonta lineata* in Hayward *et al*, 1995a; Gibson *et al*, 2001). It has a cream to greyish or reddish brown coloured shell. The distinctive tooth-like projection on the inner lip is an important feature of this species (Hayward *et al*, 1995a; Gibson *et al*, 2001). *Osilinus lineata* inhabits the middle and upper shores where it is found on rocks and boulders and feeds on algal biofilm (Hayward *et al*, 1995a; Gibson *et al*, 2001). Its distribution in the British Isles extends from the western English Channel to Wales and west coast of Ireland (Hayward *et al*, 1995a; Gibson *et al*, 2001). The development of this species involves a relatively short larval stage that extends to a few days, after which time it settles on the lower shore (Desai, 1966; Gibson *et al*, 2001; Mieszkowska *et al*, 2007).



Figure 1.4 Thick Top Shell *Osilinus lineata* (da Costa). Scale bar = 5 mm.

Grey Top Shell *Gibbula cineraria* (Linnaeus)

Gibbula cineraria (Figure 1.5) possesses a flat shell that is up to 16 mm tall and 15 mm wide, with compressed rounded whorls with fine spiral ridges ending in a sharp apex. (Hayward *et al*, 1995a; Gibson *et al*, 2001; Bruyne, 2003). It is usually found in the mid to lower rocky shore commonly under boulders or stones and seaweeds, and is thought to have less resistance to desiccation and salinity variation than its relative *O. lineata* (Hayward *et al*, 1995a; Gibson *et al*, 2001). It is abundant in all British coasts, distributed commonly on Atlantic, English Channel, and North Sea coasts of north-western Europe (Hayward *et al*, 1995a; Gibson *et al*, 2001). This species has a relatively long planktonic stage (Johnson *et al*, 2001).



Figure 1.5 Grey Top Shell *Gibbula cineraria* (Linnaeus). Scale bar = 5 mm.

Flat Top Shell *Gibbula umbilicalis* (da Costa)

Gibbula umbilicalis (Figure 1.6) reaches a maximum size of 15 mm tall and 22 mm wide, and has a smooth, rounded and conical shell shape with defined whorls (Hayward *et al*, 1995a; Gibson *et al*, 2001) that is a greenish or creamy colour with red bands (Gibson *et al*, 2001). This species can tolerate high levels of salinity variation and desiccation and inhabits the entire shore where it is commonly found in rock pools (Newell, 1979; Gibson *et al*, 2001). Its distribution extends from the Orkney Islands and Irish sea coasts to north-west Europe and western England (Gibson *et al*, 2001). This species has a relatively very short planktonic stage lasting only a few days (Desai, 1966; Johnson *et al*, 2001).



Figure 1.6 Flat Top Shell *Gibbula umbilicalis* (da Costa). Scale bar = 5 mm.

Predator Species

***Carcinus maenas* (Linnaeus)**

The European common shore or green crab *Carcinus maenas* originates from the northeast Atlantic and is distributed throughout all European coasts; it has also invaded the northwest Atlantic, the Northeast Pacific, Australia and South Africa (Hayward *et al*, 1995b; Grosholz and Ruiz, 1996; Gibson *et al*, 2001). Throughout its range, *Carcinus maenas* is considered among the most important shoreline predators (Raffaelli, 1982; Boulding and Hay, 1993; Lowell *et al*, 1994; Bertness, 1999; Trussell and Smith, 2000; Johannesson and Ekendahl, 2002; Trussell *et al*, 2002; Cotton *et al*, 2004; Kempainen *et al*, 2005). The carapace of *Carcinus* may reach a length of 6 cm and a width of 7.5 cm and its colour varies from white in juveniles to

dark green in the adult stage (Hayward *et al*, 1995b; Gibson *et al*, 2001). This species is also found in habitats such as estuarine muds, saltmarshes and splash-zone pools (Hayward *et al*, 1995b; Gibson *et al*, 2001).

Study Site

All snails were collected from the rocky intertidal at Hannafore Point, Cornwall, the United Kingdom (50° 20' N, 4° 27' W). *Carcinus maenas* individuals were collected from the Plym Estuary, Devon, U.K. (50° 22' N, 4° 6' W). All the types of alga (*Enteromorpha lactuca*, *Fucus serratus* and *Ascophyllum nodosum*) used throughout the study were also collected from the Plym Estuary.

Chapter 2

Predator-Induced Phenotypic Plasticity in Marine Gastropods: A Cross-Species Comparison

SUMMARY

Induced defences are a common form of phenotypic plasticity in species that experience varying levels of predation. In marine systems, studies of this phenomenon have focused on the responses of single or pairs of species. Here I make a cross-species comparison of induced defences for six species of intertidal gastropod, including three Littorinidae (*Littorina littorea*, *L. fabalis* and *L. obtusata*) and three Trochidae (*Osilinus lineata*, *Gibbula cineraria* and *Gibbula umbilicalis*). My main aim was to test whether species showed similar types of induced defence and if plastic responses were constrained evolutionarily, i.e. linked to species' relatedness. Snails were raised (in the laboratory) for 15 days in the presence or absence of green shore crabs *Carcinus maenas* to compare the direction of induced defences in several traits: shell thickness, shell width, shell shape (aspect ratio and aperture ratio) and total wet weight. There were significant treatment and species effects for most of the morphological traits measured and a significant species vs. treatment interaction for all traits. Hence, species differed both in terms of whether they showed an induced response and in the traits exhibiting responses and in how they responded. A multivariate (cluster) analysis of all trait plasticities showed that induced responses were not linked to relatedness. These findings suggest that there is significant variation in plastic responses between gastropod species inhabiting rocky shore habitats, which could have implications for ecological interactions in these habitats. This variation is not related to phylogeny, but may be related to susceptibility to crab predation.

INTRODUCTION

Phenotypic plasticity, the differential phenotypic expression of the same genotype in response to the environment, is a phenomenon central to the study of evolution and ecology that, in effect, aims to get to the crux of the “nature versus nurture” debate (Schlichting, 1986; Schlichting and Pigliucci, 1998; Pigliucci, 2001; West-Eberhard, 2003; DeWitt and Scheiner, 2004; Pigliucci, 2005; Brakefield, 2006). Much of our current knowledge of the potential role of plasticity in evolution draws on studies that have demonstrated adaptive plasticity (Lyytinen *et al*, 2003), selection for plasticity (Van Buskirk and Relyea, 1998; Scheiner, 2002), and have provided models that account for the genetic basis for plasticity (Price *et al*, 2003) (see review by Pigliucci, 2005). Whilst such studies have shown that plasticity is a widespread phenomenon, and provide some insight into the mechanisms underpinning plasticity, they are limited in what they can tell us about the importance of plasticity for macroevolution. Hence, the investigation of plasticity across species within phylogenies is a research priority (West-Eberhard, 2003; Pigliucci, 2005).

Induced defences are a common form of phenotypic plasticity exhibited in response to the threat of predation, and have been demonstrated in a wide range of organisms (Tollrian and Harvell, 1999; Weining and Delph, 2001; Agrawal *et al*, 2002; Callaway *et al*, 2003). In marine systems, induced defences have been demonstrated to occur in: brown and green algae (Borell *et al*, 2004; Molis *et al*, 2006; Toth and Pavia, 2007), marine zooplankton (Vaughn, 2007), sea urchins (Russell, 1998), mussels (Leonard *et al*, 1999; Beadman *et al*, 2003; Freeman, 2007), intertidal barnacles (Lively *et al*, 2000), fish (Krause *et al*, 1996) and gastropods (Bertness and Cunningham, 1981; Kemp and Bertness, 1984; Appleton and Palmer, 1988; Trussell, 2000a; Trussell and Smith, 2000; Trussell and Etter, 2001; Delgado *et al*, 2002;

Trussell and Nicklin, 2002; Hollander *et al*, 2006; Brookes and Rochette, 2007; Vaughn, 2007). Moreover, it has been demonstrated that the presence of predators can mediate effects in marine food chains through trait-mediated indirect interactions (TMII), whereby the density of a species is affected by a trait change (i.e. an induced defence) of an intermediate species (Trussell *et al*, 2003). Hence, it is clear that an induced defence is likely to have an important role to play in both the evolution and ecology of marine systems.

Marine gastropods are important components of most intertidal habitats (Trussell, 2000a; Johannesson, 2003) where they are subject to great variation in biotic factors, such as competition and predation, and abiotic factors such as temperature, salinity, wave action and desiccation (Johannesson, 2003). As such, they are excellent models for studying plasticity and, notably, induced defences (Kemp and Bertness, 1984; Appleton and Palmer, 1988; Trussell and Nicklin, 2002; Trussell and Smith, 2000; Trussell *et al*, 2003; Smith and Ruiz, 2004; Hollander *et al*, 2006; Brookes and Rochette, 2007). Defensive traits in the gastropod shell have been the focus of several plasticity studies; in order to resist predators such as crabs, gastropods may exhibit induced defences in the form of heavy shells, selective thickening of the body whorl or aperture, different sculpturing and or a reduced apical spire, narrow aperture (Vermeij, 1978; Bertness and Cunningham, 1981; Reimchen, 1982; Vermeij, 1993; Lowell *et al*, 1994). However, the morphology and structure of gastropods shells appears to be related to their predator attack mechanisms (Vermeij, 1978). Several studies have also demonstrated that different shell parameters such as density, length, height, mass, thickness, aperture size and shape are factors, which provide resistance to predators (Vermeij, 1978; Reimchen, 1982; Lowell *et al*, 1994; Delgado *et al*,

2002; Cotton *et al*, 2004). Hence, the gastropod shell offers the opportunity to assess plastic responses in several different defensive traits at the same time.

As is generally true of studies of phenotypic plasticity, studies on induced shell defences in marine gastropods have focused on single species and/or traits (Kemp and Bertness, 1984; Appleton and Palmer, 1988; Trussell, 2000a; Trussell and Nicklin, 2002; Brookes and Rochette, 2007), and rarely consider multiple phenotypic traits (Trussell, 2000b; Trussell and Nicklin, 2002; Hollander *et al*, 2006). Hence, we have a poor understanding of just how widespread a phenomenon induced shell defences are and of the relative importance of evolutionary history and ecology in shaping these defences (but see recent meta-analysis by Hollander, 2008). In this study I examined the expression of two forms of induced defences (shell morphology and strength) in six species of intertidal marine gastropod exposed to predation cues from the green shore crab *Carcinus maenas*. My main aims were to assess whether: i) induced shell defences occurred in all species and, if so, whether species exhibited plasticity in the same shell trait; and ii) whether shell plasticities were linked to species' relatedness.

MATERIALS AND METHODS

Study Organisms

I compared induced defence production in six species of gastropod common in intertidal habitats in the UK: *Littorina littorea* (Linnaeus), *L. fabalis* Turton, *L. obtusata* (Linnaeus), *Osilinus lineata* (da Costa), *Gibbula cineraria* (Linnaeus), and *G. umbilicalis* (da Costa). All snails were collected during May-June 2005, from one shore at Hannafore Point, Cornwall, UK (50° 20' N, 4° 27' W); the use of snails from a single shore reduced the influence of local adaptation on shell morphology. Twenty snails of each species were used for each treatment (control/ predator cue). The size of the snails used was standardised as far as possible, mean lengths in mm (\pm SD) were: 7.0 (\pm 1.0) for *L. fabalis*, 9.5 (\pm 1.0) for *L. obtusata*, 7.5 (\pm 1.0) for *L. littorea*, 7.0 (\pm 0.5) for *O. lineata*, 6.5 (\pm 1.0) for *G. cineraria* and 7.5 (\pm 1.0) for *G. umbilicalis*. Prior to growth trials, all snails were acclimated under laboratory conditions for 10 days. Subsequently, snails were kept under the experimental conditions for 15 days at 15° C in filtered sea water and were fed a mixture of three types of algae (*Enteromorpha lactuca*, *Fucus serratus* and *Ascophyllum nodosom*) collected from the Plym Estuary, Devon, UK (50° 22' N, 4° 06' W). This time period has been shown previously to be adequate for detecting plasticity in aquatic gastropods (Delgado *et al*, 2002; Rundle *et al*, 2004). Upon the completion of the experiment, all snails were frozen for further analysis (see below).

Carcinus maenas was used to produce predator cues as this crab is a common intertidal predator on rocky shores in the UK and feeds on gastropods (Trussell, 2000a; Trussell and Smith, 2000; Yamada *et al*, 1998; Reid *et al*, 2002; Cotton *et al*, 2004). Individuals were collected in May 2005 from the Plym Estuary, Devon, UK. All crabs used were in good condition with no signs of damage or limb loss, and had

carapace widths of 4-6 cm. The stock of crabs was maintained in sea water at temperature of 15° C and fed with frozen fillet of Coley fish (*Pollachius virens*) once every three days. Groups of crabs for use in trials were randomly chosen from this stock population and introduced to the "cue" tanks and replaced every three to four days.

Experimental set up

Experimental mesocosms were 20 l plastic aquaria (32 cm length, 18 cm width and 20 cm height) containing aerated sea water at 15° C (12:12 hr Light:Dark regime). The control treatment contained only sea water, while cue treatment tanks contained a single crab from the stock population repetition (see above). There were twenty mesocosms for each treatment (20 control/20 predator cue), interspersed randomly. Each mesocosm was stocked with 6 pots (4 cm diameter, 6 cm height and 60 ml volume) containing an individual gastropod, one from each species. Thirty-five holes (3.5 mm diameter) were made in each pot to allow water circulation. Each pot also contained one disc (1 cm²) each of *Enteromorpha lactuca*, *Fucus serratus* and *Ascophyllum nodosum* which were replaced twice each week.

Measuring Induced Defences

I measured several parameters that relate to defence in gastropods such as shell length, width, thickness, aspect ratio (length/width) and aperture ratio (aperture length/ aperture width) (Figure 2.1) (Vermeij, 1993; Trussell, 2000a; Trussell and Nicklin, 2002; Cotton *et al*, 2004; Rundle *et al*, 2004). Morphological parameters were measured at the start and completion of the experiment and calculated as the proportional difference between the final and initial measurements of the trait in

question. Images were taken using a Nikon coolpix 4500 Digital Camera attached to Kyoma optical SDZ-IR-P microscope and analysed using analySIS software. For analysis, the proportional difference between the final and initial measurements of the following traits: shell length, width, aperture length, aperture width and thickness. Shell strength related to susceptibility to predation and was measured as crush resistance by an Instron 4301 Universal mechanical testing machine (Instron Corporation, Canton, Massachusetts, USA). The measurement used was the compressive force (KN) needed to produce a fracture in the shell.

Total snail wet weight was taken at the beginning and the completion of the experiment. Each snail was left to dry on tissue paper for 10 minutes to ensure there was no water still within shell and the weight was taken using digital balance ($\pm 0.0001\text{g}$).

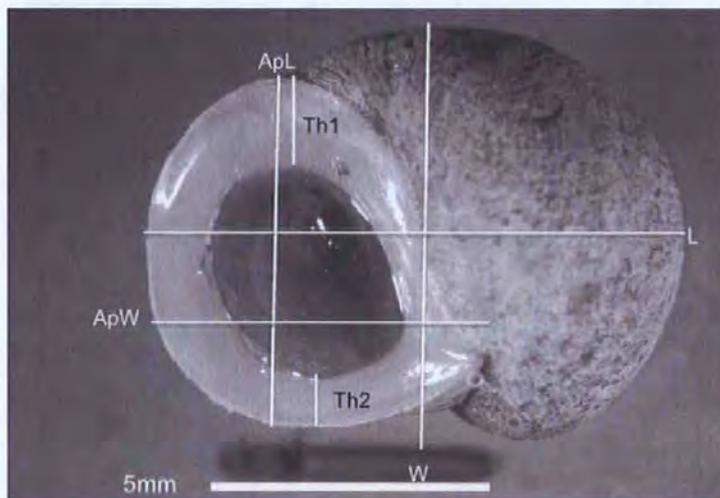


Figure 2.1 Shell measurements: shell length (L), shell width (W), shell aperture length (ApL), shell aperture width (ApW), shell thickness (Th1, Th2). The image sample used is *Littorina obtusata*, which represent the measurements in all other species.

Statistical Analysis

Repeated measures ANOVAs in SPSS 15 were used to test for differences in traits between species and treatments and their interaction with tank used as the repeated measures in the analysis. Measurements of all traits except crush resistance force were expressed as proportional increase over the trial and were arcsine transformed before the analysis (Sokal and Rohlf, 1995; Dytham, 2005). T-tests were used to test for significant treatment effects for each trait within species with Bonferroni correction (critical value for six tests $p < 0.0083$) and Levene's test was used for normality of variance of data. Cluster analysis was undertaken on multiple traits using a programme within the Primer-6 package (Plymouth Routines in Multivariate Ecological Research of PRIMER-E Ltd) to test the similarity of induced defence response between taxa. This analysis (Euclidian distance similarity measure; agglomerative clustering using group-averaging) aimed to determine whether species within the two families cluster together and to illustrate the pattern and degree of similarities or differences between the species across all measured variables (traits) in the treatments.

RESULTS

Survival rates were generally high for the duration of the study (> 95 %). There were significant treatment effects for the percentage change in several traits, including: width, aspect ratio, thickness and total wet weight, and also a significant species effect for all traits (Table 2.1). Significant species vs. treatment interactions for all traits apart from thickness and aperture ratio also indicated that species differed in their response. Individual and tests showed that *L. littorea* had a significant increase in the presence of predator cues in four traits (shell length, shell width, shell thickness and total wet weight). *Gibbula cineraria* showed significant increase in two traits (shell width and shell thickness), whereas for three species *Littorina fabalis*, *Littorina obtusata*, *Osilinus lineata* and *Gibbula umbilicalis*, showed positive increases in at least one trait (Table 2.2). Shell thickness increased significantly in *L. littorea* ($t_{38}= 4.554$, $p < 0.0083$), *L. obtusata* ($t_{36}= 5.408$, $p < 0.0083$) and *G. cineraria* ($t_{37}= 3.3$, $p < 0.0083$) (Table 2.2 and Figure 2.2 A). There were significant increases in the proportional shell length of *L. littorea* ($t_{38}= 3.365$, $p < 0.0083$), *O. lineata* ($t_{38}= 3.184$, $p < 0.0083$) and *G. umbilicalis* ($t_{38}= 3.938$, $p < 0.0083$), whereas the shell width increases significantly in *L. littorea* ($t_{38}= 4.728$, $p < 0.0083$) and *G. cineraria* ($t_{37}= 3.233$, $p < 0.0083$) (Table 2.2). The shape of the shell in *L. littorea* and *G. cineraria* also became more rounded on exposure to cues, with decreased aspect ratio ($p < 0.05$; Table 2.2 and Figure 2.2 C). The aperture ratio decreased significantly ($p < 0.05$) in *L. littorea* (Figure 2.2 D), potentially indicating a response of minimizing aperture size in this species. Proportional wet weight (Figure 2.2 E) increased in response to cues in *L. fabalis* ($t_{38}= 3.177$, $p < 0.0083$), but showed no significant difference in the rest of the studied species (Table 2.2).

Cluster analysis of all trait plasticities combined demonstrated that species' plasticities were not related to their phylogeny (Figure 2.3). Species were clustered into two main groups: *L. littorea* and *G. cineraria* in one group and *L. fabalis*, *O. lineata* and *G. umbilicalis* in the other. *L. obtusata* was an outlier and not closely associated with any other species.

Table 2.1 Results of repeated measure ANOVAs testing for differences in shell trait induced defences (length, width, aspect ratio, aperture ratio, thickness) and total wet weight between species and treatments and their interactions (with or without predation cue from *Carcinus maenas*) over 15 days in six species of gastropod: *Littorina littorea*, *L. fabalis*, *L. obtusata*, *Osilinus lineata*, *Gibbula cineraria* and *G. umbilicalis*.

| Trait | Source | DF | F | p-value |
|----------------|-------------------|--------|--------|----------|
| Length | Treatment | 1, 37 | 3.239 | 0.0801 |
| | Species | 5, 185 | 26.937 | < 0.0001 |
| | Treatment*Species | 5, 185 | 3.298 | < 0.05 |
| Width | Treatment | 1, 37 | 8.154 | < 0.05 |
| | Species | 5, 185 | 16.427 | < 0.0001 |
| | Treatment*Species | 5, 185 | 4.790 | < 0.001 |
| Aspect ratio | Treatment | 1, 37 | 8.154 | < 0.05 |
| | Species | 5, 185 | 16.427 | < 0.001 |
| | Treatment*Species | 5, 185 | 4.790 | < 0.001 |
| Aperture ratio | Treatment | 1, 34 | 3.822 | 0.059 |
| | Species | 5, 170 | 5.148 | < 0.001 |
| | Treatment*Species | 5, 170 | 2.257 | 0.051 |
| Thickness | Treatment | 1, 37 | 11.99 | < 0.05 |
| | Species | 5, 175 | 8.51 | < 0.001 |
| | Treatment*Species | 5, 175 | 0.727 | 0.604 |
| Wet weight | Treatment | 1, 36 | 15.458 | < 0.001 |
| | Species | 5, 180 | 5.313 | < 0.001 |
| | Treatment*Species | 5, 180 | 3.347 | < 0.05 |

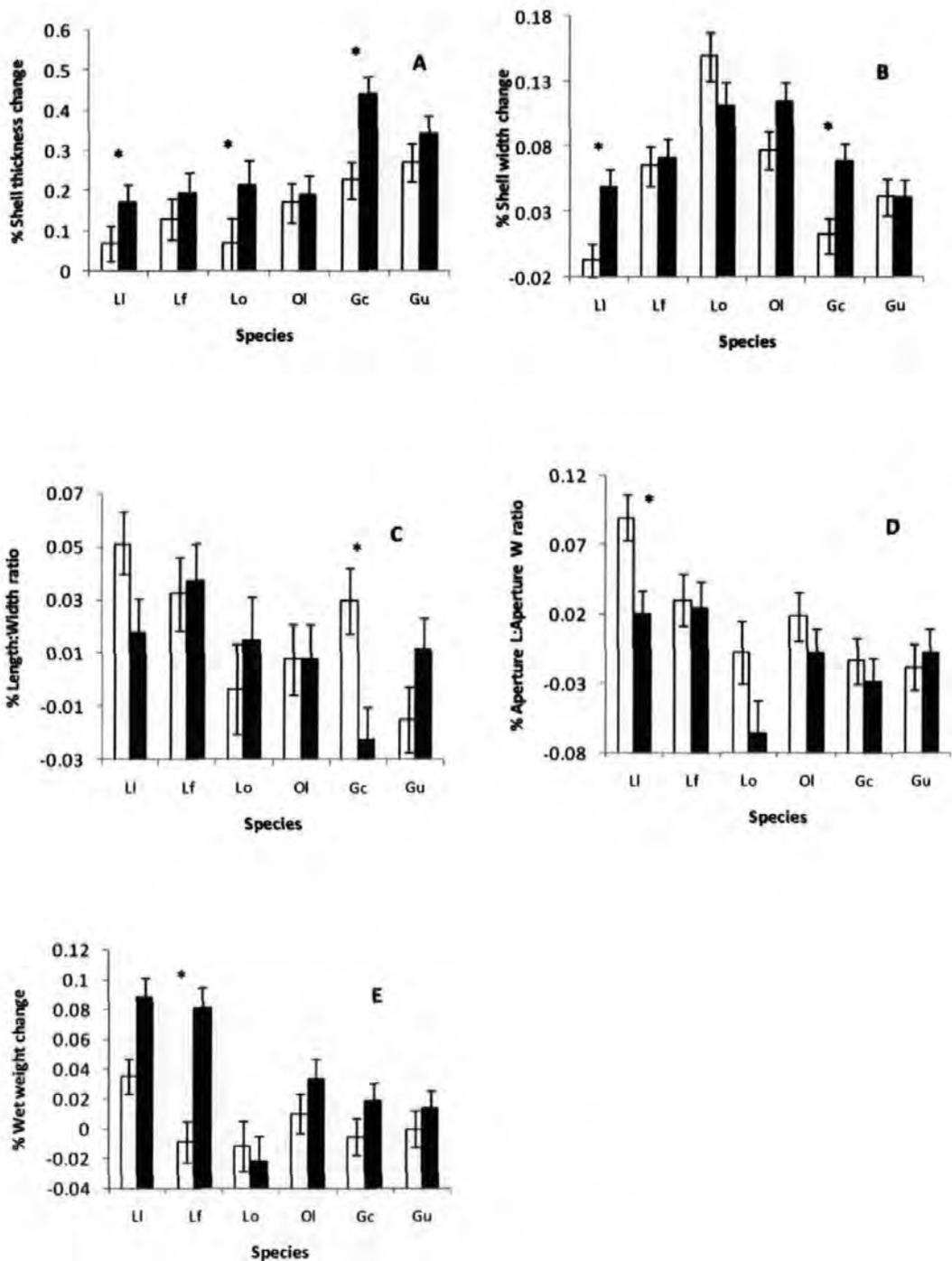


Figure 2.2 Response of six gastropods (*Littorina littorea* (LI), *L. fabalis* (Lf), *L. obtusata* (Lo), *Osilinus lineata* (Ol), *Gibbula cineraria* (Gc) and *G. umbilicalis* (Gu)) when raised with control sea water (light bars) or seawater containing *Carcinus maenas* cues (dark bars). Values are expressed as means \pm SE proportional change over the experimental period: a) Shell thickness, b) shell width, c) shell length relative to shell width (aspect ratio), shell aperture length relative to shell aperture width (aperture ratio), d) shell total wet weight. * = post hoc comparison, significant @ $p < 0.05$.

Table 2.2 Summary of induced defences for six gastropod species (*Ll- Littorina littorea*, *Lf- L. fabalis* , *Lo- L. obtusata* , *Ol- Osilinus lineata*, *Gc- Gibbula cineraria* , *Gu- G. umbilicalis* (*Gu*)) in response to crab cues: ++ significant increase compared with control ($p < 0.0083$ see text); + increase non significant compared with control ($p < 0.05$); - non- significant decrease ($p < 0.05$) ; -- significant decrease ($p < 0.0083$).

| Trait | <i>Ll</i> | <i>Lf</i> | <i>Lo</i> | <i>Ol</i> | <i>Gc</i> | <i>Gu</i> |
|------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Shell length | ++ | | - | ++ | + | ++ |
| Shell width | ++ | | - | | ++ | |
| Aspect ratio | - | | | | - | |
| Aperture ratio | -- | | - | | | |
| Shell thickness | ++ | + | ++ | | ++ | |
| Total wet weight | + | ++ | | + | + | |
| Crush force | ++ | | | | | |

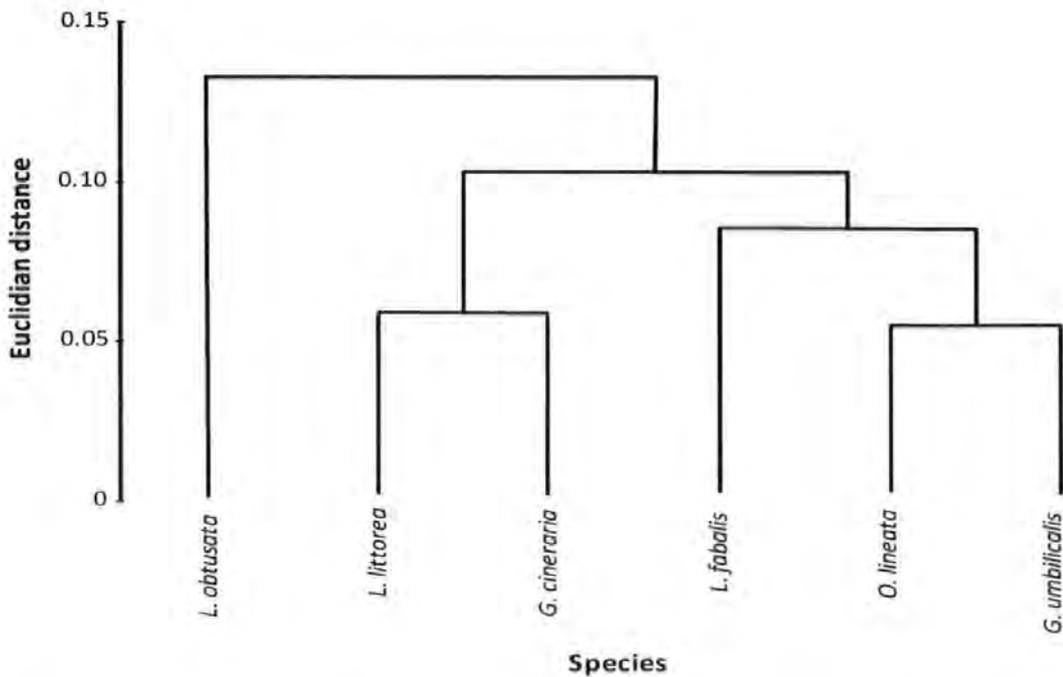


Figure 2.3 Dendrogram of Euclidian distances from Primer-6 cluster analysis that shows how *Littorina littorea*, *L. fabalis*, *L. obtusata*, *Osilinus lineata*, *Gibbula cineraria* and *G. umbilicalis* cluster in groups in terms of the similarity in their plastic responses when raised with *Carcinus maenas* cues (predator) or control water (see text for details).

DISCUSSION

Numerous studies have revealed that the presence of predators can cause species to exhibit induced defences, yet there have been few cross-species comparisons that have included more than two species (although see meta-analysis by Hollander, 2008). Here I demonstrated that six intertidal gastropods showed highly varied responses to cues from *Carcinus maenas*, from little response in the case of *G. umbilicalis*, *O. lineata*, *L. fabalis* and *L. obtusata* to significant plasticity in several defensive traits in *L. littorea* and *G. cineraria*. It was also clear that the plasticity in these species was not linked to their evolutionary relatedness, with species from the same genus in different clusters based on their combined plasticities.

The few cross-species studies of induced defences that have been made do allow some comparison with my findings. Van Buskirk (2002) examined induced defences in 16 species of tadpoles exposed to dragonfly predator cues. He found evidence for a positive phylogenetic correlation between morphological plasticity and the exposure to highly variable environments. Relyea and Werner (2000) and Relyea (2001) conducted cross-species comparison of induced defences in tadpoles and demonstrated morphological and behavioural induced defences; however, the effect of relatedness was not investigated. If evolution constrained plasticity I might have predicted that in my study closely related species would show similar induced defence responses when exposed to predator cues. However, cluster analysis (Figure 2.3) of all of the shell plasticities combined demonstrated the three littorinid species (*L. littorea*, *L. fabalis* and *L. obtusata*) had clearly different types and directions of induced shell defences. Similarly, responses in the three trochid species (*O. lineata*, *G. cineraria* and *G. umbilicalis*) were not linked to phylogeny. Indeed the two most

closely related littorinids, *L. obtusata* and *L. fabalis*, had markedly different induced responses, with *L. obtusata* an outlier in the cluster analysis. According to Lowell *et al* (1994), these two sibling littorinid species are sympatrically distributed and share similar predatory threats; however, their markedly different induced defence responses are seemingly related neither to their evolutionary nor ecological similarities.

It is possible, however, in the absence of any clear influence of evolution, that species' ecologies might relate to the expression of induced defences. A recent study on some of the gastropod species used in my study (Cotton *et al*, 2004) assessed how different shell parameters related to the risk of predation gauged by the handling time of *Carcinus maenas*. This work demonstrated that species such as *G. umbilicalis*, with a more discoid shaped shell (i.e. with a lower aspect ratio), were less susceptible to crab predation than those such as *L. littorea* with high spires (high aspect ratio). The significant reduction in aspect ratios in *L. littorea* and *G. cineraria* in my study suggests that these species respond when exposed to crab cues by growing a shell form that offers more protection from crab predators.

The comparison with Cotton *et al* (2004) also allows me to examine the susceptibilities of species to crab predation and the degree of plastic responses exhibited. They found that handling time by *Carcinus maenas* decreased as follows: *G. umbilicalis* > *O. lineata* > *G. cineraria* > *L. littorea*. Interestingly, in my study, the degree of plasticity as gauged by the number of shell traits showing an induced response (either significant or marginally, non-significant) was in the reverse order: 5 (*L. littorea*) > 4 (*G. cineraria*) > 2 (*O. lineata*) > 1 (*G. umbilicalis*). Although I cannot draw any strong conclusions from a comparison with comparatively few species, this result does suggest that further exploration of the link between susceptibility and

degree of induced defence expression might be worth further exploration in marine gastropods, for example through test of the adaptive plasticity hypothesis (Van Buskirk, 2002).

Increased shell thickness has been demonstrated to be a substantial defensive trait in marine gastropods in responses to crab predation (Vermeij and Covich, 1978; Vermeij, 1993; Trussell, 2000a; Trussell and Nicklin, 2002). *Littorina littorea* and *L. obtusata* both showed significant increases in shell thickness in my study, and *L. fabalis* and *G. cineraria* also showed increases although there were non-significant following Bonferroni correction ($p < 0.05$). These results seem in accordance with other studies, particularly as Trussell and Nicklin (2002) have reported plasticity in *Littorina obtusata* in North American populations. It may be that thickness is important in specific instances, or is a trait that is plastic later in ontogenetic development. In the UK, *Littorina obtusata* usually occurs in shallow water, tends to be very tolerant of a wide range of exposure, and generally lives on macroalgae, especially *Fucus vesiculosus* and *Ascophyllum nodosum*; by contrast, *L. fabalis* is mainly found on *Fucus serratus*, lower on the shore than *L. obtusata* (Hayward *et al*, 1995a; Gibson *et al*, 2001). The first two species of algae have spherical to elliptical air bladders that are frequently found damaged, or with a hole (Gibson *et al*, 2001) that might provide protection for small *L. obtusata* (Reimchen, 1982). Conversely, *F. serratus* has no air bladders (Gibson *et al*, 2001), thus *L. fabalis* does not have access to the same shelter on this seaweed. Similarly, with their study on *L. fabalis*, Kempainen *et al* (2005) suggested that induced defence on intertidal rocky shores is very complicated and may incorporate several factors in addition to more than the wave action and crab predation threat, for example life history optimization and algal refuges.

It is also possible that plasticity might be influenced by life history strategy or by environmental influences during early development. Species with planktotrophic larvae typically experience a greater range of environmental variability compared with those with direct development (Yamada, 1987; Johannesson, 1992; Hollander *et al*, 2006; Hollander, 2008). Hence, we might predict that planktotrophs would exhibit greater plasticity. A recent study by Vaughn (2007) also demonstrated that veligers of *Littorina scutulata* developed rounder shells with smaller shell apertures in the presence of *Cancer* zoea, which made them more resistant to predation. This study flags the possibility that plasticity in later stages might reflect the experience early in development, again raising the possibility that life history strategy might be of importance. In my study we used three species (*Littorina littorea*, *Gibbula umbilicalis* and *G. cineraria*) with relatively long planktonic stages (e.g., Kemp and Bertness, 1984; Johnson *et al*, 2001; Hollander *et al*, 2006), one (*O. lineata*), with a relatively short larval stage (Mieszkowska *et al*, 2007) and two species with direct development (*L. obtusata* and *L. fabalis*; Paterson *et al*, 2001; Kemppainen *et al*, 2005). Again, however, there is no unambiguous link between the degree of plasticity exhibited and life history strategy in my study.

Predator-prey interactions may have direct effects on prey density and prey resources in intertidal habitats (Trussell *et al*, 2003). At the same time, plastic responses may lead to indirect food chain effects through trait-mediated indirect interactions (TMIIIs) (Relyea, 2000; Dill *et al*, 2003) or behaviourally mediated indirect interactions (BMIIIs) – a specific form of TMII (Dill *et al*, 2003). In my study, all taxa studied occupy similar habitats and share the threat of predation from *Carcinus*. Crab predators may alter the foraging behaviour of gastropods either by suppressing activity (Trussell *et al*, 2002) or by prolonged manipulation during which time the

snail remains inactive in the shell (Vermeij, 1993). Crab predation plays an important role influencing intertidal snail density, and indirect resource regulation and species interactions over intertidal rocky shore through TMIs (Trussell *et al*, 2002; Trussell *et al*, 2003; Trussell *et al*, 2004). For example species with poor structure defences spend less time foraging (due to increased levels of avoidance behaviour) may be at a competitive disadvantage. Therefore, even the species that did not show any significant induced defences may still be affected positively or negatively by *Carcinus* impact through BMIs or TMIs.

Chapter 3

Flexibility of Phenotypic Plasticity in *Littorina littorea*

SUMMARY

The production of induced defences is assumed to be costly and, hence, individuals should not invest in plasticity under low risk conditions. At the same time organisms should aim to match their optimum phenotype as quickly as possible, and make up for any time lag in induced defence production. In this chapter I investigate such flexibilities in induced defence production in *Littorina littorea*. Snails were exposed to four treatments for six weeks: i) no predation threat; ii) constant predation threat; iii) the removal of predation threat after three weeks; iv) the addition of a predation threat after three weeks. Two size groups of *L. littorea* were used in this study to reveal if the plasticity and flexibility of plasticity varied with size. There was clear evidence that snails altered their degree morphological plasticity with altered predation threat. Snails exposed to predation threat half way through the trial showed a high degree of plasticity whereby they appeared to “catch up” snails continuously exposed to predator cues in terms of their shell size. Snails that experienced a removal of predation cues showed a significant reduction in growth rate following this switch in predation environment. There were no significant differences in the responses of different sized snails suggesting that at this stage in their development snails show similar adjustments in their response to predator cues.

INTRODUCTION

Despite the large number of studies documenting phenotypic plasticity in a wide and varied range of organisms (Via *et al*, 1995; Tollrian and Harvell, 1999; Trussell, 2000a; Trussell and Etter, 2001; Relyea, 2002; Gabriel, *et al*, 2005; Brookes and Rochette, 2007), most of these studies used the approach of subjecting target organisms to constant environmental conditions (Gabriel, 2005; Gabriel, 2006). In reality, however, it is likely that in many situations environmental conditions will fluctuate during the period when individuals are exhibiting plastic responses. Indeed, the degree of change in environmental conditions may actually favour reversible plasticity (Doughty and Reznick, 2004). In particular, if an organism frequently experiences changes in environmental conditions of duration shorter than their lifetime, then the reversible plasticity could be adaptive (Piersma and Drent, 2003; Doughty and Reznick, 2004; Gabriel, 2005; Gabriel *et al*, 2005).

Reversible phenotypic plasticity has been documented in some cases in response to variation in local food conditions, for example rapid adjustment of body size by positive or negative growth in response in Caribbean sea urchins *Diadema antillarum* (Levitan and Genovese, 1989), reduction in overall body size in marine iguanas *Amblyrhynchus cristatus* (Piersma and Drent, 2003), and flexible mass and the relative mass of gills and palps in Pacific oyster *Crassostreaa gigas* (Honkoop *et al*, 2003). Studies on reversible phenotypic plasticity and induced behaviours in response to predation threat have also been performed previously on organisms such as wood frogs *Rana sylvatica* and leopard frogs *R. pipiens* (Relyea, 2000), treefrog tadpoles *Hyla versicolor* (Relyea, 2003), Matsui tadpoles *Rana pirica* (Kishida and Nishimura, 2006), and the common *Anolis sagrei* Lizard (Schoener *et al*, 2002). Such examples

also exist for molluscs. Although their responses were limited to their early ontogeny, freshwater snails *Helisoma trivolvis* showed reversible induced defences when the impact of water bug predators ceased (Hoverman and Relyea, 2007). Intertidal mussels *Semimytilus algosus* also exhibited flexible inter-population morphological plasticity in response to local levels of crab and /or snail predation (Caro and Castilla, 2004).

The interaction between predatory crabs and gastropod prey has provided an excellent model for examining the evolutionary ecology of induced plastic defences (Bertness and Cunningham, 1981; Reimchen, 1982; Palmer, 1985; Appleton and Palmer, 1988; Trussell, 1996; Trussell, 1997; Trussell, 2000a; Trussell, 2000b; Trussell and Smith, 2000; Trussell and Etter, 2001; Trussell and Nicklin, 2002; Trussell *et al*, 2002; Johannesson, 2003; Trussell *et al*, 2003; Cotton *et al*, 2004; Trussell *et al*, 2006; Brookes and Rochette, 2007). The intertidal snails *L. sitkana* resumed normal feeding with the removal of predatory crab *Cancer productus* (Randall) suggesting reversible growth rate with changing environmental conditions (Yamada *et al*, 1998). However, none of these studies has investigated the flexibility of induced defences in marine gastropods. At the same time there has been no attempt to assess how plastic responses vary through their development (Lowell *et al*, 1994).

In Chapter two I demonstrated significant variation in plastic responses between gastropod species inhabiting a rocky shore. This study extends this work by assessing the flexibility of marine gastropods in the development of induced defence over time. The common periwinkle *Littorina littorea* was identified as an ideal model for such studies as it exhibited plasticity in the laboratory trials (Chapter 2), was also showing

in a preliminary trial to have a high survival for several weeks in the laboratory. The aim of this study was to examine whether plastic responses were sensitive to the disappearance and appearance of predator threat during development. Specifically, I measured a range of induced defensive traits in the intertidal gastropod *Littorina littorea* to investigate whether individuals of different sizes exhibited: i) different rates and forms of induced defence; ii) different developmental windows for induced defence expression; iii) different potential for trait reversal.

MATERIALS AND METHODS

Study Organisms

Littorina littorea (Linnaeus) individuals were collected in November 2005 from the intertidal, Hannaford Point, Cornwall, UK (50° 20' N, 4° 27' W) and were then maintained in the laboratory at 15 °C for two weeks prior to experimental trials and fed on *Enteromorpha lactuca*. *Carcinus maenas* individuals used to produce predator cues were also collected in November 2005 from the Plym Estuary, Devon, UK (50° 22' 0.7° N, 4° 6' 0.1° W). All crabs used were in good condition with no signs of damage or limb loss, and had carapace widths of 4-6 cm. The stock population of crabs were maintained at laboratory temperature of 15 °C and fed with frozen fillet of Coley fish (*Pollachinus virens*) once every three days. Groups of crabs for use in trials were randomly chosen from this stock population.

Experimental design and set up

In order to investigate flexibility of plastic responses in *L. littorea* I used a set of experimental treatments that represented four scenarios that individuals might experience in the field: i) no predation threat (CC); ii) constant predation threat (PP); iii) the removal of a predation threat (PC); and iv) the addition of a predation threat (CP). Trials were run using two different sizes of snail (see below), which allowed an investigation of whether plasticity and flexibility of plasticity varied with size; here I predicted that larger snails would respond less due to increased investment in reproduction. As well as assessing the overall effects of predator cue treatments at the end of the experiment, I also tested how snails responded to altered predation threat using differences in growth rate before and after manipulations of the cue environment. In particular I tested whether snails that had been exposed to the threat

of predation reduced their growth rate on removal of predators (due to the costs of the plastic response) or continued on the same growth trajectory, due to an “epiphenotypic problem” (DeWitt *et al*, 1998). Similarly, where a predation threat was added, I tested whether snails increased their growth rate in line with that in predator treatments (i.e. whether there were developmental windows for plasticity) or exhibited “catch up” growth (i.e. an increase in growth rate) such that snails in the CP treatment ended up similar in size to snails in PP treatment. Trials were run for six weeks with the change in predator status in the latter two trials implemented at three weeks.

Nine replicate mesocosms (20 L plastic aquaria - 32 cm length, 18 cm width and 20 cm height) were used for each treatment, each containing 6 pots (4 cm diameter, 6 cm height and 60 ml volume) housing a single gastropod of either 6.1 ± 0.05 mm (small) or 8.7 ± 0.05 mm (large) mean length; hence, each mesocosm contained three snails of each size. Each pot contained one disc (1 cm^2) of *Enteromorpha lactuca* and had thirty-five holes (3.5 mm diameter) that allowed water circulation. Mesocosms contained 20 L containing aerated seawater and were maintained at 15°C (12:12 hr Light regime). Predation threat was simulated by including an individual *Carcinus maenas* in the mesocosm; crabs were placed on the bottom of the tank and were unable to access the pots containing snails. Crabs were present in the constant predation threat treatments (PP) for the entire period, and were replaced every 3 days from the stock population. For the variable predation threat treatments, crabs were either introduced (CP) or removed (PC) at twenty-one days; crabs in the former treatment were again replaced every 3 days. Mesocosms were arranged in random

order on a single shelf in the laboratory. Upon completion of the experiment, all snails were frozen for further analysis and crabs returned back to their site of collection.

Measuring Induced Defences

Several morphological parameters that relate to defence in gastropods were measured at the start of the experiment and at 21 and 42 days: shell length, shell width, shell thickness, shell aperture length, shell aperture width, aspect ratio (shell length/shell width) and aspect ratio (aperture length/aperture width) (Vermeij, 1993; Trussell, 1997; Trussell, 2000a; Trussell and Nicklin, 2002; Cotton *et al*, 2004; Rundle *et al*, 2004). Measurements were made using images taken with a Nikon Coolpix 4500 digital camera attached to a Kyoma SDZ-IR-P optical microscope and analysed using analySIS software. Total snail wet weights were also measured before and after trials: each snail was left to dry on tissue paper for 10 minutes to ensure there was no water still in within shell and the weight was taken using digital balance Fisherbrand PS-100 ($\pm 0.0001\text{g}$).

Statistical Analysis

Two-way ANOVAs in SPSS 15 were used to test for differences between size groups and treatments for all morphological traits at the end of the experiment using the mean values for all snails in each mesocosm as replicates. All measurements were expressed as proportional increases. T-tests were also used to test for differences in the growth slopes between the second and first half of the experiment; here, the mean values for each mesocosm were again used as replicates and the slope in the first half of the experiment was tested against that in the second half for each treatment. All data were transformed where necessary to normalise their distribution (Sokal and

Rohlf, 1995; Dytham, 2005). Post hoc (SNK) tests were used to test for significant differences between individual treatments of each size group.

RESULTS

Two-way ANOVAs demonstrated overall significant effects of predator cue treatments at the end of the trial for all traits except for the shell shape (aspect ratio and aperture ratio) (Table 3.1). Percentage growth of snails was significantly higher ($p < 0.001$) in all treatments containing predators at some time (i.e. PP, PC and CP) compared with the control (CC) in terms of shell size (all traits) but no change in shell shape was seen (aspect ratio or aperture ratio) (Figure 3.1). There were also significant differences in the growth of snails in different predator treatments: overall percentage growth was significantly higher in the predator addition treatment (CP) compared with that in the constant predator (PP – for shell width, aperture length and aperture width) and predator removal (PC - all traits except shell thickness and total wet weight) treatments (Figure 3.1; Table 3. 1). Percentage growth in shell thickness and aperture size (length and width) were also significantly greater in the constant predator (PP) compared with the predator removal (PC) treatment.

Apart from shell length and total wet weight, there were no significant differences between snails of different size in terms of their percentage growth at the end of the experiment (Table 3.1) but there were significant treatment * size interactions for shell length and width (Table 3. 1, Figure 3.1 A-B). Post-hoc tests showed that this was driven by the significantly faster growth of small snails in the control treatment (CC) (Figure 3.1-A & B).

Table 3.1 Results of two-way ANOVAs testing for differences in shell trait induced defences (length, width, aperture length, aperture width, thickness) and total wet weight in two size groups of *Littorina littorea* between four treatments run over 42 days: control-control (no predator cues), predator-predator (predator cues from *Carcinus maenas* always present), control-predator (predator cues initially absent then added mid-way), predator-control (predator cues initially present then removed out mid-way).

| Trait | Source | Df | Mean Square | F | p- value |
|------------------|-----------------|----|-------------|--------|----------|
| Length | Treatment | 3 | 0.082 | 47.067 | < 0.001 |
| | Group | 1 | 0.023 | 13.333 | < 0.05 |
| | Treatment*Group | 3 | 0.007 | 3.788 | < 0.05 |
| | Error | 64 | 0.002 | | |
| Width | Treatment | 3 | 0.074 | 29.504 | < 0.001 |
| | Group | 1 | 0.001 | 0.328 | 0.569 |
| | Treatment*Group | 3 | 0.005 | 1.962 | 0.129 |
| | Error | 64 | 0.003 | | |
| Aperture length | Treatment | 3 | 0.055 | 41.162 | < 0.001 |
| | Group | 1 | 0.000 | 0.140 | 0.710 |
| | Treatment*Group | 3 | 0.001 | 0.515 | 0.673 |
| | Error | 64 | 0.001 | | |
| Aperture width | Treatment | 3 | 0.089 | 24.274 | < 0.001 |
| | Group | 1 | 0.004 | 1.145 | 0.289 |
| | Treatment*Group | 3 | 0.000 | 0.128 | 0.943 |
| | Error | 64 | 0.004 | | |
| Thickness | Treatment | 3 | 0.071 | 20.891 | < 0.001 |
| | Group | 1 | 0.002 | 0.641 | 0.426 |
| | Treatment*Group | 3 | 0.002 | 0.577 | 0.632 |
| | Error | 64 | 0.003 | | |
| Total Wet Weight | Treatment | 3 | 0.079 | 15.599 | < 0.001 |
| | Group | 1 | 0.028 | 5.437 | < 0.05 |
| | Treatment*Group | 3 | 0.004 | 0.838 | 0.478 |
| | Error | 64 | 0.005 | | |
| Aspect ratio | Treatment | 3 | 0.000 | 0.163 | 0.921 |
| | Group | 1 | 0.000 | 14.438 | < 0.001 |
| | Treatment*Group | 3 | 0.000 | 1.125 | 0.346 |
| | Error | 64 | 0.000 | | |
| Aperture ratio | Treatment | 3 | 0.004 | 16.170 | 0.230 |
| | Group | 1 | 0.002 | 0.636 | 0.428 |
| | Treatment*Group | 3 | 0.01 | 0.299 | 0.826 |
| | Error | 64 | 0.003 | | |

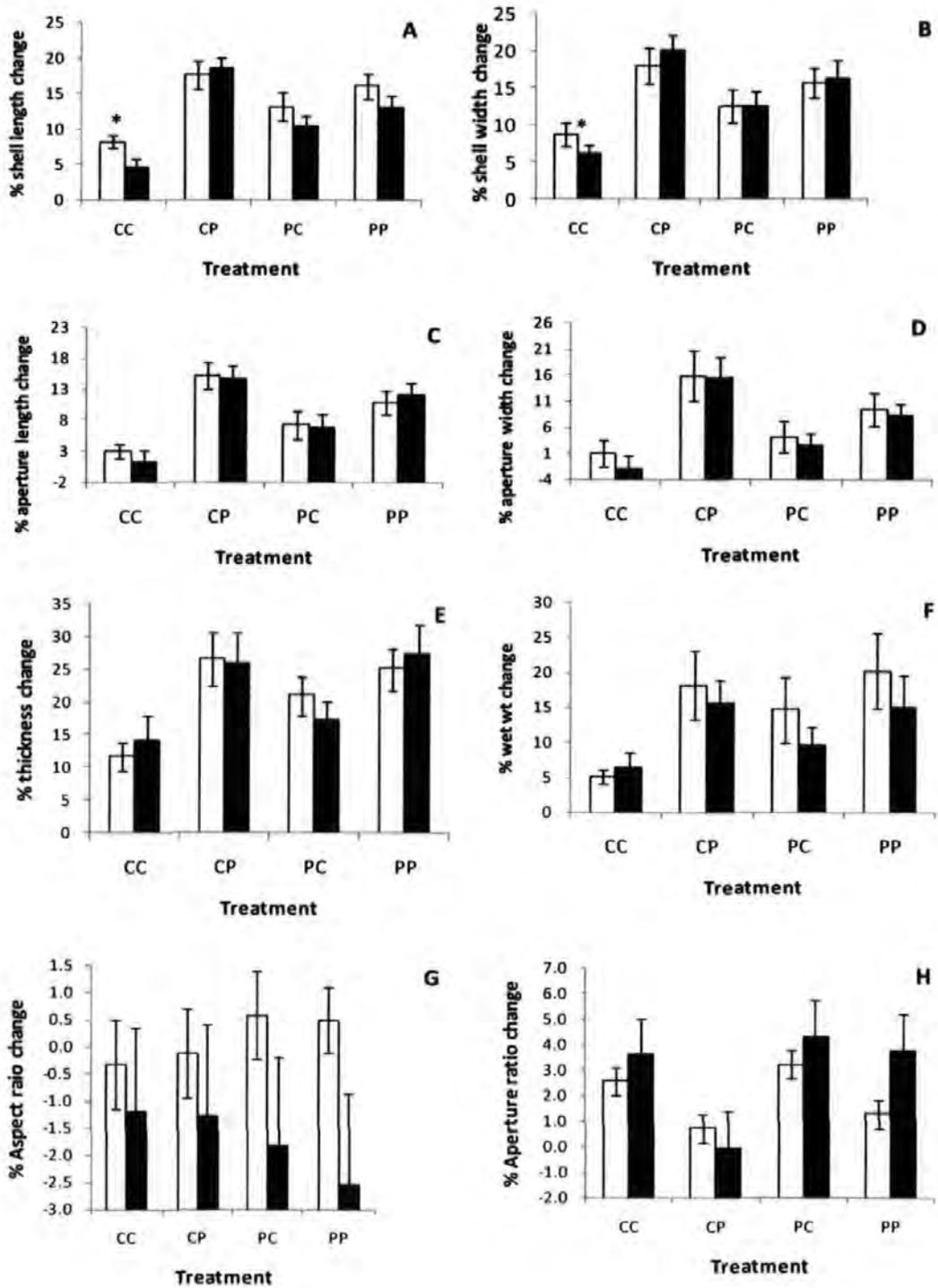


Figure 3.1 Response of shell traits of the intertidal gastropod *Littorina littorea* when raised with or without *Carcinus maenas* under four treatments: CC (predator cues always absent), PP (predator cues of always present), CP (predator cues added ad mid-way), PC (predator cues removed at mid-way). Two size groups were used: Group 1 small (5-7 mm) (light bars) or Group 2 large (8-10 mm) (dark bars). Values are means \pm SE of proportional change over the experimental period: A) length, B) width, C) aperture length, D) aperture width, E) thickness, F) total wet weight, G) length relative to width (aspect ratio), H) aperture length relative to aperture width (aperture ratio). * = post hoc comparison, significant @ $p < 0.05$.

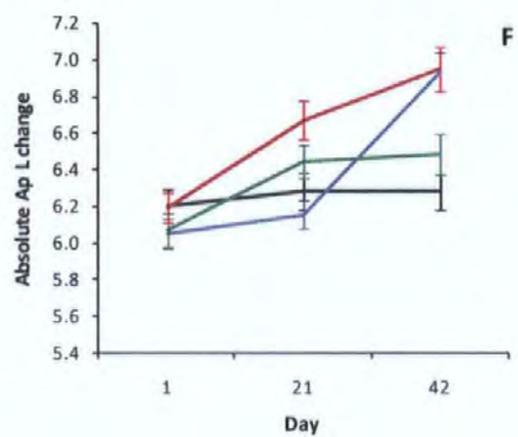
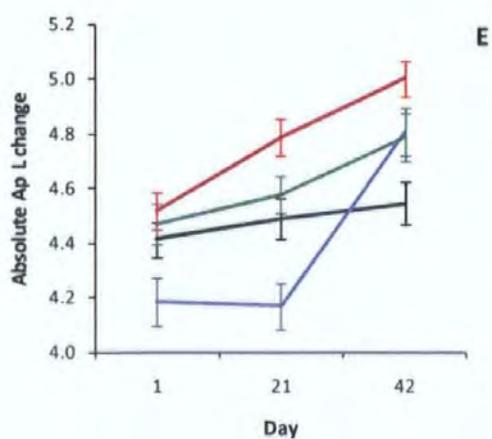
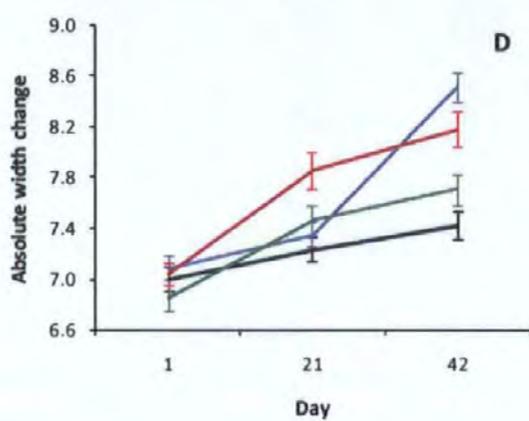
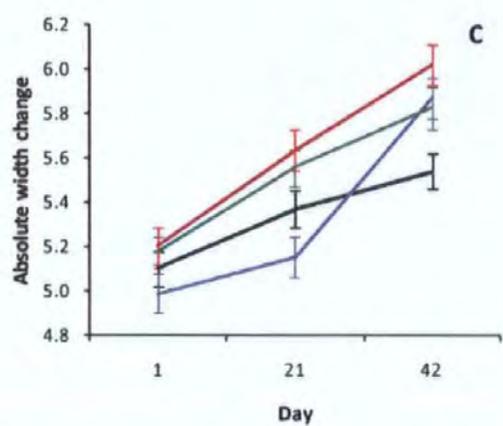
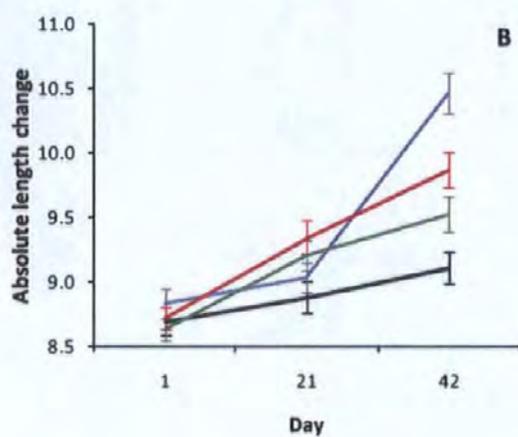
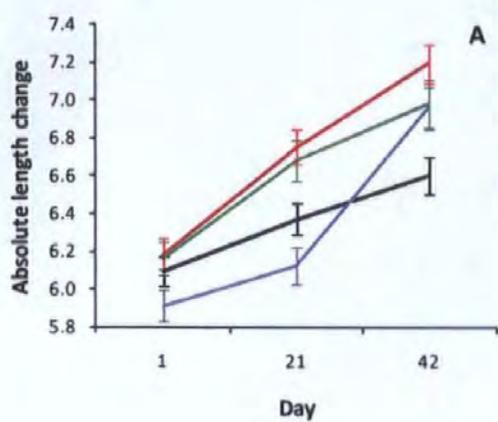
T-tests assessing differences between slopes of treatment in the first and second halves of the experiment illustrated the effect of predator addition or removal for all morphological traits and total wet weight for both size groups (Table 3. 2). In general, for the control (CC) there was no significant difference in growth rates between the first and second half of the experiment (Table 3.2; Figure 3.2 A-H). Except for shell width and aperture width, the constant predator (PP) treatments also showed no significant differences in growth rates between the first and second half of the of the experiment (Table 3.2; Figure 3.2 A-H).

In contrast, there were significant differences in the treatments that experienced a “switch” in cue conditions. The predator addition (CP) treatment showed a significant increase in growth in the second half of the trial, after exhibiting a growth trajectory parallel to the control treatment (CC) in the first half of the experiment, “catching up” with the constant predator (PP) treatment in the second half (Table 3.2, Figure 3.2 A-B). The predator removal (PC) treatment showed a significant decrease in growth rate in the second half of the trial in the shell size (length and width), shell thickness and aperture width (Table 3.2; Figure A-H) . The growth rate of the snails of both sizes in the predator removal treatment seemed to revert to a rate similar to the control.

Table 3.2 Results of t-tests testing for differences in growth slopes of shell traits induced defences (length, width, thickness, aperture length, aperture width) and total wet weight between the two periods of each treatment (control-control, control-predator, predator-control and predator-predator in two size groups of *Littorina littorea* when exposed to *Carcinus maenas* cues over the duration of study- see 3.1 legend for details).

| Trait | Treatment | | | | | | | |
|------------------|-----------|----|--------|-----------|-------|-----------|-------|----------|
| | CC | | CP | | PC | | PP | |
| | t | p | t | p | t | p | t | P |
| Length | 0.034 | NS | 10.018 | p < 0.001 | 4.126 | p < 0.001 | 1.788 | NS |
| Width | 2.207 | NS | 9.309 | p < 0.001 | 4.108 | p < 0.001 | 2.723 | p < 0.05 |
| Aperture length | 1.252 | NS | 13.468 | p < 0.001 | 1.578 | NS | 2.353 | NS |
| Aperture width | 0.915 | NS | 7.216 | p < 0.001 | 2.131 | p < 0.05 | 2.199 | p < 0.05 |
| Thickness | 1.592 | NS | 8.815 | p < 0.001 | 4.035 | p < 0.05 | 0.323 | NS |
| Total wet weight | 2.506 | NS | 3.790 | p < 0.001 | 0.979 | NS | 1.465 | NS |

df= 34



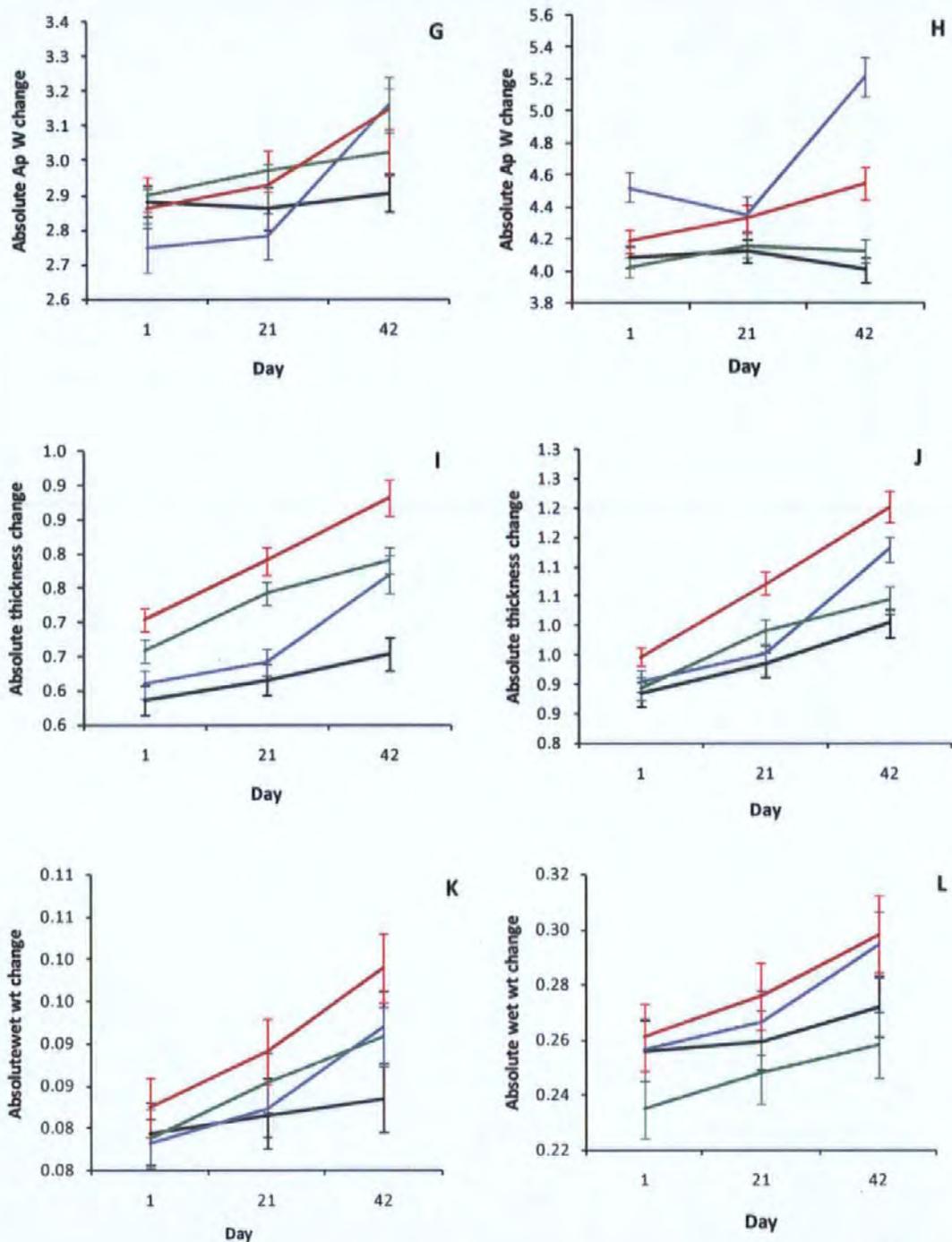


Figure 3.2 Responses of shell traits in the intertidal gastropod *Littorina littorea* when raised under four days: CC (—), PP (—), CP (—), PC (—) (no predation threat (CC), constant predation threat (PP), the removal of predation threat (PC), the addition of predation threat (CP)). Two size groups were used: Group 1 (5-7 mm) (left) or Group 2 (8-10 mm) (right). Values are expressed as means \pm SE absolute change over the experimental period: A) length, Group 1, B) length, Group 2, C) width, group 1, D) width, group 2, E) aperture length, group 1, F) aperture length, Group2, G) aperture width, Group 1, H) aperture width, Group 2, I) thickness, Group 1, J) thickness, group 2, K) total wet weight, Group 1, L) total wet weight, Group 2.

DISCUSSION

This study aimed to assess how flexible the plastic responses of the intertidal gastropod *Littorina littorea* were in the face of the threat of predation by using crab cue treatments that mimicked scenarios where the threat of predation was either introduced or removed. There was clear evidence that snails have the ability to alter their degree of morphological plasticity in line with the current predation threat they are experiencing. When the threat of predation was removed, snails reduced their growth rate to levels found in the control treatment, suggesting that the production of induced morphological defences was costly. At the same time, snails experiencing the addition of a predation threat increased their growth rate dramatically, to greater levels than those seen in the constant predator treatment; in effect, snails appeared to exhibit a response, which meant that they “caught up” with those snails experiencing a constant predator regime. Finally, there were no clear differences in the growth responses of different sized snails.

The ability to exhibit reversible morphological defences is determined by three main factors: i) termination of ecological conditions that initiate the induced defences; ii) simplicity of the morphology that is subject to the reversibility, for example the modification of relative shape but not removal or changes in a complex structure such as spines; and iii) the trait should not be of modular type which could inhibit the reversibility such as stem elongation in plants (Relyea, 2003). In my study, all these factors are applicable, however, the defensive traits were not actually reduced or reversed; it was the growth rate which was reversed. Animals in general have the ability to assess their risk of predation and have the ability to decide the optimal activities accordingly (see Lima and Dill, 1990). Induced defensive responses among

tadpoles *Rana lessonae* are very flexible and these animals are capable of matching graded predator cues and respond to subtle variations of these cues (Van Buskirk and Arioli, 2002). Such reversible phenotypic plasticity can be advantageous if an organism is exposed to differences in selection regimes during its lifetime (Gabriel, 1999; Gabriel, 2005; Gabriel *et al*, 2005) and the rocky intertidal is a highly variable habitat where such reversibility may be favoured. The intertidal snails *L. sitkana* resumed normal feeding with the removal of predatory crab *Cancer productus* (Randall) suggesting reversible behaviour and growth rate with changing environmental conditions (Yamada *et al*, 1998). Some intertidal gastropods such as *Nodilittorina australis* have shown flexible phenotypic plasticity by changing the distinctive appearance of the shell between distinct nodulose and striate morphs even at late stages of their life (Yeap *et al*, 2001).

In this experiment, snails exposed to predator threat in the second stage of the experiment (i.e. in the predator addition treatment CP) grew bigger, thicker and heavier shells with larger apertures; indicating that the introduction of a predator in the later stage of the trial induced stronger morphological defences than early introduction of the predator. In plants, the development of plasticity also differs through their ontogeny and the timing of the plasticity may be plastic (Pigliucci, 1997; Sultan, 2000). Snails of both size classes under the CP treatment appeared to illustrate “catch up” with those snails experiencing a constant predator treatment. A similar type of response was found by Relyea (2003) with gray treefrog tadpoles *Hyla versicolor* which continue to develop induced morphological defence and on this without using behavioural defences at later stage of stages of their life. According to Relyea (2003) this suggests a selective pressure that influences the removal of any

developmental constraints and allowing development of morphological defences throughout this tadpole's ontogeny. However, this result was not observed in the freshwater snail *Helisoma trivolvis* in which the ability to reverse induced defence was limited to the early ontogeny and later introduction of the predator was associated with costs with modular growth of shell leading to reduced shell thickness (Hoverman and Relyea, 2007). However, snails used by Hoverman and Relyea (2007) did not experience predators in their natural habitat. On the other hand, the intertidal snails used in my study were collected from the natural habitat and so might be more likely to have an innate flexible response to predator cues.

This study illustrated that there was no significant differences between small and large sized snails in their proportional growth when exposed to the different treatments. Based on their relative sizes, these two size group of snail are highly to be 2- 4 months different in age (Fretter and Graham, 1980). However, the study revealed that by introducing the predator in the second period of the experiment (CP), the larger snails demonstrated greater elevation of the slope of growth than the small snails (Figure 3.2 A-H). *Littorina littorea* growth rate has been shown to vary depending on population density (Petraitis, 2002), season (Fretter and Graham, 1980; Hughes and Answer, 1982) and location or habitat (Fretter and Graham, 1980). In general, growth rates of *L. littorea* are higher in the early stage of their life and decreases rapidly in year 2 (Fretter and Graham, 1980; Reid, 1996). This might suggest that energy resources are shifted from growth to investment in reproduction. However, my results suggest that this doesn't appear to affect the ability of the snail to exhibit induced defences. It would be interesting to combine an investigation of reproductive

investment with such a study of plasticity to assess if trade offs between reproduction and growth exists.

Chapter 4

The Effects of Variable Risk of Predation on Phenotypic Plasticity in the Marine Gastropod *Littorina littorea*

SUMMARY

Most studies investigating induced defences do so under conditions mimicking a constant predation threat, yet in nature the risk of predation is likely to vary through time. Here I assess the relative importance of dose (i.e. the duration of exposure to predator cue) and frequency (i.e. the temporal pattern of exposure) of chemical cue addition on induced defences in the intertidal gastropod *Littorina littorea*. Snails were exposed to four treatments: predation-free control (C); constant predation threat (CP); systematic, predictable, predation threat (SP); and unpredictable, intermittent predation threat (IP). I predicted that, if frequency was of overriding importance, that the variable predator treatments would produce a greater induced response compared with treatments with constant predation threat or no predation threat. I also tested if unpredictable predator threat (IP) would induce a greater response than with predictable cue addition (SP) in line with predictions of the risk allocation hypothesis. The results supported the first prediction: the variable predator treatments exhibited the greatest increase in shell size and total wet weight versus the constant predator threat and the control. However, the results did not reveal any significant difference between the variable treatments, i.e. there was no difference in plastic responses between unpredictable and the predictable predator cue treatments.

INTRODUCTION

The threat of predation is a biotic factor that plays a very important role in the life history, morphology, behaviour, development and fitness of prey organisms (Lima, 1998; Lima and Bednekoff, 1999; Lima and Dill, 1990; Tollrian and Harvell, 1999; Laurila *et al*, 2004; Kishida and Nishimura, 2006). If successful, predation leads to the death of the prey which has obvious implications for prey population dynamics and, ultimately, the entire ecosystem (Lima, 1998). However, through the production of induced defences, such as protective morphology or avoidance behaviour, prey may avoid or escape the threat from predators (Lima, 1998; Pigliucci, 2001; DeWitt and Scheiner, 2004; Kishida and Nishimura, 2006). Therefore, through its non-lethal impact, predation may play a major role in the evolution of morphological and behavioural traits (Lima and Dill 1990; Lima, 1998).

Most previous studies on induced defences have focused on the effect of a constant predation threat, yet in the natural environment the threat of predation is often unpredictable and varied in intensity (Kishida and Nishimura, 2006). Coping with an unpredictable and variable predator threat may be a major force selecting for the evolution of induced defences (Clark and Harvell, 1992; Harvell and Tollrian, 1999, Trussell, 2000b). Even when predation varies systematically over time, such as diel or seasonal cycles, threat of predation on any individual may be unpredictable (Clark and Harvell, 1992). At the same time, the development of phenotypically plastic traits is constrained by several costs and constraints (DeWitt, 1998; DeWitt *et al*, 1998; Relyea, 2002); hence, in order to reduce such costs, prey organisms should not continue to exhibit induced defences when the inductive force (i.e. the predator cue) is

not present. Flexible induced defences would therefore offer the optimal strategy for prey organisms.

The ability of animals to assess risk, adjust their various activities and behaviourally respond to predator impact was reviewed in detail by Lima and Dill (1990). The use of anti-predator behavioural avoidance is one aspect of induced defences that has received a great deal of attention (e.g. Lima and Dill, 1990; Sih, 1992; Turner, 1997; Lima and Bednekoff, 1999; Hamilton and Heithaus, 2001; Sih and McCarthy, 2002; Laurila *et al*, 2004). However, with the exception of Laurila *et al* (2004), there have not been any studies that have attempted to assess how morphological induced defences respond under scenarios where predation threat is variable and unpredictable.

As morphological induced defences are the central theme of this thesis, it seemed pertinent to include a study of how temporal variation in predation threat influenced this form of prey response. Following the previous experiment (Chapter 3), which demonstrated the long term flexibility of plastic induced defences in *L. littorea* in response to crab predation threat, here I extend my work to examine the effects of varied and infrequent predator cues on induced defences in this species.

The risk allocation hypothesis predicts that maximum antipredator behaviour should be exhibited by prey during short and infrequent pulses of high predation risk (Lima and Bednekoff, 1999). It is questionable, however, whether responses to variable predation risk in morphological induced defences are likely to correspond with this prediction, as induced morphological responses take longer to form than behavioural responses. Indeed, the only study that has tried to apply the risk allocation model to

morphological plasticity suggested that the model did not apply in this case when studying *Rana temporaria* tadpoles (Laurila *et al*, 2004). According to Laurila *et al* (2004), the morphological defence responses are continuous rather than threshold traits. Here I provide a test of Lima and Dill's (1990) model using induced defences in the intertidal gastropod *Littorina littorea*. The design I used involved contrasting the morphological responses of these snails to variable versus constant predation cue threat. At the same time I used two types of cue addition that represented predictable and unpredictable (i.e. random) predation threat in order to assess whether responses were affected by temporal variation in predation threat. Specifically I tested the proposal by Caro and Castilla (2004) and Harvell (1986) that species experiencing unpredictable environmental cues should show greater induced defences than those in predictable environments. The main predictions that I tested in this experiment are: i) induced morphological responses would be ranked in the order: control < constant predator cue < variable predator cue; and ii) random cue addition unpredictable cue presentation would elicit a greater response than a systematic predictable cue addition (SP < IP).

MATERIALS AND METHODS

Study Organisms

All snails were collected in early July 2007, from the intertidal at Hannafore Point, Cornwall, UK (50° 20° N, 4° 27° W). The mean shell length of the snails used was 7.3 (\pm 0.47 SE) mm. Prior to the study procedures, all snails were acclimatised under laboratory conditions of 15° C and salinity 27 ‰ for 10 days. Subsequently, snails were kept under experimental conditions for 28 days.

Green crabs *Carcinus maenas* were used to produce predator cues as this is a common intertidal predator on rocky shores in the UK where it is known to feed on gastropods (Cotton *et al*, 2004; Hughes and O'Brien, 2001; Spooner *et al*, 2007). Crabs were collected in early July 2007 from the Plym Estuary, Devon, UK (50° 22° 0.7° N, 4° 6° 0.1° W). All crabs were in good condition with no signs of damage or limb loss, and had a carapace width of 4-6 cm. The stock of crabs was maintained in seawater at a temperature of 15° C and fed with frozen fillet of Coley fish (*Pollachius virens*) once every three days. Groups of crabs for use in trials were randomly chosen from this stock population and introduced to the cues tanks for three to four days. Upon completion of the study, all snails were frozen for further analysis and crabs were returned to their natural habitat.

Experimental set up

Experimental mesocosms were 20 l plastic aquaria (32 cm length, 18 cm width and 20 cm height) containing aerated seawater at 15° C (12:12 hr Light: Dark regime). Four treatments were used in this study: (1) control treatment (C); (2) predator and conspecific cue treatment (P); (3) systematic (i.e. predictable) predator and conspecific cue treatment (SP); (4) intermittent (i.e. unpredictable) predator and

conspecific cue treatment (IP). For the conspecific cue I used two crushed snails of *L. littorea* of the same average size used in the treatments. The control treatment (C) contained only sea water while the constant predator treatment (P) contained a single crab and crushed *L. littorea* (added once with every introduction of crab and new water) present throughout the duration of the trial. In the systematic predator treatment (SP), a predator and crushed snails were added and removed every seven days for the duration of the experiment (28 days). In the intermittent predator treatment (IP), a predator and crushed snails were added and removed randomly (over 14 days selected randomly out of total 28 days) to create an unpredictable pattern of predator appearance and disappearance. Snails under both systematic predator cue (SP) and intermittent predator cue (IP) were exposed to predator and crushed conspecific for the same total exposure throughout the trial (14 out of 28 days).

For all predator cue treatments (P, SP and IP), a single crab from the stock population (see above) was used to produce the predator cue threat; this predator was replaced depending on the treatment schedule and all treatments treated the same to avoid any disturbance or stress effects. There were three replicate tanks for each treatment with a total of 12 tanks for the whole study, interspersed randomly. Each mesocosm was stocked with 20 pots (4 cm diameter, 6 cm height and 20 ml vol.) containing an individual gastropod, with a total of 20 snails per tank and a total of 240 snails for the whole experiment. Thirty five holes (3.5 mm diameter) were made in each pot to allow water circulation. Each pot also contained one disc (1 cm²) of *Enteromorpha lactuca* that was replaced twice each week.

Measuring Induced Defences

Several morphological parameters were measured that relate to defence in gastropods: shell length, shell width, shell thickness and total wet weight in addition to aperture length, aperture width (Vermeij, 1993; Trussell, 1997; Trussell, 2000a; Trussell and Nicklin, 2002; Cotton *et al*, 2004; Rundle *et al*, 2004). Measurements were made at the start and completion of the experiment from images taken with a Nikon Coolpix 4500 digital camera attached to Kyoma SDZ-IR-P optical microscope and analysed using analySIS software. Total snail wet weight was taken at the beginning and the completion of the experiment. Each snail was left to dry on tissue paper for 10 minutes to ensure there was no water still within shell and the weight was taken (\pm 0.0001 g) using a Fisherbrand PS-100 digital balance.

Statistical Analysis

One-way ANOVAs in SPSS 15 were used to test for differences in traits between different predator cue treatments using the mean value in mesocosm as replicates. Measurements of all traits were expressed as proportional increase over the trial, and were arcsine square root transformed (Sokal and Rohlf, 1995; Dytham, 2005) before the analysis. Post-hoc (SNK) tests were used to assess significant differences between individual treatments and Levene's test was used to confirm homogeneity of variance.

RESULTS

Gastropod survival rates were generally high for the duration of the study (> 98 %). One-way ANOVAs showed that there were highly significant treatment effects ($p < 0.001$) for shell size (length and width) and shell thickness, aperture size (length and width) and total wet weight ($p < 0.05$) (Table 4.1). There were no significant differences in shell shape or the aperture shape (i.e. aspect ratio and aperture ratios).

Table 4.1 Results of one-way ANOVAs testing for differences in shell trait induced defences (length, width, aperture length, aperture width, thickness) and total wet weight in *Littorina littorea* between four treatments: C (control), or *Carcinus maenas* cues added in P (continuous predator cue), SP (systematic predator cue), IP (intermittent predator cue) over 28 days.

| Trait | Source | Df | MS | F | p |
|------------------|-----------|----|--------|--------|---------|
| Length | Treatment | 3 | 0.003 | 29.88 | < 0.001 |
| | Error | 8 | 0.000 | | |
| Width | Treatment | 3 | 0.003 | 25.529 | < 0.001 |
| | Error | 8 | 0.000 | | |
| Aperture Length | Treatment | 3 | 0.005 | 7.271 | < 0.05 |
| | Error | 8 | 0.001 | | |
| Aperture Width | Treatment | 3 | 0.010 | 4.209 | < 0.05 |
| | Error | 8 | 0.002 | | |
| Aspect ratio | Treatment | 3 | 0.0006 | 1.47 | 0.295 |
| | Error | 8 | 0.0004 | | |
| Aperture ratio | Treatment | 3 | 0.001 | 0.346 | 0.793 |
| | Error | 8 | 0.003 | | |
| Thickness | Treatment | 3 | 0.05 | 33.48 | < 0.001 |
| | Error | 8 | 0.002 | | |
| Total Wet Weight | Treatment | 3 | 0.006 | 13.731 | < 0.05 |
| | Error | 8 | 0.000 | | |

Post-hoc comparisons showed significant differences between predator treatments compared with the control, demonstrating the significant effects of predator cues irrespective of the manner of presentation. This significant treatment effect occurred for shell size (shell length and shell width), aperture length and shell thickness in the constant predator treatment (P) (Table 4.2); snails raised in this treatment had significantly longer, wider and thicker shells with larger apertures on completion of the trial compared with those raised in the control treatment (Figure 4.1 and Table 4.2). There were also significant differences between the control and both the systematic predator treatment (SP) and the intermittent predator treatment (IP) (Table 4.2, Figure 4.1). Again, snails raised under these two treatments exhibited longer, wider and thicker shells with larger aperture and a significant increase in the total wet weight when compared with controls.

Table 4.2 Post-hoc comparisons (SNK) between four experimental treatments: C (control), P (constant predator cue), SP (systematic predator cue), and IP (intermittent predator cue) applied to *Littorina littorea* for 28 days.

| Trait / Treatment | C vs P | C vs SP | C vs IP | P vs SP | P vs IP | SP vs IP |
|-------------------|-----------|-----------|-----------|---------|---------|----------|
| Shell length | P < 0.05 | P < 0.001 | P < 0.001 | NS | NS | NS |
| Shell width | P < 0.05 | P < 0.001 | P < 0.05 | NS | NS | NS |
| Aperture length | P < 0.05 | P < 0.05 | P < 0.05 | NS | NS | NS |
| Aperture width | NS | NS | NS | NS | NS | NS |
| Shell thickness | P < 0.001 | P < 0.001 | P < 0.001 | NS | NS | NS |
| Total wet weight | NS | P < 0.05 | P < 0.05 | NS | NS | NS |
| Aspect ratio | NS | NS | NS | NS | NS | NS |
| Aperture ratio | NS | NS | NS | NS | NS | NS |

In general, induced defence production may appear less in the constant predator cue treatment (P) compared with both systematic predator cue treatment (SP) and intermittent predator cue treatment (IP) (Figure 4.1), however, no significant difference was seen between any predator involved treatments (P, SP and IP) (Table 4.2).

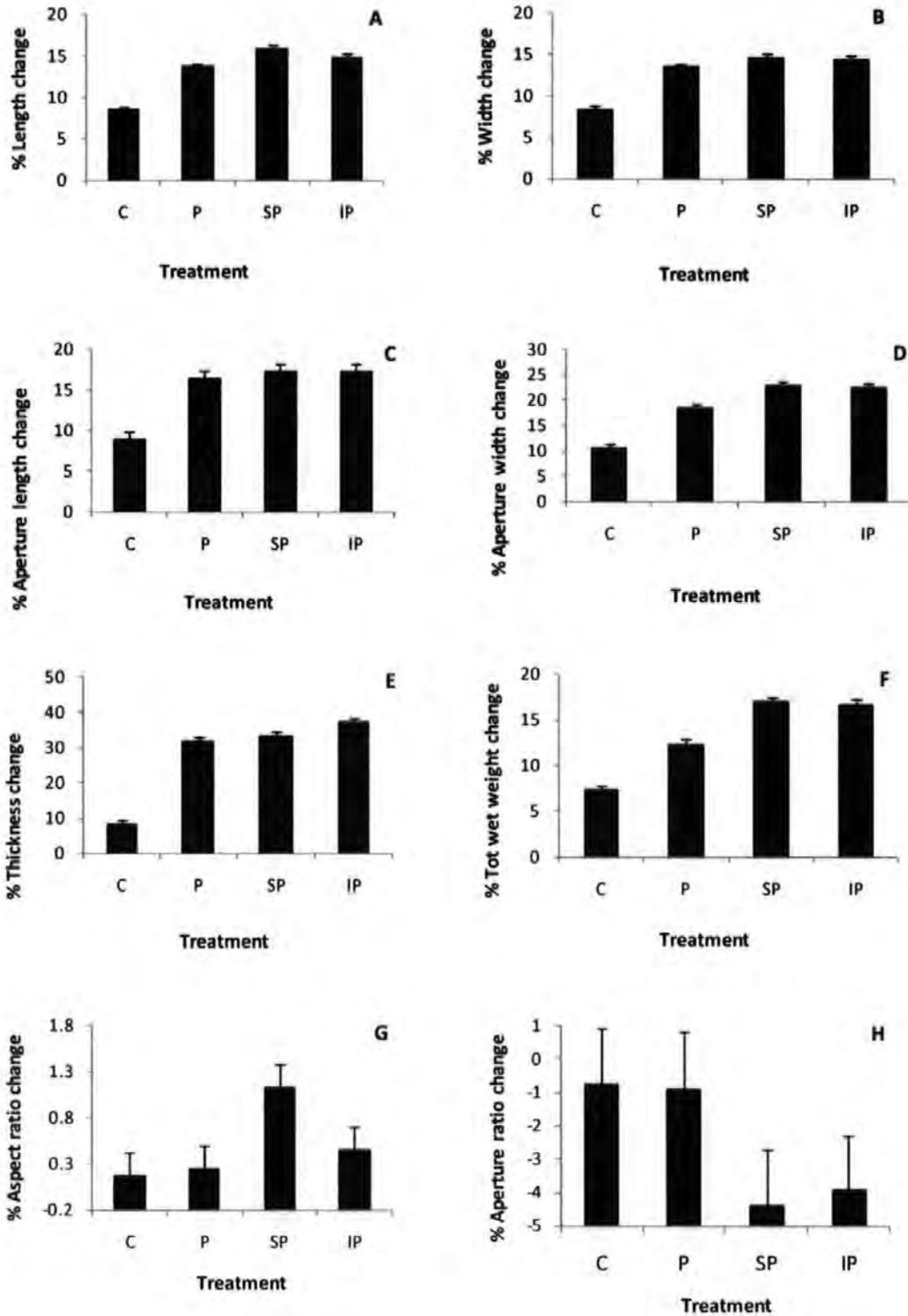


Figure 4.1 Response of intertidal gastropod *Littorina littorea* when raised with control sea water (C) or seawater contains *Carcinus maenas* cues added in three manners: continuous (P), systematic (SP), or intermittent (IP). Values are expressed as means (\pm SE) proportional change in shell traits over the experimental period: A) length, B) width, C) aperture length, D) aperture width, E) thickness, F) total wet weight, G) aspect ratio, H) aperture ratio.

DISCUSSION

This experiment demonstrated that *L. littorea* consistently developed morphological induced defences when kept in the presence of *Carcinus* crab cues and crushed conspecifics, in comparison to those kept under control conditions, regardless of the manner of predator cue presentation. The overall proportional growth in all predator treatments showed that shell size (length and width) increased significantly by 14-16 % more than the 8 % increase of the control group (Figure 4.1). Aperture width and length showed a greater increase for predator treatments; however, shell thickness demonstrated the greatest increase (31-37 %) in predator treatments, while in the control group this increase was only 8.5 %. These results are consistent, in general, with those in Chapters two and three which demonstrated significant induced defences in snails exposed to predator cue in contrast to the control treatments.

Clearly, these results are also in accordance with several previous studies demonstrating such plasticity (Vermeij, 1978; Appleton and Palmer, 1988; Vermeij, 1993; Trussell, 1996; Trussell, 1997; Trussell, 2000a; Trussell, 2000b; Trussell and Smith, 2000; Trussell and Nicklin, 2002; Hoverman and Relyea, 2008).

The main prediction under test was that snails experiencing the greatest variability in predator cues, those in the predictable, systematic (SP) and unpredictable, intermittent (IP) predator environment, would exhibit the greatest level of induced defences. This was not supported in this experiment; snails experiencing variable cue addition treatments showed no significant difference in any shell morphological traits in those kept under constant predator cue conditions (Table. 4.2). Furthermore, within the two variable addition treatments (SP vs. IP), the results demonstrated no significant difference, contrary to my second prediction which assumed that random

unpredictable predator cue presentation would elicit a greater induced responses than systematic predictable predator cue addition.

These results do not support the risk allocation hypothesis (Lima and Bednekoff, 1999) in that the variable (infrequent) treatment demonstrated the greatest antipredator responses versus the constant predator treatment. However, the results do not appear in accordance with the only previous study that has tried to apply the risk allocation model to morphological plasticity. Laurila *et al* (2004) suggested that the constant predator risk demonstrated the greatest morphological response and modification in the life history among *Rana temporaria* tadpoles. Their results, however, were not consistent; some traits such as relative body size and tail fin depth showed the greatest response towards constant predator cue, on the other hand body length and depth and tail length showed the greatest response to the non-continuous treatment.

My second prediction, that snails experiencing an unpredictable predation threat would show a greater response than those experiencing predictable predation threat was not supported; there were no significant differences between the systematic predator threat (SP) and the random threat treatments (IP). These results contradict studies of anti-predator behaviour which suggested that the greatest induced defence was produced with the unpredictable predator cue and intermittent presence of predator in time and space (Harvell, 1986; Lima and Bednekoff, 1999; Caro and Castilla, 2004).

The lack of a difference between the treatments with different temporal patterns of cue addition may indicate that foraging activities during the periods of time when predators were absent in both treatments were equal and that snails were able to compensate for the time when they were inactive (Sih and McCarthy, 2002). It would have been good to have had data on foraging activity during the trials but that this was not possible using my experimental set up. Unlike behavioural responses, induced morphological defences are not immediate and may also take longer to evolve (Laurila *et al*, 2004). Hence, in my trial, the time scale used did not reflect the time frame that occurs in the natural habitat. *Carcinus maenas* as a major predator on the wave-protected rocky shore of the intertidal community (Trussell, 1996; Yamada and Boulding, 1996; Trussell, 1997; Trussell, 2000b; Cotton *et al*, 2004) and its major foraging activity takes place during the high tide (Yamada and Boulding, 1996). Thus the variation driving the induced morphology is wider and the response might not be predicted to be short term (Laurila *et al*, 2004). Further studies are required to tackle this matter of how the scale of temporal variation in predation threat scales in relation to induced defences.

This study did not support the predictions of the risk allocation hypothesis and appear in agreement with Laurila *et al* (2004), in that the morphological defence responses are continuous rather than threshold. Furthermore, the results suggest that experiments on induced defences that use a constant predator environment may be underestimating the potential strength of trait mediated indirect effects.

Chapter 5

The Effects of Temperature on Phenotypic Plasticity in the Marine

Gastropod *Littorina littorea*

SUMMARY

Most of the previous studies on phenotypic plasticity in intertidal gastropods have focused on the development of induced defences where animals are exposed to predator cues in isolation. It is likely, however, that such induced changes will be influenced by other environmental factors. In this chapter, I assess how temperature affects the expression of induced defences in the intertidal gastropod *Littorina littorea*. I used a factorial design in which snails were exposed to either control or predator cue (*Carcinus maenas*) conditions at one of three temperatures (16, 20, or 24° C) for 14 days. Snails maintained at 16 and 20° C showed significant predator-induced defences by growing larger and thicker shells, but there was no significant difference in the degree of induced defence production between these temperatures. At 24° C, growth was significantly lower than at 16 and 20° C and the expression of induced defences was also reduced. Furthermore, significant negative effects was observed in two of the shell traits at 24° C, which suggests that growth might be inhibited under the double stress of high temperature and predation threat.

INTRODUCTION

The phenomenon of global warming is caused by human-induced increases in greenhouse gases such as water vapour, freon, methane and ozone; with carbon dioxide (CO₂) accounting for more than 50% of the warming effects (Libes, 1992; IPCC, 1995; IPCC, 2001; Kaiser *et al*, 2005). These gases trap the Earth's outgoing thermal radiation, and the increase in their concentrations has increased the world's temperature by about 0.6° C over the past century (IPCC, 2001; Bale *et al*, 2002; Kaiser *et al*, 2005). This increase in temperature has resulted in the 20th century being the warmest during the last 1000 years (Bale *et al*, 2002) and sea surface temperature has increased by between 0.4- 0.8° C in the last century (IPCC, 1995; IPCC, 2001; Harley *et al*, 2006). Climate change may have large scale consequences such as increasing sea temperature and sea level, decreasing sea-ice cover (which may alter salinity regimes), increased flooding, increased coastal erosion and loss of wetlands and mangroves (IPCC, 2001; Harley *et al*, 2006).

Temperature is one of the most important abiotic environmental factors that can have a strong affect on the metabolism, development, abundance and the survival rate of wide range of species and biota (Vermeij, 1978; Bale *et al*, 2002; Massot *et al*, 2002; Sokolova and Portner, 2003). Temperature also influences the local and geographical distributions of species as well as temporal population dynamics and behaviour (Vermeij, 1978; Helmuth *et al*, 2005). In marine taxa, temperature has been shown to affect a variety of biological factors such as physiological performance (intertidal mussels *Mytilus californianus*, Helmuth and Hofmann, 2001); reproductive cycle (intertidal gastropods *Melarhappe neritoides*, Cronin *et al*, 2000) and spawning times (bivalve *Macoma balthica*, Harley *et al*, 2006). Marine gastropods show variations in

shell morphology (*Cypraea annulus* - Irie, 2005) and shell colour (two species of *Batillaria* - Miura *et al*, 2007) which have been influenced by temperature.

Temperature also has strong effects on predator-prey interactions in some intertidal organisms (Pienkowski, 1983); for example predation strength in the sea star *Pisaster ochraceus* has been found to vary with temperature (Harley *et al*, 2006). Also, temperature plays an important role in the geographical distribution of invasive *Carcinus maenas* and interacts with the effects that this predator has on intertidal gastropods (Trussell, 2000a; Trussell and Smith, 2000; Trussell and Etter, 2001).

Despite this well documented importance of temperature for bioic processes in intertidal systems, very little is known about how temperature and, hence global warming, might affect the production of induced defences in this environment. Intertidal rocky shore species can be useful models for studying climate-driven ecological change because they are relatively long lived, and are often easily observed as they have limited ranges and are slow (Helmuth and Hofmann, 2001; Stillman, 2002; Tomanek and Helmuth, 2002; Sokolova and Portner, 2003; Helmuth *et al*, 2006; Lima *et al*, 2006). For example, the phenotypic flexibility of metabolic rate in the marine limpet *Cellana tramoserica* was observed to be affected by water temperature (Sinclair *et al*, 2006). At the same time, induced defences in the intertidal gastropod *Littorina littorea* have previously been found to be affected by another environmental parameter, lowered seawater pH (Bibby *et al*, 2007), which suggests that the expression of plasticity in marine systems may be affected by multiple parameters. Therefore, the main focus of this chapter was to investigate how the expression of induced defences in the intertidal gastropod *Littorina littorea* was

influenced by temperature with a view to discussing how potential sea temperature increases might affect plasticity.

MATERIALS AND METHODS

Study Organisms

Littorina littorea (Linnaeus) individuals were collected during September 2006, from Hannafore Point, Cornwall, UK (50° 20' N, 4° 27' W). The length of the snails ranged between 8-10 mm. All snails were acclimated under laboratory conditions for 10 days prior to experiments.

Carcinus maenas were used to produce predator cues as this is a common intertidal predator on rocky shores in the UK and has been shown to feed on gastropods (Cotton *et al*, 2004; Hughes and O'Brien, 2001; Spooner *et al*, 2007). Individual crabs were collected in August 2006 from the Plym Eastury, Devon, UK (50° 22' 0.7" N, 4° 6' 0.1" W). All crabs were in good condition with no signs of damage or limb loss, and had a carapace width of 4-6 cm. The stock of crabs was maintained in seawater at a temperature of 15° C and fed with frozen fillet of Coley fish (*Pollachius virens*) once every three days. Groups of crabs for use in trials were randomly chosen from this stock population and introduced to the cues tanks for three to four days.

Experimental set up

Experimental mesocosms were 20 l plastic aquaria (32 cm length, 18 cm width and 20 cm height) containing aerated seawater and stocked with 15 pots (4 cm diameter, 6 cm height and 60 ml Volume) each containing an individual gastropod. Thirty five holes (3.5 mm diameter) were made in each pot to allow water circulation. Each pot

also contained one disc (1 cm²) of *Enteromorpha lactuca* that was replaced twice a week.

The interactive effects of temperature and predator cues were investigated by maintaining snails at 3 different temperatures, 16° C, 20° C and 24° C for 15 days in either the presence or absence of predator cues. Cue treatment tanks contained a single crab from the stock population (see above) replaced every 3-4 days. There were two tanks for each treatment in each of the temperature regimes with total of 12 tanks for the whole study, interspersed randomly. Tanks were maintained under a 12:12 hour light: dark regime. The temperature effects on the salinity and the pH were eliminated due to the frequent change of water and were monitored on daily bases throughout trial and the mean of these parameters was (37 ± 1 ‰, 8 ± 0.1) respectively. Calcium and magnesium as two important salinity parameters and essential in the shell-forming of marine gastropods (Vermeij, 1993; Klein *et al*, 1996; Foster and Cravo, 2003) were also tested (see below).

Measuring Induced Defences

I measured several morphological parameters that relate to defence in gastropods: shell length, width, thickness, aspect ratio (length/width) and aperture ratio (aperture length/ aperture width) (Vermeij, 1993; Trussell, 1997; Trussell, 2000a; Trussell and Nicklin, 2002; Cotton *et al*, 2004; Rundle *et al*, 2004). Measurements were made at the start and completion of the experiment using images taken with a Nikon Coolpix 4500 Digital camera attached to Kyoma optical SDZ-IR-P microscope and analysed using analySIS software. For the analysis, the proportional difference between the final and initial measurements of the trait in question was used. Total snail wet weight was taken at the beginning and the completion of the experiment. Each snail was left

to dry on tissue paper for 10 minutes to ensure there was no water still within shell and the weight was taken using a Fisherbrand digital balance (± 0.0001 g).

Statistical Analysis

Two-way ANOVAs in SPSS 15 were used to test for significant differences in all traits between temperature and predation cue treatments and their interaction using mean values from each mesocosm as replicates. Measurements of all traits were expressed as proportional increase over the trial and were arcsine square root transformed (Sokal and Rohlf, 1995; Dytham, 2005) before the analysis. Post hoc (SNK) test were used to test for significant differences between individual treatments and Levene's test was used to confirm homogeneity of variance.

RESULTS

Survival rates were generally high for the whole duration of the experiment (> 98 %) in both 16° C and 20° C treatments, but were slightly lower at 24° C (88 %). Two-way ANOVAs showed that there were significant treatment (i.e. predator cues) effects ($p < 0.001$) for shell thickness and for shell size (length and width) and aperture length (all $p < 0.05$) (Table 5.1). There were also significant temperatures*cue interactions for shell thickness, aspect ratio and aperture width (Table 5.1). The results also showed significant temperature effects ($p < 0.05$) for shell length, aperture width, shell thickness and total wet weight (Table 5.1).

Table 5.1 Results of two-way ANOVAs testing for differences in induced defences in *Littorina littorea* between control (no predator cue) treatment and predator cue treatment (predator cue from *Carcinus maenas*) both kept under three different temperature regimes: 16° C; 20° C; and 24° C over 14 days.

| Traits | Source | Df | Mean square | F | p-value |
|-----------------|-------------------------|----|-------------|--------|----------|
| Shell length | Treatment | 1 | 0.014 | 6.20 | < 0.05 |
| | Temperature | 2 | 0.015 | 6.723 | < 0.05 |
| | Treatment * Temperature | 2 | 0.007 | 2.956 | 0.128 |
| | Error | 6 | 0.002 | | |
| Shell width | Treatment | 1 | 0.016 | 10.936 | < 0.05 |
| | Temperature | 2 | 0.007 | 4.579 | 0.062 |
| | Treatment * Temperature | 2 | 0.002 | 1.397 | 0.318 |
| | Error | 6 | 0.001 | | |
| Aperture length | Treatment | 1 | 0.022 | 13.106 | < 0.05 |
| | Temperature | 2 | 0.005 | 2.903 | 0.131 |
| | Treatment * Temperature | 2 | 0.004 | 2.432 | 0.168 |
| | Error | 6 | 0.002 | | |
| Aperture width | Treatment | 1 | 0.005 | 2.121 | 0.196 |
| | Temperature | 2 | 0.018 | 7.212 | < 0.05 |
| | Treatment * Temperature | 2 | 0.014 | 5.797 | < 0.05 |
| | Error | 6 | 0.002 | | |
| Shell thickness | Treatment | 1 | 0.065 | 62.127 | < 0.0001 |
| | Temperature | 2 | 0.016 | 15.648 | < 0.05 |
| | Treatment * Temperature | 2 | 0.015 | 13.889 | < 0.05 |
| | Error | 6 | 0.001 | | |
| Wet weight | Treatment | 1 | 0.017 | 4.682 | 0.074 |
| | Temperature | 2 | 0.049 | 13.497 | < 0.05 |
| | Treatment * Temperature | 2 | 0.000 | 2.352 | 0.176 |
| | Error | 6 | 0.004 | | |
| Aspect ratio | Treatment | 1 | 0.000 | 0.433 | 0.535 |
| | Temperature | 2 | 0.003 | 5.455 | < 0.05 |
| | Treatment * Temperature | 2 | 0.000 | 9.012 | < 0.05 |
| | Error | 6 | 0.000 | | |
| Aperture ratio | Treatment | 1 | 0.000 | 0.007 | 0.938 |
| | Temperature | 2 | 0.001 | 2.245 | 0.187 |
| | Treatment * Temperature | 2 | 0.002 | 4.225 | 0.072 |
| | Error | 6 | 0.001 | | |

Post-hoc comparisons showed that there were significant predator cue effects for all traits at 16° C and for shell length and width and aperture length at 20° C (Figure 5.1). Snails exposed to predator threat at 16° C grew bigger (increases of 8 % in length, 6 % in width) and thicker shells (24 %) with larger apertures compared with control snails. Snails responses at 20° C were similar in terms of the difference between predator cue and control snails, although the increase in shell thickness in predator cues was not as great as observed at 16° C (14 % compared with 24 % at 16° C). Snails at 24° C did not show any significant increase in growth in the predator cue treatment, in fact there was a significant reduction in shell length and aperture width relative to control snails (Figure 5.1 A, D). At 24 ° C growth rate of all traits was significantly lower in predator cues than at 16 and 24° C and there was significant cue effect at this temperature. In other words it appears that the production of induced defences was impaired at this higher temperature.

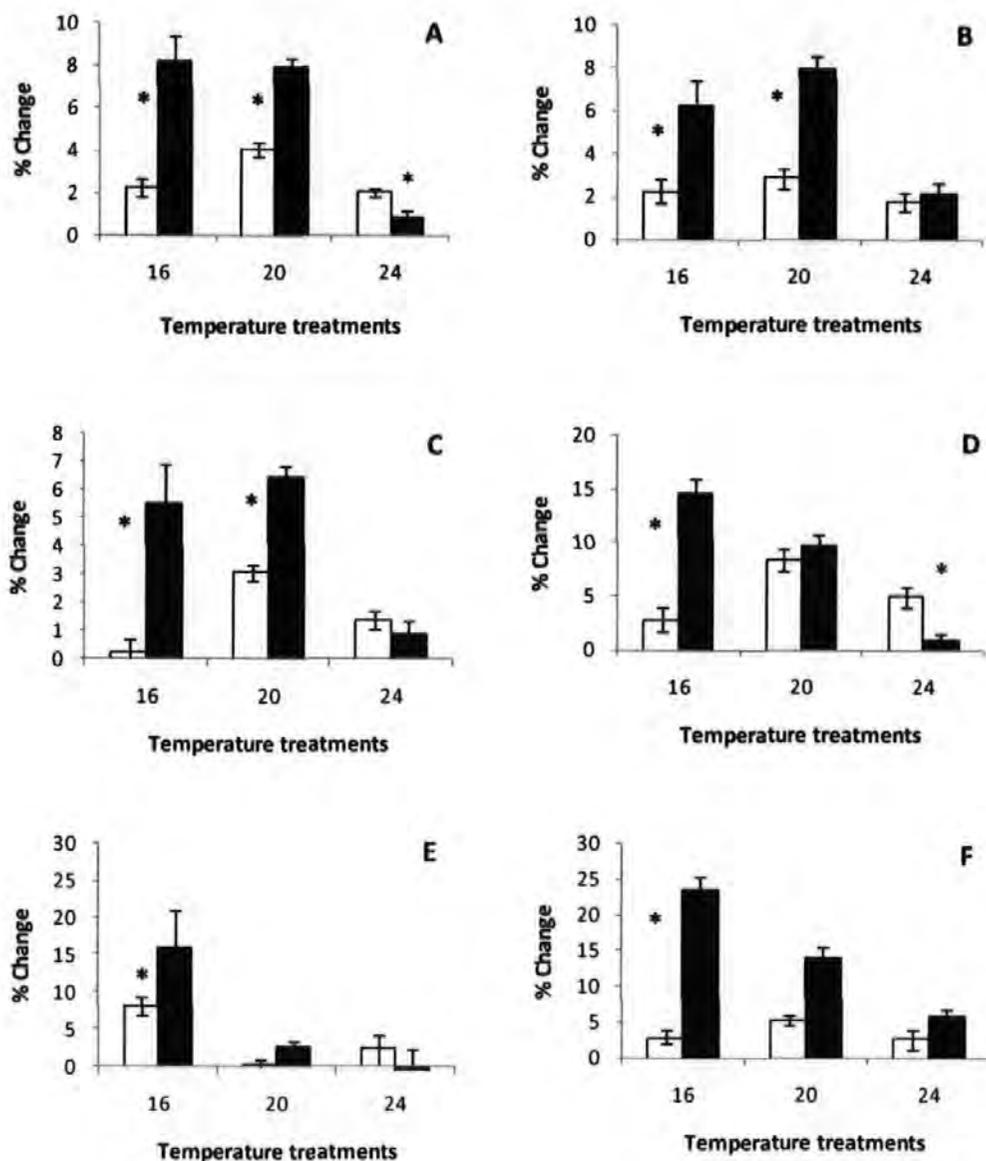


Figure 5.1 The response of intertidal gastropods (*Littorina littorea*) when raised in normal sea water (light bars) or sea water containing *Carcinus maenas* cues (dark bars) at three temperatures: 16° C, 20° C, and 24° C. Values are expressed as means (\pm SE) proportional change in shell traits over the experimental period: a) length, b) width, c) aperture length, d) aperture width, e) total wet weight, f) thickness. * = post hoc tests comparison, significant @ $p < 0.05$.

Post hoc tests between temperature treatments also showed that there were no significant differences in induced traits in 16° C and those in 20° C; total wet weight was exception and was higher at 16° C (Table 5.2). There were also significant differences between both 16° C and 20° C treatment and the 24° C treatment in the

shell size (length), shell thickness, aperture size and total wet weight (the one exception being shell length for 20 versus 24° C) (Figure 5.1 and Table 5.2).

Table 5.2 Post-hoc comparisons (SNK) between three temperature experimental treatments: 16 C, 20° C, and 24° C applied to *Littorina littorea* kept in sea water (control) or with predator cue (cue from *Carcinus* crab) over 14 days.

| | Shell length | Shell width | Aperture length | Aperture width | Shell thickness | Total wet weight | Aspect ratio | Aperture ratio |
|-------------|--------------|-------------|-----------------|----------------|-----------------|------------------|--------------|----------------|
| T 16 * T 20 | N.S. | N.S. | N.S. | N.S. | N.S. | < 0.05 | N.S. | N.S. |
| T 16 * T 24 | N.S. | N.S. | N.S. | < 0.05 | < 0.05 | < 0.05 | N.S. | N.S. |
| T 20 * T 24 | < 0.05 | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. |

Temperature and Salinity Interactions

The salinity was monitored on daily bases throughout the trial and the mean was 37 ± 1 ‰ (see above). However, as there was a chance that salinity may have varied between the temperature treatments in my experiment, due to potential evaporation, I tested explicitly for this possibility. Three replicate water samples were taken from one experimental mesocosm for each treatment along with saltwater from the supply tank (control) and were analysed for calcium and magnesium using Atomic Absorption Spectrophotometry (AAS) (Figure 5. 2AB). A two-way ANOVA was used to test for differences between temperature treatments in (calcium and magnesium concentrations). Although there is significant difference between temperature treatments ($P < 0.05$, $F = 7.99$), there was no significant effect of predator cue within these treatments or cue*temperature interaction. A further trial was conducted to investigate if biological activity had affected ionic concentrations. Three mesocosms were stocked with 15 dead shells (i.e. the same as the number of animals

used in experimental trials) and exposed to same water temperature regimes, i.e. 16, 20 and 24° C were undertaken. In this case, the results of AAS for calcium and magnesium showed no significant difference between different treatments (Figure 5. 2 C).

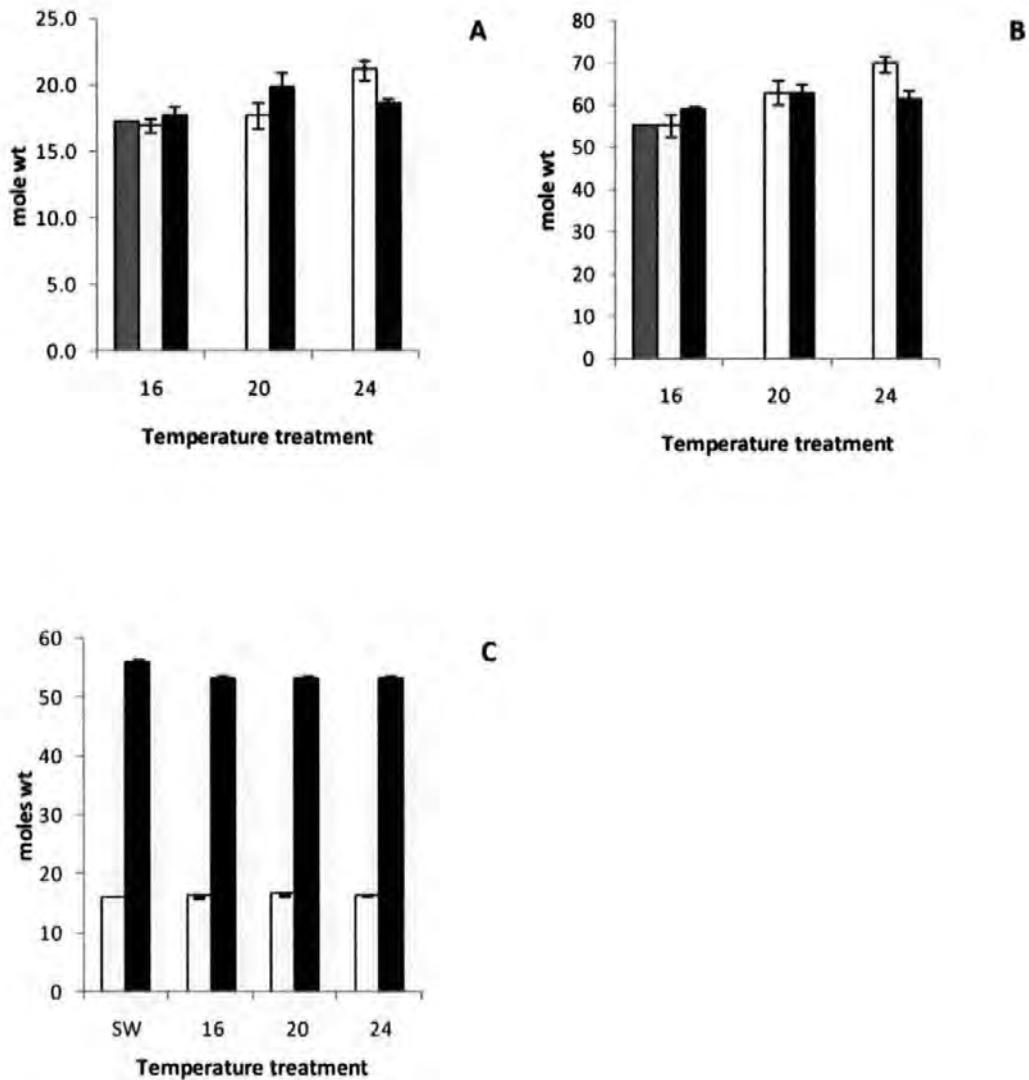


Figure 5.2 The mean of water samples from three temperature treatments: 16° C, 20° C and 24° C analysed for (A) calcium concentration (B) magnesium concentrations of three replicates of the water samples from experimental mesocosms (dark bars ■) and seawater (control- light bars □) tested using atomic spectrophotometer (AAS) (C) calcium and magnesium concentrations of water samples from mesocosms containing gastropod shells at the experimental temperature regimes and only seawater as control (SW ■).

DISCUSSION

The main aim of this study was to assess how temperature affected the production of morphological induced defences in the intertidal gastropod *Littorina littorea* exposed to predator (*Carcinus maenas*) cues. At 16 and 20° C snails exhibited induced defences in the form of increase shell size (shell length and shell width) and thickness with the same magnitude (8 % length and width, 15-24 % thickness) of response at both temperatures. Induced defences are significantly lower at 24° C compared with the other temperature treatments, however, two traits, shell length and aperture width, showed significantly negative responds compared to the control. This may suggest that induced defences (morphological) production was inhibited at this temperature.

Bibby *et al* (2007) showed that acidification of sea water driven by carbon dioxide (CO₂) also negatively affected the production of induced defences in *L. littorea*. They showed that induced growth in shell thickness was reduced by around 5% under low pH and predator cue relative to those kept under same predator threat and normal pH. This is similar to my results for *L. littorea* kept at 20° C while this reduction was much higher around 20 % at 24° C. They also showed that respiration was reduced under the double stress of crab cues and low pH and that snails lacking induced morphological defences in the crab cue and low pH treatment apparently increased their avoidance behaviour. A similar increase in the respiration of snails in my experiment may also have occurred with the double stress of predators and high temperature stress on the snails. This is suggested by lack of responses in most of the traits and significant reduction in shell length and aperture width (Figure 5.1 A, D) at 24° C.

The fact that induced defences were expressed in a similar way at the two lower temperatures of 16 and 20° C in my study suggests that this species of snail is able to respond to predators in their environment within this range. Although these snails experience a mean annual seawater temperature on average around 15° C in their natural habitat in the UK coast (Newell *et al*, 1971) they will also be subject to higher temperatures when exposed at low tide; rock pool temperatures, for example, can reach temperatures close to 30° C during low tide (Evans, 1948; Newell *et al*, 1971; Newell, 1979) and temperatures on uncovered on rocks and boulders can be as high as 40° C within the range of *L. littorea* (Evans, 1948).

The ways that intertidal gastropods respond to temperature can be influenced by several factors such as season, geographical distributions and shore height (Evans, 1948; Newell *et al*, 1971; Clarke *et al*, 2000a; Clarke *et al*, 2000b; Tomanek, 2005). In addition, other factors like acclimation temperature, thermal history and salinity were also reported to have impact on their response to temperature (Clarke *et al*, 2000b). Previous studies on the thermal tolerance of *L. littorea* suggested that it can remain active until 32° C, with an upper critical temperature of 39° C, and death around 45° C (Evans, 1948). *Littorina littorea* has also been reported to show low mortality of 5 % within 14 days in the laboratory at 25° C (Newell *et al*, 1971), which is similar to mortality in my experiment (12 % at 24° C). For *L. littorea*, position on the shore has also been shown to affect its thermal tolerance with upper shore individuals showed higher limit of thermal tolerance than the lower shore individuals (Newell *et al*, 1971). However, Clarke *et al* (2000a) suggested there is no effect of shore height on the upper critical thermal limits (heat-coma) although snails in this

study were acclimated under 12° C for 10 days which might have in fact eliminated the shore height effects which was clearly indicated in Newell *et al* (1971). In addition, animals such as *L. littorea* that live under relatively variable temperature regimes have a greater ability to respond by acclimation and to adjust many physiological activities (Tomanek, 2005). Therefore, induced defences may be influenced by small scale variation between populations in association with temperature.

It is also likely that different populations of *L. littorea* would respond differently to temperature. On the coast of Portugal for example, this species may experience seawater temperatures of up to 21° C (Wells, 1965). Other studies suggested that this species extends throughout the intertidal zone of the UK and some can survive under experimental acclimation at very high temperatures around 35° C (Newell *et al*, 1971; Newell, 1979). The low growth rate of both control and predator treatment of *L. littorea* maintained in 24° C indicates that this temperature exceeds the range suitable for the UK populations. Increasing temperature above the natural physiological temperature limits has very strong negative impacts on an organism's physiology affecting heart rate and cardiac function in addition to the synthesis of heat-shock protein (Harley *et al*, 2006; Stenseng *et al*, 2005).

Temperature plays a major role in metabolism, development, abundance, population dynamics, geographical distributions and the survival rate of wide range of species and biota (Vermeij, 1978; Bale *et al*, 2002; Massot *et al*, 2002; Sokolova and Portner, 2003; Helmuth *et al*, 2005). In marine taxa temperature has also been shown to affect factors such as the physiological performance of the intertidal mussels *Mytilus*

californianus (Helmuth and Hofmann, 2001); spawning time (in the bivalve *Macoma balthica*) (Harley *et al*, 2006), migration in flounder *Platichthys flesus* (Sims *et al*, 2005) and veined squid *Loligo foresi* (Sims *et al*, 2001), spawning and breeding cycle in the New Zealand abalone *Haliotis australis* (Moss, 1998), eggs hatching in several marine fishes (Review- Pauly and Pullin, 1988), the omnivorous ability in *Girella nigricans* (Behrens and Lafferty, 2007).

The global temperature is predicted to rise by 0.28-0.58° C by the year 2020 (IPCC, 1995; Thomson *et al*, 2002). According, to the results of my study, this range seems to be within the tolerance range of *L. littorea*. However, the interactions between temperature and other physical factors is the most important in dealing with global warming (Thompson *et al*, 2002). Temperature plays an important role in the distribution of intertidal gastropods hence clear gradient was shown from temperate to tropical latitudes (Vermeij, 1973). This could be driven by the calcification in marine gastropods which is correlated positively with temperature (Vermeij, 1978). Several other life activities in marine gastropods such as oxygen consumption, metabolic activities (Sinclair *et al*, 2006; Sokolova and Portner, 2003), growth rate, morphological differentiation (Zimmerman and Pechenik, 1991) are all temperature dependent. However, the results of this study suggested that the temperature differences within the chosen ranges have little effect in the development of morphological induced defences among intertidal gastropods *L. littorea*.

Chapter 6

Discussion

My general aim in this thesis was to forward our understanding of phenotypic plasticity and, in particular, induced defences in intertidal organisms. I used the intertidal gastropod *Littorina littorea* to address specific questions within this general aim including: how induced defences in this species compared with those shown by other gastropods which occur in the same habitat (Chapter 2); whether *L. littorea* exhibited developmental windows for and flexibility of plasticity (Chapter 3); if plastic responses varied depending on the type of temporal presentation of risk (Chapter 4); and if the production of induced defences was influenced by temperature (Chapter 5).

The main objective in the first experiment of this thesis (Chapter 2) was to undertake a controlled, cross-species comparison of induced defences in six gastropods species from the same rocky shore to investigate if they varied in a predictable way.

Specifically I was interested to discover if the type of induced defence shown were the same in all species and, if there was variation between species, whether this was related to life history, their relatedness or their potential susceptibility to predation.

My main conclusion was that susceptibility to crab predation appeared to be the main factor relating to the degree of induced responses shown. Cotton *et al* (2004) suggested a negative correlation between shell morphological defences and predator-avoidance behaviour shown in four marine gastropods when exposed to *Carcinus* predator cue. The handling time by *Carcinus maenas* in their study was decreasing as follows: *G. umbilicalis* > *O. lineata* > *G. cineraria* > *L. littorea* (Cotton *et al*, 2004), in my study however, the degree of morphological plasticity in number of shell traits in response to crab cue was in the reverse order: 5 (*L. littorea*) > 4 (*G. cineraria*) > 2 (*O. lineata*) > 1 (*G. umbilicalis*). This approach of making a cross-species comparison

is an important tool for looking at how traits have evolved in clades of organisms. My study was fairly limited in terms of the number of species that I used and a profitable area of future research could be to increase the number of species in such a cross-species comparison and to include an analysis that is phylogenetically controlled (Harvey and Pagel, 1991). Also, it would be interesting to extend the approach taken in my study to include direct comparisons between induced morphological defences and behavioural responses.

Direct and indirect interactions between species that share predators and resources have been suggested to have a significant impact within communities (Abrams, 1987; Van Tamelen, 1987). Trait-mediated indirect interactions (TMIIIs), whereby plastic responses in one species (such as changes to development, morphology, physiology, life history or behaviour) affect other species in any given food web (Chapter 1), are an important phenomenon in the intertidal marine community (Trussell *et al*, 2002; Trussell *et al*, 2003; Trussell *et al*, 2004; Trussell *et al*, 2006). The results from Chapter 2 could be used to inform future studies that aim to study TMIIIs by selecting species that show contrasting responses to predator cues and, which may thus be predicted to show differing levels of behavioural strategies in the presence of a predation threat.

In Chapters 3 and 4 I was interested in assessing how *Littorina littorea* responded to variation in the presentation of predator cues. In effect, these studies were carried out within the context that marine intertidal systems are typically highly dynamic and, hence, that organisms inhabiting these habitats are likely to experience fluctuations in the level of predation risk. The development of plasticity is associated with costs

(DeWitt, 1998; DeWitt *et al*, 1998; Relyea, 2002; Relyea, 2004) and morphological induced defences in marine gastropods have been demonstrated to be costly to produce (Palmer, 1992; Trussell, 2000a; Trussell, 2000b). Therefore, intertidal gastropods should express induced defences only when it is vital to do so.

Bearing this in mind the main focus in Chapter 3 was on investigating how *Littorina littorea* responded in terms of its induced defences when predator cues were either added or removed during its development. Flexibility or reversibility are two of the most important aspects of phenotypic plasticity (Gabriel, 1999; Relyea, 2000; Relyea, 2003; Gabriel, 2005; Miner *et al*, 2005; Hoverman and Relyea, 2007).

The main findings of Chapter 3 suggested that this gastropod did have flexibility to adjust the expression of morphological induced defences with altered predation threat. The introduction of *Carcinus* predator threat half way through the trial led to snails exhibiting a high degree of morphological plasticity in terms of shell size, such that they appeared to “catch up” snails continuously exposed to predator threat. At the same time, a clear reversal of induced defences was obvious with the removal of predator cue – snails were able to adjust to their new environmental conditions and showed a significant reduction in shell growth rate. There was no clear difference in the response of different sizes of snail which might indicate that the snail size (5-10 mm shell length) that I used in my study was in fact too small to show clear differences in responses. It seems important to extend such work in future using full range of development to include embryonic stages and going through reproductive adults to assess how reproduction is affected. A comparison of *L. littorea*, which has a relatively long planktonic stage (Kemp and Bertness, 1984) with other species with direct development such as *L. fabalis* and *L. obtusata* (Paterson *et al*, 2001;

Kemppainen *et al*, 2005) would also be instructive in assessing how life history strategy influences flexibility.

Chapter 4 extended the study of how temporal variation in predation risk affected the expression of induced defences in *Littorina littorea* aiming to test the risk allocation hypothesis (Lima and Bednekoff, 1999). This has previously been tested on behavioural responses (Hoverman and Relyea, 2007), but not applied to morphological responses (Laurila *et al*, 2004). Two main predictions were tested in this experiment: i) the morphological response would vary in rank order as follows: control < constant predator cue < variable predator cue; and ii) random unpredictable cue presentation would induce a greater response than a systematic predictable cue addition. Findings illustrated that gastropods exhibited no significant difference in their morphological traits when exposed to variable predator cue compared to constant predator cue. There was also no impact of the predictability of predation threat on the expression of induced morphological plasticity was observed. This study could be extended to further investigations on the adaptive value of phenotypic plasticity and the cost associated with its expression (see Chapter 1). It has been proposed that some of the costs of plasticity are associated with the information acquisition for assessing the variability of the environmental conditions (DeWitt *et al* 1998). It might be predicted that such costs would be reduced if predators feed in a systematic manner. In addition to morphology, behavioural avoidance may be adapted choice by snails in response to variable threat which in turn incurs other behaviour costs such as reduced feeding activities or fewer mating opportunities. Clearly one future strategy would be to investigate the link between behavioural and morphological defensive traits together in relation to the risk allocation model.

The main aim in Chapter 5 was to assess how temperature affects the expression of induced defences. *Littorina littorea* demonstrated significant induced defences when maintained at temperature of 16° C or 20° C, but it was obvious that growth and induced defence production was inhibited at 24° C. Hence, there was no systematic effect of temperature on the induced defences at this temperature. This part of my study was stimulated by the expected impact of global warming on ecosystems (Chapman and Reiss, 1992; Helmuth and Hofmann, 2001; IPCC, 2001; Helmuth *et al*, 2005; Kaiser *et al*. 2005; Helmuth *et al*, 2006). Temperature plays a very important role on different levels of biological activity such as physiological, behavioural and developmental factors and species interactions and distributions (Newell 1979; Bertness 1999; Helmuth and Hofmann, 2001; Stillman, 2002; Johannesson 2003). Therefore, temperature can exert a major influence on the populations on intertidal rocky shores (Johannesson, 2003).

In addition to raised temperature, increased atmospheric CO₂ affects other ecological and physical processes leading to ocean acidification which may have effects on the expression of induced defences in *L. littorea* (IPCC, 1995; IPCC, 2001; Bibby *et al*, 2007; Byers *et al*, 2008). According to Bibby *et al* (2007) *L. littorea* in more acidific conditions could not develop thick shells in response to crab cues, thus, the snails had to rely more heavily on behavioural avoidance. This is, however, the only study of this kind, and further investigations on the effects of the ocean acidification on the expression of induced defences, and preferably cross species comparisons that include interactive effects of temperature, would an important issue for future research.

Clearly there are many different factors other than just temperature that might influence the production of induced defences in intertidal gastropods. The influence of parasite infections on the expression of induced defences in *L. littorea* is one environmental variable that might be investigated in future research. Studies on *L. littorea* show that it is frequently infected by parasites, in particular platyhelminthes (Huxham *et al*, 1993; Huxham *et al*, 2001; Byers *et al*, 2008). Huxham *et al* (1993) suggested that parasite infections resulted in decreased survivorship and reduced reproduction in addition to reduced shell height in some individuals. According to Byers *et al* (2008), once *L. littorea* had become infected it is likely that this infection will last for its entire life. Obtaining data on the effect of parasite infections on the expression of induced defences in *L. littorea* would enhance the link between laboratory experiments and their natural habitat, and hence increase understanding of the intertidal community.

In my study, the snails used were selected and judged to be healthy based on their general appearance. There is a small possibility that some of the individuals used in my studies were parasitized. If present these parasites could have dramatically altered the type of induced responses reported in this study because they are known to either reduce or enhance ('gigantism') growth rate (Huxham *et al*, 1993). It is unlikely that this would have led to bias, however, as infection rates on open shorelines are usually low and , hence, for most experiments infected snails were equally likely in all treatments.

The dispersal strategy which determines the grain size (i.e. heterogeneity) of the environment experienced by marine taxa such as gastropods is another important ecological factor affecting the expression of the phenotypic plasticity (Hollander *et al*,

2006; Hollander, 2008). A coarse-grained environment associated with species having low dispersal rate, whereas fine-grained environment is associated with species having high dispersal rate (Hollander, 2008). According to Hollander (2008) it might be predicted that taxa with high dispersal rates should experience a high degree of environmental variation and, hence, should exhibit more plasticity. He tested this proposal in a meta-analysis of plasticity studies on marine molluscs, annelids and echinoderms and found that, in all three cases, plasticity was higher in species with high dispersal rates. Therefore, more studies aiming to test the effects of dispersal rate on the expression of induced defences within intertidal community are important aspect of future studies.

Applications of Induced Defences

As well as having fundamental implications for marine intertidal communities, phenotypic plasticity may also have applications in commercial activities, such as aquaculture and stock enhancement. Morphological plasticity is used as a strategy for enhancing survival in the queen conch *Strombus gigas* (Delgado *et al*, 2002). This commercially important tropical gastropod has been shown to survive better when juveniles were experimentally exposed to spiny lobster *Panulirus argus* predation cues. Predator induced morphological defences in the mussel *Mytilus edulis* after exposure to the predator threat of starfish *Asterias rubens* leads to increased survival in the mussel, furthermore the meat per shell volume was increased even though the shell size was reduced (Reimer and Tedengren, 1996). Similarly, a difference in induced phenotypic plasticity in the skull morphology associated with feeding was found between hatchery-reared largemouth bass *Micropterus salmoides floridanus* and their wild counterparts (Wintzer and Motta, 2005). The wild bass quickly

developed morphological changes, such as a fusiform head and elongated jaw when reaching 80-90 mm in total length; however, those reared in hatcheries developed later when reaching a total length of 135 mm (Wintzer and Motta, 2005). There is no direct impact on the commercial yield was suggested but it may indicate an important aspect of phenotypic plasticity.

The development of techniques to use induced defences in aquaculture is certainly worth pursuing, although it should be born in mind that despite the example above, induced defences have been associated with a reduction in body mass (Trussell, 2000a; Trussell and Nicklin, 2002) in some species, while other studies such as on *Littorina subrotundata* showed that growth rate was not affected by the development of induced defences (Dalziel and Boulding 2005). This demonstrates that special care must be taken if dealing with the induced defences the stock enhancement.

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