An interdisciplinary approach to understanding predator modification of antipredator traits in prey



A thesis presented for the degree of Doctor of Philosophy

By

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**Thesis Summary**

Predators have a profound impact on the fitness of prey, able to consume them directly or affect them indirectly, through modulation of several physiological, morphological or behavioural traits intended to minimise the negative impacts of predation risk. Recent studies have shown that the outcome of predator-prey interactions are context dependent, with prey subject to different abiotic and biotic factors responding differently to the perception of risk. As short-term fluctuations in predation risk combine with more predictable large-scale environmentally driven differences in predation pressure, understanding the drivers of antipredator traits in prey is complex. Antipredator responses are further complicated by the ability of some parents to influence the antipredator responses of subsequent generations via parental effects. Therefore, in order to fully appreciate the drivers and implications of antipredator responses for prey, investigation must consider the impacts of risk in different contexts.

Through a combination of field and laboratory experiments I investigated the antipredator responses of prey exposed to different predation risk regimes throughout their development and the potential for parental effects to alter them. More specifically, my thesis examined the antipredator responses of the intertidal dogwhelk *Nucella lapillus* to the predation risk posed by the green shore crab *Carcinus maenas*. One of the knowledge gaps addressed in this thesis is the potential for individual vulnerability to direct predation to impact the physiological response of prey to the detection of a predator. Although ontogenic growth in dogwhelks can result in them reaching a size refuge from crab predation, little is known about the potential for the indirect effects of predation to persist past this point. Thus, the first part of my thesis (Chapter 2) investigates the potential pervasive influence predators have on prey, by comparing changes in oxygen consumption of vulnerable and invulnerable size classes of *Nucella* under predation risk. I was able to show that even once *Nucella* grow to a size refuge exposure to crab effluent is still able to elicit a metabolic response, showing that in some cases the indirect effects of predation risk can outlast the impacts of direct predation.

The second part of my thesis (Chapters 3,4,5) uses a long term breeding experiment to examine the combined influences of parental habitat (exposed versus sheltered rocky shores) where dogwhelks are exposed to different predation pressure and the risk regime under which newly hatched dogwhelks develop to examine physiological, morphological and behavioural responses. Findings presented in Chapter 3 showed, for the first time in this species, that the risk posed by crabs affects aspects of the long-term energy budget of *Nucella*, in addition to confirming previously demonstrated impacts on their growth. Using the Cellular Energy Allocation technique to investigate the energy allocation at the cellular level, my analysis revealed that the way in which *Nucella* store and utilise energy was influenced by developmental experience of risk, but responses of offspring from each parental habitat varied. Moreover, *Nucella* offspring invested proportionally more in shell growth than in somatic growth as the amount of time spent under risky conditions throughout development increased.

In Chapter 4 behavioural assays were used to examine the impact of risk on several short-term behavioural responses of *Nucella* bred under the long-term programme. There was surprisingly little influence of the parental background of offspring, the risk regime under which development occurred or risk perceived in experimental mesocosms on short term responses. However, the amount of time spent under risky conditions did influence refuge use in these individuals.

Finally, in Chapter 5 I examined how the patterns of risk faced by offspring of two populations of *Nucella* affected long-term foraging decisions. Although no robust conclusions can be drawn as to the importance of parental habitat in the responses of the two populations of *Nucella* used, I showed that *Nucella* from these two populations responded differently depending on the risk they experienced during development as well as that experienced throughout the foraging experiment.

My thesis as a whole, in addition to illuminating the importance of predation risk on the antipredator responses of prey at size refuge, adds to our understanding of how individual experience of risk can combine with parental effects to inform the antipredator responses of prey. Collectively, my results highlight the importance of incorporating individual experience with that of previous generations when aiming to investigate the impacts of predation risk on prey.

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**Table of Contents**

[Chapater One General Introduction 1](#_Toc52387615)

[1.1 Overview 2](#_Toc52387616)

[1.2 Predator-prey interactions 4](#_Toc52387617)

[1.2.1 Detection 4](#_Toc52387618)

[1.2.2 Predator-induced stress response 6](#_Toc52387619)

[1.2.3 Metabolic response to predation risk 7](#_Toc52387620)

[1.2.4 Behavioural responses of prey 9](#_Toc52387621)

[1.2.5 Morphological adaptations of prey 11](#_Toc52387622)

[1.2.6 Foraging implications of predation risk 13](#_Toc52387623)

[1.2.7 Parental effects and predation risk 14](#_Toc52387624)

[1.2.8 Ecological impacts of indirect predation - TMIIs 15](#_Toc52387625)

[1.3 Project overview 16](#_Toc52387626)

[1.3.1 Predation risk and the intertidal dogwhelk *Nucella lapillus* 16](#_Toc52387627)

[1.3.2 Parental effects 17](#_Toc52387628)

[1.3.3 Wave-exposure and its impacts on *Nucella* 17](#_Toc52387629)

[1.3.4 Ecological impacts of *Nucella* under predation risk 18](#_Toc52387630)

[1.4 General methodology and experimental design 19](#_Toc52387631)

[1.4.1 Site selection and field experiment 20](#_Toc52387632)

[1.4.2 Laboratory reared *Nucella* 21](#_Toc52387633)

[1.5 Thesis Outline 22](#_Toc52387634)

[Chapater Two Prey vulnerability and predation pressure shape predator-induced changes in O2 consumption and antipredator behaviour 24](#_Toc52387635)

[2.1 Abstract 25](#_Toc52387636)

[2.2 Introduction 26](#_Toc52387637)

[2.3 Materials and Methods 28](#_Toc52387638)

[2.3.1 Predator prey system 28](#_Toc52387639)

[2.3.2 Site selection and predator numbers 29](#_Toc52387640)

[2.3.3 Field measurements 29](#_Toc52387641)

[2.3.4 Laboratory measurements 31](#_Toc52387642)

[2.3.5 Statistical analysis 32](#_Toc52387643)

[2.4 Results 33](#_Toc52387644)

[2.4.1 Field results 33](#_Toc52387645)

[2.4.2 Laboratory results 35](#_Toc52387646)

[2.4.3 Laboratory behavioural responses 36](#_Toc52387647)

[2.5 Discussion 38](#_Toc52387648)

[2.5.1 Oxygen consumption in vulnerable *Nucella* 39](#_Toc52387649)

[2.5.2 Oxygen consumption in invulnerable *Nucella* 40](#_Toc52387650)

[2.5.3 Antipredator behaviour 40](#_Toc52387651)

[2.5.4 Potential ecological importance 41](#_Toc52387652)

[2.6 Conclusions 41](#_Toc52387653)

[Chapater Three Parental effects and individual experience of predation risk informs growth and the energy budget of prey 44](#_Toc52387654)

[3.1 Abstract 45](#_Toc52387655)

[3.2 Introduction 46](#_Toc52387656)

[3.3 Methods 48](#_Toc52387657)

[3.3.1 Study System and general approach 48](#_Toc52387658)

[3.3.2 *Nucella* collection and breeding 49](#_Toc52387659)

[3.3.3 Risk Manipulation 51](#_Toc52387660)

[3.3.4 Data collection 51](#_Toc52387661)

[3.3.5 Data Analysis 53](#_Toc52387662)

[3.4 Results 53](#_Toc52387663)

[3.5 Discussion 60](#_Toc52387664)

[Chapater Four Parental and individual experience of predation risk informs short-term antipredator responses in the dogwhelk, *Nucella lapillus*. 64](#_Toc52387665)

[4.1 Abstract 65](#_Toc52387666)

[4.2 Introduction 66](#_Toc52387667)

[4.3 Methods 68](#_Toc52387668)

[4.3.1 *Nucella* collection and breeding 68](#_Toc52387669)

[4.3.2 Risk Regimes 68](#_Toc52387670)

[4.3.3 Behavioural assay 69](#_Toc52387671)

[4.3.4 Data Analysis 70](#_Toc52387672)

[4.4 Results 70](#_Toc52387673)

[4.5 Discussion 73](#_Toc52387674)

[Chapater Five Parental effect and individual experience of predation risk informs the foraging behaviour of a wave-exposed and a wave-sheltered population of *Nucella lapillus*. 77](#_Toc52387675)

[5.1 Abstract 78](#_Toc52387676)

[5.2 Introduction 79](#_Toc52387677)

[5.3 Methods 81](#_Toc52387678)

[5.3.1 Site selection and *Nucella* Breeding 81](#_Toc52387679)

[5.3.2 Risk Regimes 82](#_Toc52387680)

[5.3.3 Foraging experiment 83](#_Toc52387681)

[5.3.4 Data Analysis 85](#_Toc52387682)

[5.4 Results 86](#_Toc52387683)

[5.5 Discussion 92](#_Toc52387684)

[Chapater Six General Discussion 95](#_Toc52387685)

[Chapater Seven References 97](#_Toc52387686)

# **General Introduction**

**CHAPTER ONE**

**General Introduction**

## Overview

The impacts predators have on prey and the ecological systems they inhabit are indisputable, and as such are one of the corner stones of ecological research (Volterra 1928, Connell 1961a, Paine 1974a, Menge 1976, 1978a, 1983). Many early investigations into the impacts of predation viewed prey as passive units of energy to be consumed by a predator (Paine 1966) and as such investigations were conducted from the perspective of the predator. Their focus was therefore on the feeding rates of predators within different environmental and biological scenarios (Paine 1969, 1974b) and generally required the removal of predators from an area and the reporting of subsequent changes in distribution and abundance. These studies also highlighted the importance of direct predation on community composition, inter- and intraspecific competition (Connell 1961b, 1961a), species diversity, as well as showing impacts on the resources of seemingly unconnected species through trophic cascades (Carpenter et al. 1985).

Due to its considerable influence on an organism’s fitness, most prey are not passive in their interaction with predators, instead able to modify various behavioural and physiological traits in order to increase their chances of survival. Recent theoretical (Dill 1987, Lima and Dill 1990, Lima and Bednekoff 1999, Ferrari et al. 2009), experimental (Trussell et al. 2004, Viant 2007, Hawlena and Pérez-Mellado 2009, Matassa and Trussell 2014) and technological advances (Santini et al. 2000, Kamenos et al. 2006) have turned the focus of investigations towards the influence predators have on the traits of prey. It has become more apparent that the detection of a predator, is enough to affect the physiology (Hawlena et al. 2011, Van Dievel et al. 2016), behaviour (Lima and Dill 1990, Lima and Bednekoff 1999), morphology (Heller 1976) and life history (Laurila et al. 1998) of prey as well as the outcome of predator-prey interactions (Ydenberg and Dill 1986). These non-lethal impacts of predators based on how prey respond to the perception of predation risk, have been termed the indirect effects of predation (Dill 1987). Finally collective changes at the individual level can influence populations level processes (Beckerman et al. 1997, Schmitz et al. 1997, Matassa and Trussell 2011) and in some cases have been shown to be as important ecologically as the impacts of direct predation (Werner and Peacor 2003, Trussell et al. 2006a, 2017, Peacor and Werner 2014).

The response to a potential attack begins once the predator is detected. The inherently high costs of not responding to the detection of a predator, has resulted in prey evolving physiological stress responses which increase their chances of surviving the initial moments of the encounter (Beckerman et al. 2007, Van Dievel et al. 2016). For many animals the detection of a predator initiates a series of biochemical reactions designed to liberate energy to deal with an impending attack. The release of stress hormones enables energy to be mobilised to locomotory structures and increases the chance of escape (Steiner and Van Buskirk 2009). Stress hormones also regulate metabolic processes (Munck et al. 1984, Sapolsky et al. 2000) and increase heart rate to transport oxygen and metabolic reagents around the body, enabling prey to respond early enough to avoid being eaten. Although this “fight or flight” response is vital in surviving the immediate threat (Cannon, 1915), it comes at an energetic cost to prey. High metabolic rates require prey to replenish any used energy and this in turn entails increasing foraging rates which inevitably lead to more exposure to predation risk (Lima and Bednekoff 1999). Prey therefore modulate their behaviour (for example changes in microhabitat and the use of refuges or influences on foraging strategies) in order to minimise the impacts which predation risk has on their energy budget. Prolonged exposure to predation risk in many animals also results in more permanent morphological modifications which also reduce their chances of being injured or consumed by a predator (Hughes and Elner 1979).

In the next section I will attempt to describe in more detail the process by which indirect predation impacts prey and the ecosystems they inhabit. This description will begin with the detection of a predator, the resulting physiological response, the influences this may have on the short-term energetics of prey and the importance of compensatory mechanisms as an adaptive response to repeat or chronic exposure to predation risk. I will then describe the potential behavioural responses that arise under predation risk both as short- and long-term solutions to the threat posed by predators, with a focus on escape and avoidance responses as well as the individual and ecological influence of predator induced changes to prey foraging strategies. After a description of the influence of predation risk on the morphological traits of prey, I will conclude by attempting to illuminate the importance of the effects of indirect predation on the ecological processes that were once believed to be influenced solely through direct predation. A final section will provide information on how predation risk is not necessarily confined to a single generation, but rather can influence many traits of future generations.

## Predator-prey interactions

### Detection

Due to the unforgiving nature of failing to detect a predator early enough to escape, there is an inherent fitness inequality in early detection between predators and prey (Ydenberg and Dill 1986). If a predator misses the chance to consume a prey item, it will have little impact on its lifetime fitness. However, failure by prey to anticipate an attack by a predator could result in being eaten or injured. Therefore, as early detection of a predator has a potentially enormous impact on prey fitness, prey species are generally characterised by a high level of antipredator vigilance and have evolved a variety of early detection mechanisms (Beauchamp 2015), adapted to best detect predation risk in the environmental context they live in. Therefore, prey have evolved to use and potentially combine visual, acoustic, mechanical, electrical or chemical cues from predators in order to gain an edge prior to an encounter. The use or combined use of these modes of predator detection, can depend on the medium through which information is being transmitted, as well as the type of predator being detected. In addition to simple detection of predation risk, many prey are able to distinguish between different predators and to respond accordingly to the threat posed by each. For example, the nomadic wood warbler, *Phylloscopus sibilatrix,* responds by drastically increasing the number of calls at its nest following the detection of the Eurasian jay (*Garrulus glandarius*), its main nest predator. When warblers are instead exposed to visual cues from the least weasel (*Mustela nivalis*) which only poses a moderate threat to their nests, they only produced a moderate increase in calls at the nest (Maziarz et al. 2018).

The environmental conditions as well as the medium through which signals are sent and received by prey, ultimately affects the detection and reliability of the information transmitted (Lima and Dill 1990, Hay 2009). For animals relying on vision to detect a predator, for example, changes in light intensity at dusk and dawn are detrimental to the detection of a predator and their perception of risk (Lima 1988, 1998a). Once the light diminishes, prey that rely on visual detection are disadvantaged and therefore, many reduce the time they are exposed to predation risk. In one example, the dark-eyed junco (*Junco hyemalis*), modifies risk taking behaviour in accordance with the level of risk they perceive (Lima 1988). Juncos adjust the level of risk they are exposed to during foraging by selecting to forage in relatively safe areas when light conditions are poor, increasing their chances of escape in the event of an attack. As light conditions improve, Juncos increase their use of more exposed but potentially energetically superior foraging areas.

In the aquatic and marine realms many species rely on the detection of kairomones, that are inadvertently released by other taxa (Chivers and Smith 1998, Zimmer and Butman 2000, Hay 2009). These unintended chemical cues are carried through the water column by means of hydrodynamic mixing. Many prey rely on differences in kairomone composition, concentration and flux to locate the sender (see reviews by Chivers & Smith, 1998; Zimmer & Butman, 2000; Hay, 2009). These chemical signals form plumes, creating an olfactory seascape that animals must utilise to locate resources and avoid adversaries (Nevitt et al. 1995). As these chemical signals rely on water movement to be transmitted, they are affected by environmental conditions such as wave-action which can impact their reliability (Freeman and Hamer 2009). Weissburg and Zimmer-Faust (1993) argued that within an environment of differing hydrodynamic regimes (i.e. waves and tidal flux), prey may have a hydrodynamic refuge from predators during periods of high turbulence (Rochette et al. 1994). As prey also rely on kairomones to detect predators, these adverse hydrodynamic conditions will likely provide predators with a similar hydrodynamic refuge. This has been demonstrated by Large *et al.* (2011), who showed that the dogwhelk, *Nucella lapillus*, only adopted antipredator behaviour (cessation of movement) to effluent from the green shore crab, *Carcinus maenas*, under intermediate flow velocities, whereas no such behaviour was seen in slow- or fast-moving water.

The importance of predator detection and the potential influence that abiotic conditions may have on its reliability means that prey need to be flexible and able to adjust their responses based on previous experiences. In some cases, the plasticity of antipredator responses enable prey to “learn” from previous encounters and react differently in future ones (Lima and Dill 1990, Lima 1998a, Johnston et al. 2012). In one example, when the whelk, *Nucella lamellosa*, was preconditioned for 30 days with the scent of a predatory crab, it reduced the threshold concentration of crab effluent required to elicit a future antipredator response (Edgell 2010). Preconditioned whelks were able to react to dilute concentrations of crab effluent (50%), allowing them to adopt antipredator behaviours earlier than predator naïve individuals. Moreover, prey that depend on the safety of groups to avoid being eaten, do not have to rely on their own experience of risk, but rather are able to combine it with that of other group members (Powell 1974, Lima 1995). In an experiment designed to compare individual and group experience of risk on the response to predation risk of group members, Crane and Ferrari (2015) showed that the minnow, *Pimephales promelas*, preferred to rely on the behaviour of conspecifics when in a group rather than on their own learned experiences. This preference to rely on the collective behaviour of others over its own perception of predation risk indicates that minnows utilise a hierarchy of reliability when it comes to the detection and response to predation risk (Crane and Ferrari 2015).

### Predator-induced stress response

As stated earlier, the detection of a predator commonly elicits a stress response (Rovero, Hughes, & Chelazzi, 1999) broadly consisting of changes to metabolism, the production of stress hormones as well as an increase in heart rate (Hertz et al. 1982, Slos and Stoks 2008, Creel et al. 2009, Clinchy et al. 2013, Chu et al. 2014). Shifts in these biochemical processes have evolved to enable prey to utilise energy faster, allowing them to better respond to an imminent threat (Angell 1915, Lima 1998b). Upregulation of the breakdown of energy reserves, mainly through the liberation of glucose, combines with an increase in heart rate, resulting in more energy reaching locomotory structures faster (Cooper and Blumstein 2015). However, these physiological stress responses of prey are not necessarily limited to the instant a predator is detected, but rather can persist long after the encounter has ended (Boonstra 2013). Behaviourally this may be more obvious, as animals may remain hidden even after a predator has gone (Morgan 1988), whereas the less conspicuous physiological changes may also persist for minutes or even hours after the predation risk has abated (Cooke et al. 2003). For example, when damselfly larvae (*Enallagma spp.*) are exposed to cues from a stickleback (*Gasterosteus aculeatus*), they show increases in oxygen consumption in line with the fight-or-flight response (Slos and Stoks 2008). However, they also produce stress proteins (Hsp70) to combat the adverse impacts of increased metabolism (Slos and Stoks 2008). These energetically costly stress hormones remain in the damselfly system long after the stress has been alleviated and are thought to add to the general growth inefficiencies seen in prey under predation risk (McPeek et al. 2001, McPeek 2004, Slos and Stoks 2008).

Intriguingly in some species, stress responses are not fixed and may be influenced by the context in which a predator is detected. The heart rate of the queen scallop, *Aequipecten opercularis*, for example, increases in the presence of its starfish predator (*Asterias rubens*), but this response depends on the quality of nearby refuges (Kamenos et al. 2006). Scallops provided with substrata suitable for use as a refuge in a laboratory experiment had a significantly lower heart rate than those detecting a predator without suitable refuge close by. The authors provide two possible explanations for this difference. It may be that the lack of a suitable refuge close-by increases scallop stress levels or that the increase in heart rate itself may be adaptive, enabling scallops to swim faster to locate a safe space to hide. Although ubiquitous in the interaction between a predator and its prey, stress responses can vary between species, but also between the different situations prey may find themselves in.

### Metabolic response to predation risk

Predator induced changes in metabolic rate in the short-term may differ from those of prey exposed to predation risk over prolonged periods. In the short-term, increases in metabolic rate may help initial responses, but they generally come at an energetic cost and can therefore not be sustained indefinitely (Lima and Bednekoff 1999, Steiner and Van Buskirk 2009). To combat this, some prey have evolved to reduce the impacts predators have on their metabolic rate when exposure is prolonged. In one example, tadpoles (*Rana temporaria*) exposed to short-term risk show predictable increases in metabolic rate, leading to an enhanced ability to escape predators (Steiner and Van Buskirk 2009). When exposure is prolonged, tadpoles begin to acclimate to the levels of predator risk and their metabolism returns to pre-exposure levels. Reducing energetic demands when exposed to predation risk for prolonged periods, reduces the amount of time during which foraging is necessary, which in turn will reduce further exposure to predation risk (Steiner and Van Buskirk 2009).

These adaptive responses to prolonged exposure to predation risk are not always beneficial in the long term and can have large negative implications for other life history traits (Clinchy et al. 2004, Adamo and Baker 2011). These chronically induced indirect effects can impact prey fitness in many ways including a reduction in reproductive output (Creel et al. 2007), weakened immune response (Navarro et al. 2004) as well as hereditary reproductive performance passed on to offspring from parents exposed to chronic predation stress (Sheriff et al. 2010). For instance, the short-term exposure of the long-winged cricket (*Gryllus texensis)* to predation risk, results in enhanced escape behaviours through an increase in the concentration of blood stress hormones which subsides following the removal of risk (Adamo and Baker 2011). Repeat exposure results in prolonged increase in basal blood stress hormone levels. Predator conditioned crickets were able to sustain flight for far longer than unconditioned counterparts but elevated basal stress hormone levels over long periods also resulted in reductions in growth efficiency and it has been argued that it may also explain, at least in part, a reduction in reproductive investment in crickets under chronic risk (Adamo and Baker 2011). In another example, a short 3-day exposure of the tobacco hornworm caterpillar, *Manduca sexta*, to predation risk by the stink bug, *Podisus maculiventris*, showed that caterpillars increased energy assimilation efficiency to compensate for a 30-40% reduction in feeding rates compared to controls (Thaler et al. 2012). This compensation allowed the caterpillars to maintain similar growth rates to the unexposed controls. However, short-term increases in assimilation efficiency were compensated for by negatively impacting assimilation efficiency later in development. This indicates that the tobacco hornworm caterpillar uses a temporally dynamic compensatory mechanism allowing them to increase fitness during risky periods in the short-term and defer the negative impacts of predation risk to more safer periods in the future (Thaler et al. 2012). The example of the tobacco caterpillar provides evidence that short-term responses to risk may represent fitness trade-offs over the lifetime of prey, and that the real implications of short-term trade-offs between safety and risk may only become apparent in longer-term experiments.

The discovery of the ability of prey to compensate for the negative effects of predation in one instance by deferring them to the future, raises important questions regarding how prey respond to changes in the patterns of risk they experience. Theoretical and experimental investigations have shown that the temporal pattern of risk experienced by prey is an important driver in shaping their antipredator responses (Lima and Bednekoff 1999, Ferrari et al. 2008b, Matassa and Trussell 2014). As highlighted earlier, exposure to predation risk generally results in reductions in growth and impacts on post-consumptive efficiencies, although these may be compensated for in some cases (Van Dievel et al. 2016, Tigreros et al. 2018). For example, growth of the common dogwhelk, *Nucella lapillus*, under predation risk decreases relative to the amount of time spent under risky conditions and this difference is mainly driven by reductions in energy acquisition (Palmer 1990). However, when Trussell et al. (2011) exposed dogwhelks to variable and unpredictable patterns of predation risk through the manipulation of the amount of time spent under risk, their growth efficiencies do not follow the same linear pattern. Instead, analysis of dogwhelk growth efficiency (energy acquired converted into new growth) under different variable risk treatments, shows that although growth efficiencies are reduced by even a short exposure to predation risk (25% of the time), when exposure to risk was constant (100% of the time), growth efficiencies were higher than in treatments that provided some “safe times”.

The exact processes that underpin the impacts of unpredictable predation risk on prey growth rates are not fully understood, but some insight may come from another study highlighting the compensatory mechanism used by prey under risk. The beetle, *Leptinotarsa decemlineata*, is known to express intraclutch egg cannibalism when reared under adverse conditions, a process that increases their initial nutritional state (Tigreros et al. 2017). In their investigation, Tigreros et al., (2018) exposed cannibal and non-cannibal beetles to predation risk and then measured both physiological and behavioural changes in response to risk. The difference in nutritional state resulted in cannibal and non-cannibal beetles adopting different strategies to deal with the levels of risk. Low nutritional state non-cannibals responded physiologically by increasing metabolism but showed no changes in behaviour. On the other hand, high nutritional state cannibals responded behaviourally to risk and suspended feeding with metabolic rate remaining unaffected. The result was that both groups of beetles attained similar growth rates. This highlights the ability of prey to compensate physiologically and behaviourally depending on their own nutritional state. Investigations like this, provide insights into the potential mechanism that control the antipredator strategies adopted by prey.

### Behavioural responses of prey

Once detected, the physiological reactions described above result in behavioural responses that will ultimately affect the chances of survival. Prey may choose to avoid predators if they detect them early enough to evade detection themselves or are unlikely to escape following an encounter (Cooper and Blumstein 2015). Avoidance behaviours can typically include changes of microhabitats and the use of refugia (Witman 1985). For instance, the small-mouthed salamander, *Ambystoma texanum*, is a highly active mesopredator, that rarely utilises refuges in the absence of predatory fish (Sih et al. 1988, Sih and Kats 1991). Refuge use increases dramatically however, when salamanders are exposed to chemical cues from the bluegill sunfish (*Lepomis* *macrochirus*), with up to 80% of individuals selecting the safety of a refuge over remaining exposed to predation. The presence of risk from the bluegill also affects the amount of times salamanders enter and exit refuges, with individuals more likely to remain within the confines of a refuge while the risk is still present (Sih et al. 1988). When predation pressure is less extreme (low probability of attack), but more persistent, avoidance behaviour can have impacts on broad scale distribution patterns of prey. For example, clear long-term predator avoidance behaviour can be seen in the vertical distribution of many intertidal gastropods, that face predation pressure from subtidal predators and increased desiccation risk with tidal height (Phillips 1976). These avoidance behaviours will inevitably impact the pressure placed on prey resources in the less risky microhabitats and hence can have important effects on community level processes (Yamada and Boulding 1996).

When early detection fails or if the chance of escaping an encounter are high, prey may adopt more drastic escape behaviours (Wirsing et al. 2010), such as shifts in speed and direction, body contortions, jumping or falling away from a predator (Wirsing et al. 2010). In general, escape responses have evolved predominantly to rapidly increase the distance between prey and predator (Cooper and Blumstein 2015). As with physiological responses, many escape behaviours of prey show a level of plasticity which enables them to adapt to different contexts (Mery and Burns 2010). In one instance, kangaroo rats (*Heteromyidae spp.*) previously exposed to a simulated attack by rattlesnakes (*Crotalus spp.*) altered their escape behaviour to increase their chances of survival when compared to non-exposed rats (Freymiller et al. 2017). With recent experience of an attack, kangaroo rats decreased their response times, increased their take-off velocity and combined with a steeper angle of jump greatly improved their chances of escaping future attacks. Similar enhancements in escape behaviour can be seen in the adaptive jumping response in the grasshopper, *Melanoplus femurrubrum*, when reared in the presence or absence of predatory cues (Hawlena et al. 2011). When reared under risk conditions, grasshoppers altered their technique to reduce the take-off time by 1.2 times. This combined with inflight changes increased their jumping distance by 2.6 times. These small changes in jumping technique doubtlessly enables chronically exposed grasshoppers to increase their chances of survival (Hawlena et al. 2011).

Prey express plasticity in various aspects of learned escape behaviours as well, including responding to new predators (neophobia, Ferrari et al., 2015), lower response thresholds (Edgell 2010) and acquiring new escape behaviours (Rochette et al. 1998). Much insight into the plasticity of the behavioural responses of prey has been gained from the study of antipredator behaviour in the common whelk, *Buccinum undatum*, in response to cues from predatory asteroids. Although whelks express avoidance behaviours and avoid areas populated by asteroids (Rochette et al. 1994), they can occasionally be found in high densities close to feeding asteroids. This behaviour enables them to take advantage of the inefficient feeding of the asteroid and gain a substantial part of their energy in this way (Rochette et al. 1995). However, this dangerous food source is only exploited by experienced mature whelks, with smaller immature whelks avoiding asteroids completely. Furthermore, adult whelks are able to distinguish between moving and stationary asteroids and only express escape behaviours to the former, thus minimising the energy lost from adopting unnecessary escape behaviours (Rochette et al. 1997). Further evidence of the whelks’ ability to adjust its response to the threat posed by a predator has been highlighted in experiments comparing populations of whelks naturally exposed to different asteroid predators (Rochette et al. 1996). Whelks from areas sympatric with the asteroid, *Leptasterias* *polaris*, increase mucus production and show enhanced levels of foot contortions (the most effective escape behaviour against *L. polaris*), which increase their chances of survival. Moreover, although sympatric juveniles do not express foot contortions, they nevertheless express less effective escape behaviours indicating that they recognise *L. polaris* as a threat. Enhanced escape behaviours are not present in adult whelks from populations not subject to predation by *L. polaris*, and juveniles show no response to this allopatric predator (Rochette et al. 1996).

### Morphological adaptations of prey

Despite the potential effectiveness of antipredator behavioural responses, when exposure to risk is prolonged or repeated and attacks are unpredictable, many prey also develop predator-induced morphological defences (PIMDs; Bourdeau & Johansson, 2012) adapted to increase their likelihood of surviving a predatory encounter. PIMDs are designed to reduce the predation risk experienced by prey thus making it harder for predators to find, catch and/or consume them. PIMDs can include spikes, helmets and bulbus shapes of daphnia (Weiss 2019), changes in the body shape of fish (Díaz-Gil et al. 2020), development of bent morphs in barnacles (Lively 1986a, Murua et al. 2014), and increased shell thickening in gastropods (Hughes & Elner, 1979; Bourdeau, 2010). The development of PIMDs comes at a cost, with reduced energy available for growth and reproduction and increased metabolic costs due to potentially cumbersome morphologies accounting for much of the fitness losses associated with PIMDs (Bourdeau and Johansson 2012).

Although in some cases morphological changes can be reversed (Díaz-Gil et al. 2020), generally they represent more permanent responses to risk than those expressed behaviourally. For example, many gastropods increase the amount of shell deposition in relation to body growth as a response to exposure to predation risk (Hughes and Elner 1979). Potential differences in the predation pressure felt by separate populations can result in the formation of ecotypes, with thick shelled gastropods found in areas of high predation pressure and thin shelled individuals where predation is less intense (Appleton and Palmer 1988). Pascoal et al. (2012) showed that even once these ecotypes have been formed, they are able to, at least partially, alter their morphology if the predation risk they experience changes. However, the exact mechanism underpinning the development of these PIMDs is unclear. Although originally thought of as a direct result of the predatory cues themselves (Appleton and Palmer 1988), recent studies have argued that many of the inducible defences are a by-product of the behavioural responses of prey (Bourdeau 2010). Opposition to the direct link between cues and PIMDs point to strong correlations between inactivity and shell deposition, arguing that reductions in feeding rates relating to inactivity could account for their development.

Arguably, the most effective morphological defences against being attacked by a predator is growing to a size that is too large for them to exploit. The vulnerability of smaller individuals to predators is a well-established concept in predator-prey interactions (LaBarbera 1989, Scharf et al. 2000), with larger individuals generally harder to catch (Lundvall et al. 1999), harder to handle (Hughes and Elner 1979), or even potentially able to injure a predator during an attack. As prey size increases, the size classes of gape-limited predators that are able to consume them decreases and in some prey species individuals are able to grow to a large enough size that they are no longer susceptible to being eaten by a predator, therefore reaching a size refuge. For many predatory fish, for example, the gape size of their mouths defines the size of prey they are able to consume (Werner 1974, Scharf et al. 2000). Once prey surpass a predator’s maximum gape size, they are essentially safe from direct predation from that predator. For intertidal gastropods, reaching a size refuge means growing to a size at which the shell is impervious to attacks from crushing predators, such as crabs. As individuals increase in size, they reduce the size classes of crabs that have the strength to break their shell, ultimately obtaining sizes that render them impervious to attack by crabs of any size (Hughes and Elner 1979). For example, as the limpet, *Patella* *vulgata*, increases in size, it reduces the size classes of the crab *Necora puber*, that are able to consume them (Silva et al. 2010). However, a small percentage (6%) of *P. vulgata* obtain sizes larger than 41mm in length which is too big for even the largest *N. puber* to exploit.

Apart from reducing susceptibility to predation, increases in prey body size can also have profound effects on an individual’s ecological performance (LaBarbera 1989). Body size has been shown to influence inter- and intraspecific competition, foraging as well as reproduction, with larger individuals having a disproportionate impact on these population level processes (De Roos et al. 2003). Despite the relative acceptance of this ontogenic scaling of ecological performance, most of the literature focuses on the growth/risk trade-offs of prey trying to reach a size refuge. By contrast little attention has been paid to the potential for the indirect effects of predation to persist even after prey are no longer susceptible to direct predation. In one of the few studies to investigate the persistence of indirect effects of predation in prey at size refuge comes from studies of sea urchin grazing. In their study Pessarrodona et al., (2019) showed that the indirect impacts of risk posed by the presence of the omnivorous fish *Diplodus sargus*, were strongest on larger size classes of sea urchins than on smaller ones. Importantly they showed that predation risk influenced the foraging of sea urchins that had reached a size refuge even though they were no longer susceptible to direct predation from *D. sargus*. This important finding highlights the potential for indirect predation to influence both prey and the wider ecosystem even after pressure from direct predation has diminished.

### Foraging implications of predation risk

One of the most important influences of predation risk on prey, is its impacts on foraging (Lima and Dill 1990, Lima 1998a). Foraging in many taxa comes with an inherent reduction in antipredator vigilance and therefore prey must strike a balance between the risk of an attack and the risk of starvation (Lima and Bednekoff 1999). As such, reductions in energy acquisition during risky periods is ubiquitous in prey species (Lima and Dill 1990, Lima 1995). In one instance, the number of oysters consumed by the carnivorous snail, *Stramonita haemastoma*, is reduced by 50% when exposed to predatory cues from the stone crab, *Menippe adina*. Moreover, as prey are forced to find other foraging opportunities, energetically rich foraging patches may be situated in more exposed and therefore more risky areas, as the safest patches are exploited first (Elner and Hughes 1978). For instance, the insectivorous lizard, *Acanthodactylus beershebensis*, reduces its foraging activity in more profitable open ground when in high risk situations, opting to take shelter under shrubs (Hawlena and Pérez-Mellado 2009). This behavioural change results in a change of diet, as lizards are exposed to a different suite of prey when sheltering under shrubs.

These predator-induced changes in microhabitat place a strain on the resources within refuges as prey are forced to exploit the resources within a refuge during extended periods of risk. Intertidal dogwhelks (*Nucella lapillus*) need to seek the security of a refuge to escape both predation by crabs and the potential of being dislodged by wave-action. By doing so they exert pressure on the resources surrounding their refuges. As such, the likelihood of encountering a median sized barnacle (dogwhelk prey) increases with distance from the refuge (Johnson et al. 1998). The quality and availability of refuges have been shown to have an important influence on the indirect effects of predation risk on prey. Donelan et al (2017) have shown that potential energetic gains due to the combined effect of the ability to continue foraging as well as the safety provided by the refuge can alleviate up to half the negative impacts of predation risk on dogwhelks. Therefore, prey must weigh up the potential benefits and costs of foraging in an area as often the more profitable foraging grounds may also be accompanied by higher levels of predation risk (Lima and Dill 1990).

### Parental effects and predation risk

Importantly, the impacts of predation risk are not necessarily confined to a single generation, but instead may modify traits in subsequent generations as well (Mousseau and Dingle 1991, Mousseau and Fox 1998). One clear negative impact predation risk can have on the offspring generation of prey is through impacts on reproductive investment. The shield bug (*Elasmucha ferrugata*) is able to invest different amounts of energy in their eggs in accordance with the amount of predation risk they are likely to face (Mappes et al. 1997). Eggs at the edge of broods are at highest risk from predatory attack and therefore receive the least amount of energy, when compared to those laid on the centre of broods. This lower investment translates into lower levels of offspring fitness in shield bug offspring hatched from the edge of broods. Negative impacts of predation risk on offspring are not solely due to differences in maternal reproductive effort. Offspring of risk experienced parents of the threespined stickleback (*Gasterosteus aculeatus*) were less likely to respond to predation risk and had higher levels of mortality than offspring of unconditioned parents (Mcghee et al. 2012), despite hatching from similar sized eggs.

There are also, however, several other ways in which predation risk experienced by parents can be more beneficial to the fitness of their offspring. These parental effects tend to be strongest when the parental experience of risk is closely correlated with that which their offspring are likely to experience (Vermeij 1982, Mousseau and Dingle 1991, Pascoal et al. 2012a). When females of the common lizard (*Zootoca vivipara*) were exposed to predatory cues from adult green whip snakes (*Hierophis viridiflavus*) during gestation, they produced offspring that expressed higher levels of antipredator traits as well as more pronounced antipredator behaviour, than those of nonexposed females (Bestion et al. 2014). Predator exposed mothers produced offspring with longer tails which increases their escape ability once attacked. The same offspring dispersed on average three times as far as offspring of non-exposed mothers, increasing their chances of avoiding the high-risk environment experienced by their mothers. However, despite the obvious benefits to offspring, not all parental antipredator traits are as transmitted to the next generation. For example, offspring of predator conditioned parents of the freshwater snails (*Physa acuta*) retain high crush resistance shell, but do not show any of the antipredator behavioural traits exhibited by their parents (Beaty et al. 2016). Studies into the potential for parental experience to inform the traits of offspring will allow us to begin to fully appreciate the pervasive effects of predation risk as well as the potential adaptive responses of prey.

### Ecological impacts of indirect predation - TMIIs

In addition to the impacts that predators have on their prey, they are also able to indirectly influence other taxa through trophic cascades (Vanni and Findlay 1990). A consequence of direct predation is the reduction of the density of prey which in turn will reduce the pressure placed on prey resources. These are referred to as Density Mediated Indirect Interactions (DMIIs) and for many years were thought to be the main indirect impact predators have on their community. However, over the last four decades, ecologists has also demonstrated that indirect impacts on prey traits can also cause cascade effects, similar to those caused by DMIIs (Trussell et al. 2004). When community level changes are brought about through modification of prey traits (physiological, morphological or behavioural) by the perception of predation risk, they are known as Trait Mediated Indirect Effects (TMIIs). A compelling example comes from the PIMDs developed by the barnacle, *Chthamalus anisopoma*, exposed to cues from the carnivorous gastropod, *Acanthina angelica* (Raimondi et al. 2000). When exposed to predatory cues this species of barnacle develops into a “bent morph”, which reduces its probability of being consumed by *A. angelica* (Lively 1986b). When Raimondi and colleagues (2000) in turn manipulated the densities of bent morphs, they found that it affected the competition for dominance between mussels (*Brachiodontes semilaevis)* and encrusting algae (*Ralfsia sp*). If *A. angelica* consumed barnacles (non-bent morphs) then this promoted the dominance of the mussels, whereas if it induced the bent morph rather than consume them, then it promoted the growth of the encrusting algae (Raimondi et al. 2000). In another example, Trussell et al., (2017) used a intertidal rock pool food web to demonstrate that the presence of a crab predator influenced the cover of the fucoid algae (*Ascophyllum nodosum*), through modifications of both herbivorous and carnivorous gastropod foraging. Although predictably they demonstrated increases in the fucoid cover when herbivore feeding was suppressed, they also found that suppression of both herbivorous and carnivorous foraging resulted in the highest levels of fucoid cover. This they argue is a result of the persistence of fucoids on the shells of barnacles which were not consumed by the carnivorous gastropods that supressed their foraging in the presence of risk from their crab predator. Finally, although the strength of DMIIs and TMIIs are likely to be context dependent (Luttbeg et al. 2003) there is some evidence that at least in some cases TMIIs may rival or even exceed the strength of DMIIs (Trussell et al. 2006a). It will be important to incorporate the ecological implications of both DMIIs and TMIIs if we are to fully appreciate the impacts predation and its risk have on ecological communities.

## Project overview

### Predation risk and the intertidal dogwhelk *Nucella lapillus*

The intertidal dogwhelk *Nucella lapillus* (*Nucella* hereafter) is a carnivorous gastropod that plays an important role as an intermediate consumer, influencing the demography of its mussels and barnacles prey (Menge 1978a, 1978b, Hughes et al. 1999), but also impacting community dynamics by clearing space and enabling the recruitment of other species (Crothers 1985, Trussell et al. 2017). *Nucella* is also an important prey species of the green shore crab, *Carcinus maenas* (hereafter *Carcinus*) and both inhabit rocky shore habitats along a gradient of wave exposures, across most of the North Atlantic (extensive description see Crothers, 1985). This predator-prey system has been used extensively in investigations into the impacts of direct and indirect predation (Hughes and Elner 1979, Aschaffenburg 2008, Matassa and Trussell 2014), with a combination of field and laboratory investigations enabling a deep understanding of their interaction.

As with most intertidal invertebrates, *Nucella* is able to identify the presence of *Carcinus* through the detection of kairomones that are inadvertently released by crabs and transported through the water column. As such the reliability of these chemical signals can be affected by the prevailing hydrodynamic conditions (Chivers and Smith 1998, Zimmer and Butman 2000, Hay 2009). When flows are either too fast or too slow, *Nucella* do not respond to *Carcinus* effluent, and instead only adopt antipredator behaviours under intermediate flows (Large et al. 2011). Details relating to a potential stress response of *Nucella* following detection of *Carcinus* are limited, with only a single study showing that if the stress response is indeed present, it is not related to the upregulation of Hsp stress molecules (Chu et al. 2014), as is the case for other prey species (Slos and Stoks 2008). Despite this lack of knowledge relating to the stress response of *Nucella*, they do express a range of behavioural and morphological responses to the detection of *Carcinus*. Reductions in movement (Large and Smee 2010), predator avoidance (Vadas et al. 1994), increases refuge use (Donelan et al. 2017), reduced foraging rates (Freeman et al. 2014, Matassa and Trussell 2014), decreases in growth (Trussell et al. 2006b), increases in shell deposition (Trussell 1996, Pascoal et al. 2012a, Large and Smee 2013) are all responses that are typical of *Nucella* exposed to *Carcinus* effluent. (Palmer 1990, Aschaffenburg 2008). Furthermore, investigations into how changes in the pattern of risk experienced by *Nucella* have highlighted its importance in their responses to predation risk. After initially showing that predation risk negatively impacts *Nucella* growth (Trussell et al. 2006b), Trussell et al. (2011) showed that although foraging and growth rates were negatively correlated with time spent under risky conditions, *Nucella* had higher levels of growth efficiency (converting energy consumed into new growth) when risk was constantly high than when it was unpredictable.

### Parental effects

As *Nucella* are direct developers and there is little migration between distinct populations, *Nucella* parents are able to increase the fitness of their offspring by forewarning them about the prevailing predation pressure through parental effects. Parental influence of offspring antipredator traits include influences on shell morphology (Pascoal et al. 2012b, 2012a), impacts on growth and assimilation efficiencies (Donelan and Trussell 2018a, 2019) as well as foraging (Donelan and Trussell 2015) and antipredator behaviour (Donelan and Trussell 2018b). For example, Pascoal et al., (2012) showed that laboratory reared *Nucella* retained their parental shell morphologies, but that the heritability of these morphologies diminished with subsequent generations. Other studies have shown the ability of *Nucella* to inform the behaviour of their offspring (Donelan and Trussell 2018b, 2019). When parental couples of *Nucella* were exposed to predation risk, they produced offspring that were 30% more likely to utilised refuges when exposed to predatory cues, compared to responses of offspring from risk naïve parents (Donelan and Trussell 2018b). Studies like these highlight the importance that parental effects have on the development of antipredator traits in *Nucella* and the need to incorporate them in any investigation in order to fully appreciate their adaptive advantages.

### Wave-exposure and its impacts on *Nucella*

Although on small temporal and spatial scales abiotic factors are likely to fluctuate, when such factors are viewed over larger scales they become more predictable (Ballantine 1961, Underwood and Chapman 1996, Burrows et al. 2008a). With wave exposure being a function of the average wave fetch of each site, wave-action differences between sites are one of the most predictable and influential abiotic factors faced by intertidal species (Burrows, Harvey, & Robb, 2008). Investigations have shown that both direct (Menge 1978a) and indirect predation (Freeman and Hamer 2009) by *Carcinus* are influenced by wave-action. Increases in wave action increase the chance of being dislodged and this results in low *Carcinus* abundances in wave-exposed locations (Hughes and Elner 1979). As this reduction in predator numbers is combined with a decrease in the reliability of predator cues in high wave-action areas, it means the influence of direct and indirect predation on *Nucella* can differ greatly between populations. These differences result in populations being exposed to different levels of predation pressure (Smee et al. 2010, Large and Smee 2013), which combined with persistent large-scale differences in wave-action has resulted in *Nucella* forming ecotypes (Hughes 1972). *Nucella* in wave-exposed shores tend to form short thin shells with a large aperture to reduce drag and the chance of dislodgement (Elner and Hughes 1978, Trussell 2000). At wave-sheltered sites where wave-action is consistently low, predator numbers and predation pressure are high, *Nucella* develop crush resistant shells in response. By transplanting individuals between wave-exposed and wave-sheltered populations (Gibbs 1993), and later testing under laboratory conditions (Pascoal et al. 2012a, 2012b), studies have shown that *Nucella* increase shell deposition in the presence of crab effluent. Interestingly, the plasticity of shell structure seems not to be equal between exposed and sheltered shore populations of *Nucella*, with exposed shore individuals expressing higher levels of plasticity than their counterparts (Palmer 1990, Pascoal et al. 2012a). This may be related to the durability of morphological adaptations, as thin shelled exposed-shore *Nucella* may produce more pronounced changes in shell morphology (increasing shell thickness) than their sheltered shore counterparts, whose reduction in shell deposition may be harder to measure (Pascoal et al. 2012a).

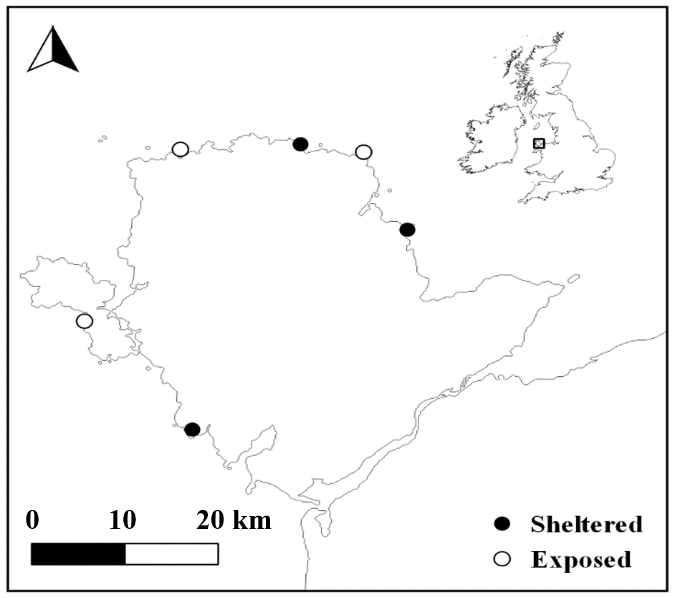
### Ecological impacts of *Nucella* under predation risk

*Nucella* have also been used as a model organism to investigate the potential impacts of DMIIs and TMIIs (Trussell et al. 2008, 2017), and as stated previously the suppression of *Nucella* foraging has been shown to facilitate fucoid algal cover, by not removing barnacle shells that provide space for fucoid recruitment (Trussell et al. 2017). Previous studies have also argued that the potential TMIIs brought about through the presence of *Carcinus* may equal or exceed those produced through DMIIs as a consequence of direct predation (Trussell et al. 2006a). Intriguingly, the strength of DMIIs and TMIIs is influenced by wave action and therefore the influence of each may differ depending on local hydrodynamic conditions (Freeman and Hamer 2009).

## General methodology and experimental design

The previous sections highlighted our understanding of the indirect effects predators have on prey and later *Nucella* more specifically, from detection, to the various responses of prey and ultimately the impact on community level processes. As is clear from this summary, in order to fully appreciate the influenced of predation risk on the traits of prey as well as how changes in these traits can influence the impacts of predation risk itself, investigations should attempt to consider as many of the potential influencing factors. In this thesis I aim to test how responses to predation risk by *Nucella* are influenced by prey vulnerability, parental effects as well as the temporal pattern of risk they are exposed to. An initial experiment will investigate the predator-induced physiological responses of prey at size refuge and those still vulnerable to direct predation of populations of *Nucella* naturally exposed to different levels of predation pressure. By conducting laboratory experiments on both wild and laboratory bred *Nucella* I will seek to illuminate the potential for individual and parental experience of risk to influence the antipredator traits of *Nucella* from the same populations differing in their levels of predation pressure.

All *Nucella* used throughout this thesis were collected from three wave-exposed and three wave-sheltered site around the coast of Anglesey (Fig. 1.1). These sites were initially selected and their levels of wave-exposure were initially identified using distinct biological zonation characteristic of each exposure (Ballantine 1961) and later confirmed using the protocol developed by Burrows et al., (2008b). The map based approach developed by Burrows et al., (2008b) provides a wave-exposure index based on calculations of average wave fetch for each site. In order to measure the potential differences in predation pressure experienced by *Nucella*, I also measured the abundance of *Carcinus* at each site using baited crab traps (60 x 40 x 35 cm, with 500g of fish). For each site, crab numbers per trap were counted on three consecutive days. I placed a single baited crab trap in the midzone and left for two complete tidal cycles. I then counted the number of crabs in each trap and then redeployed a further two times, each time deploying ~30 m along the shore. This allowed me to calculate the average crab numbers across the three days for each site and provide a mean crab number per trap per site.



***Figure 1.1*** *Map of study sites across the Isle of Anglesey, Wales, UK. Open circles = Exposed sites, closed circles = sheltered sites.*

### Site selection and field experiment

My thesis is split into an initial investigation using *Nucella* directly collected from the six sites in Figure 1.1 and then the following three investigation utilise offspring of *Nucella* from the same six sites reared under laboratory conditions. For my initial investigation into the potential impacts of predation risk on the oxygen consumption rates of vulnerable and invulnerable *Nucella* from wave-exposed and wave-sheltered populations, I initially took measurements in the field and then used the same individuals in a laboratory experiment. Oxygen consumption rates of field collected *Nucella* were conducted within 30 mins of collection, providing an approximation of how *Nucella* respiration would respond to risk under *natural* conditions. Field collected *Nucella* were then fed and housed in a laboratory for one month before measuring oxygen consumption again as well as measuring changes in movement of *Nucella* exposed to crab effluent and filtered sea water. In the case of the field experiment and all subsequent laboratory experiments crab effluent was created in the same way. In total 8-10 large male crabs (mean carapace length ± SD, 56.6 ± 4.8 mm) were placed into 20 L of filtered and aerated water for 1 hour.

### Laboratory reared *Nucella*

As stated earlier the second set of experiments were conducted using laboratory bred *Nucella* from each of the six sites. The last three chapters in this thesis investigate the influence of the developmental experience of predation risk (i.e. that occurring throughout the development of offspring) and the parental habitat (wave-exposure) on different traits in *Nucella*. I manipulated the temporal pattern of risk experienced by *Nucella* offspring throughout their development by exposing them to one of three predation risk treatment: a no risk treatment, a constant risk treatment and a variable risk treatment. Although detailed methodologies exist in each chapter, as an overview, I will describe how I bred and manipulated the risk experienced by *Nucella* offspring, providing the animals I used in my last three chapters. In chapter 3, I analysed the influence of these factors on the energy budget and growth of *Nucella* offspring; In Chapter 4, I looked at their effects on the short-term escape behaviour of *Nucella* offspring; and finally In Chapter 5, I investigated the influence of the developmental experience of risk on the foraging behaviour of offspring from a wave-exposed and a wave-sheltered population.

Adult *Nucella* (approx. 150) were collected in February 2017 from the six sites (Fig. 1.1) and placed into site specific tanks, on a 12:12 light dark cycle, with water temperatures that reflected local ambient seawater temperatures*.* *Nucella* are internal fertilisers and produce egg capsules (10s per female) contained 10-30 fertilised embryos and 100s of nurse eggs (Pechenik et al. 1984) which they attach to moist rocky overhangs and crevices (Crothers 1985). As hatchling emerge as fully formed snails and do not move great distances throughout their lives, there is little migration between populations (Rolán et al. 2004, Dingemanse et al. 2007, Carro et al. 2012). *Nucella* breeding peaks in March around the coast of North Wales (Crothers 1985, Morton 2009), when snails form large reproductive aggregations. Under laboratory conditions, *Nucella* formed breeding aggregations in February and egg capsules were deposited by *Nucella* from all 6 sites within a 2-week period in mid-March. Egg capsules from each of the six sites were randomly assigned to a no risk, a variable risk or a constant risk regime resulting in a total of 18 treatment combinations (n = 6 mesocosm replicates per treatment combination). Using a scalpel blade, egg capsules were carefully removed, and 8 egg capsules were randomly assigned to one of three risk regimes. Hatchlings were initially provided with rocks, covered in newly recruited barnacles, and then subsequently with larger barnacles as they grew in size. In addition to food, the rocks provided a refuge throughout the 8-month rearing process. Risk exposurewas based on the addition, to individual mesocosms, of a perforated crab pot which housed a large male crab (mean carapace length ± SD, 56.6 ± 4.8 mm). Housing crabs in such pots allowed the free dispersion of kairomones without allowing predators direct access to *Nucella*. Under the no-risk regime crab pots remained empty, whereas under the constant risk regime crabs were present throughout the experiment. For the variable risk regime, crabs were added and removed from the crab pot every three or four days, resulting in *Nucella* in this regime being exposed to risk for 50% of the time. *Nucella* offspring were therefore reared under these three risk regimes from the egg capsule phase and throughout the initial 8 months of development.

## Thesis Outline

In Chapter 2, I address the potential relationship between local predation pressure and prey vulnerability on prey metabolic rate and antipredator behaviour responses. I measured the oxygen consumption rate of prey at size refuge and therefore no longer susceptible to direct predation, in order to investigate the potential for the indirect effects of predation to persist in these size classes. I used *Nucella* from three wave-exposed and three wave-sheltered populations to investigate if differences in crab densities at each habitat may influence the metabolic response of vulnerable and invulnerable *Nucella*. The same individuals were then used in a laboratory investigation one month later, measuring both oxygen consumption rates and movement in response to *Carcinus* effluent.

Karythis S, Cornwell T, Gimenez L, McCarthy ID, Whiteley NM, Jenkins SR, (2020). Prey vulnerability and predation pressure shape predator-induced stress responses and antipredator behaviour. *Animal Behaviour*

In Chapter 3, I investigate the potential influence of the experience of risk throughout development and parental habitat on the growth and long-term energetics of *Nucella*. Using laboratory bred *Nucella*, I calculated and compared the growth as well as the energetic budget (using the Cellular Energy Allocation technique) of offspring reared under three different risk regimes.

Chapter 4 focused on comparing the short-term behavioural responses of *Nucella* from wave-exposed and wave-sheltered parents bred under different risk regimes. The aim was to investigate the potential for either parental experience of risk or that experienced by the individual throughout development on the antipredator behaviour adopted. This was achieved by using *Nucella* offspring in series of behavioural assays using crab effluent and filtered seawater and comparing the behavioural outcomes.

Finally, in Chapter 5 I compared the foraging behaviour of *Nucella* offspring from a wave-exposed and a wave-sheltered population under different temporal patterns of risk. The aim of this experiment was to investigate how differences in the patterns of risk experience during development may affect the foraging behaviour under predictable and unpredictable risk of *Nucella* offspring.

# **Prey vulnerability and predation pressure shape predator-induced changes in O2 consumption and antipredator behaviour**

**CHAPTER TWO**

Prey vulnerability and predation pressure shape predator-induced changes in O2 consumption and antipredator behaviour

## Abstract

Predators regulate prey abundance (direct predation) as well as influencing their metabolism and behaviour (indirect effects) through the perception of risk. Anti-predator traits are informed by individual experience of risk, which may vary over environmental gradients and through ontogeny. As prey grow in size, individual vulnerability generally diminishes, and the reduction in individual vulnerability with ontogenetic growth can potentially lead to size refugia, ultimately nullifying the impacts of direct predation. Despite the ecological importance of the indirect effects of predation and the disproportionate influence that larger individuals have on ecological level processes, there has been little focus on the potential indirect effects of predation risk on invulnerable prey. Using a combination of field and laboratory experiments, we measured the changes in routine oxygen consumption of vulnerable and invulnerable size classes of the intertidal snail *Nucella lapillus,* exposed to effluent from its crab predator *Carcinus maenas*. To test the potential influence of prior experience of predation risk, measurements were conducted on populations naturally exposed to different levels of predation pressure. Field results showed that only invulnerable snails modified their routine oxygen consumption in the presence of risk, and this occurred across all populations. Oxygen consumption rates in the laboratory however, contradicted the pattern, with only vulnerable prey responding to the perception of risk. Metabolic responses of both vulnerable and invulnerable prey under field and laboratory conditions are discussed in the context of asset protection and prey energetic state. Observations of snail behaviour in the laboratory showed that dogwhelks from sheltered shores, where predatory risk is higher, exhibited a higher propensity to exhibit anti-predatory behaviour. Importantly our findings provide evidence that the indirect effects of predators remain influential even after prey are no longer susceptible to direct predation and add to the growing body of evidence highlighting the ecological importance of indirect predation.

## Introduction

In many species, exposure to a predatory cue elicits a series of coordinated, adaptive physiological responses, which influence antipredator behaviour (Hawlena et al. 2011, Van Dievel et al. 2016) and may come at an energetic cost (Kamenos et al. 2006, Slos and Stoks 2008, Hawlena and Schmitz 2010a). Such physiological and behavioural responses, as well as promoting prey survival, lead to localised reductions in foraging rates or changes in habitat use that can cause trophic cascades with lasting effects on local population densities and community structure (Schmitz et al. 1997, 2004, Werner and Peacor 2003, Trussell et al. 2004). The energetic costs of predator-induced stress responses have been suggested as potential explanations for reductions in prey fitness, and consequently changes in prey demography (Boonstra et al. 1998, Creel et al. 2007, Preisser et al. 2007, Slos and Stoks 2008), ecosystem nutrient dynamics (Hawlena and Schmitz 2010a), energy flow through trophic levels (Matassa and Trussell 2014) and may possibly account for food chain length in some systems (Trussell et al. 2006b).

The threat of predation varies both temporally and spatially at the individual and population level (Lima and Dill 1990, Lima and Bednekoff 1999). Natural variation in ambient predation pressure among populations has led to the evolution of adaptive physiological and behavioural responses to predation, which may be adjusted by local conditions (Holopainen et al. 1997, Handelsman et al. 2013, Donelan and Trussell 2018a). For example, the resting metabolic rate (MR) of tadpoles (*Rana temporaria*) exposed to short-term risk increases, leading to an enhanced ability to escape predators. However, with longer exposure MR rates drop, showing acclimation to predator risk by reducing energetic demands which may underpin risk-averse behaviour whilst foraging (Steiner and Van Buskirk 2009). This individual experience of local predation pressure can also inform the subsequent generation, through epigenetic programming (Jablonka and Raz 2009, Love et al. 2013). This trait is particularly important for direct developing offspring, whose experience of risk is likely to strongly correlate with that of their parents (Dixon and Agarwala 1999, Poethke et al. 2010). For instance, offspring dispersal (a predator avoidance trait) in the lizard *Zootoca vivipara,* increases as a consequence of maternal predator-related stress, decreasing the potential predation pressure experienced by offspring during the most vulnerable stages of development (Bestion et al. 2014). In this way, parental input and individual experience combine to produce more suitable adaptive ecotypes (Giesing et al. 2011, Donelan and Trussell 2015) with natural selection acting to reinforce local adaptations (Mäkinen et al. 2008, Guerra-Varela et al. 2009).

At the level of the individual, several factors including learned behaviours and ontogenetic somatic growth can result in a change in the suite of predators that threaten prey and, in many cases, result in a reduction in overall predation pressure (Paradis et al. 1996, Scharf et al. 2000). Larvae of the three-spine stickleback (*Gasterosteus aculeatus*), use predator size relative to their own as a measure of predation risk and modify their foraging behaviour accordingly, thus optimizing energy intake whilst minimising the risk of being eaten (Bishop and Brown 1992). This type of threat-sensitive behaviour is further enhanced when prey are able to adjust their behaviour relative to their encounter rates with different predators (Rochette et al. 1999). Legault & Himmelman (1993) showed that this kind of threat-sensitive behaviour exists in several marine invertebrate prey, but that correlations between encounter rates and antipredator behaviour vary between species.

Ontogenetic somatic growth can be an escape strategy in and of itself, with many prey species able to grow to size refugia and escape direct predation completely (Chase 1999). Little is known regarding the way in which prey that have reached a size refuge respond to the threat of predation (Werner et al. 1983, Lundvall et al. 1999), although links between size related vulnerability and antipredator behaviour have been shown in certain aquatic gastropods (Rochette and Himmelman 1996, DeWitt et al. 1999). In their study into the potential for the aquatic snail, *Physa gyrina*, to express behavioural compensation for morphological vulnerability to a crayfish predator (*Orconectes rusticus*), DeWitt et al(1999) showed that larger less vulnerable snails demonstrate reduced levels of antipredator behaviour. Considering the disproportionate impact larger individuals have on demography and resources (Paine 1976, Etter 1989), it is perhaps surprising that little interest has been shown in the indirect effects predators have on prey that have reached size refugia.

The purpose of this study was to investigate how differences in prey size and hence vulnerability to direct predation affects the physiological and antipredator response in prey. In addition, we examined how these effects were modified by prior experience of predation risk, based on habitat (sheltered or exposed shores). We used a widely adopted intertidal predator-prey system and implemented a series of field and laboratory experiments, to examine both physiological and behavioural responses of vulnerable and invulnerable prey to predation risk.

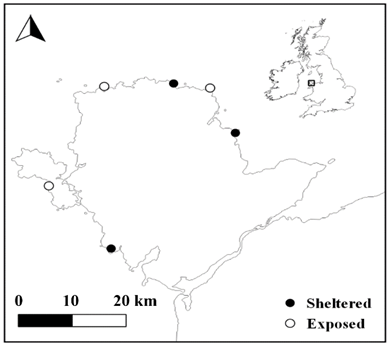
## Materials and Methods

### Predator prey system

The green shore crab *Carcinus maenas* is an important predator of the dogwhelk *Nucella lapillus* (hereafter referred to as *Nucella*)*.*  Both species are found extensively across the North Atlantic and co-occur along a gradient of wave exposure (Crothers 1985). *Nucella* reaches a size refuge from crab predation at 27mm shell length (Hughes and Elner 1979). As with many gastropod species, *Nucella* are able to assess predation risk through the detection of differences in concentration of kairomones, waterborne chemical cues inadvertently released by predators (Vadas et al. 1994, Edgell 2010, Matassa and Trussell 2011). *Nucella* use differences in the concentration of these chemicals to assess the proximity of a predator (Freeman and Hamer 2009, Large et al. 2011) and therefore are influenced by local hydrodynamic conditions (Freeman and Hamer 2009, Large et al. 2011). For example, in high flow, wave-exposed environments, characterised by an elevated degree of mixing, the homogenisation of the olfactory seascape created by predatory kairomones, affects the chemoreceptive ability of prey (Zimmer and Butman 2000, Weissburg et al. 2003, Large et al. 2011). Large et al (2011), showed that antipredator behaviour in *Nucella lapillus* is strongly influenced by hydrodynamic mixing and that *Nucella* chemoreception ability is reduced in very slow or fast flowing turbulent water. They argue that on exposed shores, due to the homogenisation of different concentrations of chemical cues, *Nucella* are unable to perceive predation risk. Wave action also directly impacts the densities of crabs, with wave exposure being negatively correlated with crab densities (Hughes & Elner, 1979; Large & Smee, 2013; Menge, 1983; Rochette, Smee, & Trussell, 2011). Hence populations of *Nucella* can experience varying levels of predation risk depending on local wave action regimes (Menge 1976, Freeman and Hamer 2009, Large et al. 2011, Tyler et al. 2014). The effects of wave action combined with inherent differences in predator densities result in the formation of distinct ecotypes of *Nucella*, with plasticity present in both morphological and behavioural antipredator traits (Crothers 1983, Rolán et al. 2004, Guerra-Varela et al. 2009, Large and Smee 2013).

### Site selection and predator numbers

Our field study was conducted at six sites, 3 wave-exposed and 3 wave-sheltered, around the coasts of Anglesey, North Wales, UK (Fig. 2.1). Sites were initially selected as wave-sheltered or wave-exposed based on community structure (Ballantine 1961) and later exposure was calculated using the map-based method to estimate mean wave fetch for each site (Burrows et al. 2008a). In order to estimate differences in crab abundance, baited crab traps (60 x 40 x 35 cm, with 500g of fish) were used at each of the 6 sites. For each site, crab numbers per trap were counted on three consecutive days. A single baited crab trap was placed in the midzone and left for two complete tidal cycles, sampled and then redeployed a further two times, each time deploying ~30 m along the shore. This allowed us to average crab numbers across the three days for each site to provide a mean crab number per trap per site.



**Figure 2.1** *Map of study sites across the Isle of Anglesey, Wales, UK. Open circles = Exposed sites, closed circles = sheltered sites.*

### Field measurements

We compared field oxygen consumption rates of two size classes of *Nucella* from exposed (low predator abundance) and sheltered (high predator abundance) shores with and without predator cue. Small *Nucella*, considered vulnerable to predation (*N* = 7 at each site, mean shell length 14.6 ± SD 1.3 mm) and large, considered invulnerable (*N* = 7 at each site, mean shell length 29.0 ± SD 1.6 mm) were collected from the same tidal height to control for any unknown shore-level size gradients (Elner and Hughes 1978). Field measurements were conducted between 11.00 and 17.00 on 4-8 October 2017, apart from one sheltered shore which had to be completed during the next tidal cycle (17 October 2017) due to adverse weather conditions. Animals were collected before being exposed to air, as the tide was receding, and subsequently were kept submerged to avoid any potential impacts on oxygen consumption rates (Stickle et al. 1985, McMahon 1988). Care was taken not to select individuals that were actively feeding. Individual *Nucella* were carefully placed into closed system respirometers (70 x 70 x 50 mm) containing fully aerated filtered seawater to determine oxygen consumption rates. All water used in field measurements was sourced from the laboratories at the School of Ocean Sciences, Bangor University, Menai Bridge, Wales. Changes in water oxygen partial pressure (PO2) were measured using an optical fluorescence technique (PreSense, Fibox 4 trace, Fiber optic Trace Oxygen Meter). Each respirometer was equipped with a single oxygen sensor spot (PreSense) located on the inside of the lid, which allowed for non-intrusive measurement of seawater PO2 levels at regular intervals. The seawater was filtered (0.45 μm) to reduce contaminating effects of biological activity from microbes and algae, and two controls consisting of respirometers without snails were included during each trial (*N*=16). Controls for filtered sea water and crab effluent were used to assess any background (microbial) oxygen consumption rates and this was then subtracted from all other measurements in that trial. Sea surface temperature was measured at each site at the beginning of each experiment and respirometers were placed into temperature-controlled water baths to ensure that *in situ* temperatures were maintained throughout the period of oxygen consumption measurement (mean14.18 ± SD0.06 oC across all sites) to prevent temperature-related changes in metabolic rates (Dahlhoff et al. 2008).

Preliminary trials showed that rates of oxygen consumption were initially elevated when *Nucella* were first placed in the respirometers due to handling stress, but levels fell over the next 25 minutes as snails settled in the respirometers. Over the next 45 minutes PO2 levels fell within the respirometers in a linear fashion which we considered to be routine rates of oxygen consumption as the snails were free to move around within the respirometers. Several studies have shown that exposure to crab effluent influences *Nucella* behaviour and therefore the use of routine rates of oxygen uptake are more appropriate when determining the natural reaction of *Nucella* to the detection of a predator (including the effects of movement). Based on these initial observations, individual snails were inserted into their respiration chambers, sealed and left for 25 mins before the initial PO2 reading was taken. Repeated PO2 readings were then taken every 5 min for the next 45 min to determine the linear fall in PO2 over time. Snails therefore spent 70 min in total in their respective respirometers. In each case, care was taken to avoid hypoxia from developing within the respirometers by ensuring that PO2 levels remained above 17 kPa throughout this period. Rates of oxygen consumption were determined from the drop in PO2 over 45 min by linear regression, minus the background fall in PO2 from the respective controls. This value was multiplied by the solubility coefficient for oxygen adjusted for salinity and temperature to give whole-body values in ml O2/h. Values were corrected to STPD and expressed as umol O2/h.

Once a baseline oxygen consumption rate had been established for each individual, they were then subjected to the predation risk treatment. Each individual that had been monitored for baseline oxygen consumption was exposed to predation risk by exchanging the water in the respirometer for water treated through exposure to crabs. This ‘predation risk’ water was created in the field by adding 8-10 large male crabs (mean carapace length ± SD, 56.6 ± 4.8 mm) to 20 L of filtered and aerated water for 1 hour. Care was taken to keep *Nucella* submerged at all times during the water change. *Nucella* were then allowed a further 25 minutes to acclimatise to the new treatment. Once measurements were complete, all *Nucella* were marked and returned to the laboratory in order to assess their oxygen consumption rates and behavioural responses to predation risk under controlled laboratory conditions.

### Laboratory measurements

Individuals collected from the field were housed in a temperature-controlled aquarium at similar temperatures to those in the field (mean 13.9 ± SD 0.9 oC) in fully aerated, recirculated, natural seawater for one month prior to running the second experiment. *Nucella* were not exposed to predation risk during this period. They were fed mussels and barnacles *ad libitum* and then starved for 48 hours prior to taking oxygen consumption measurements, in order to standardise digestive state (Matassa and Trussell 2014). Laboratory measurements followed the same protocol as the field experiment with routine oxygen consumption rates being established prior to measuring them under predation risk conditions. Water temperature was maintained at the respective *in situ* temperatures. After 25 minutes acclimatisation, oxygen consumption rates were measured every 5 minutes for 45 minutes. As with the field experiment, the same individuals were then exposed to crab effluent and their oxygen consumption rates were measured.

To determine whether the two size classes of *Nucella* demonstrate typical antipredator responses (cessation of movement, Freeman, Dernbach, Marcos, & Koob, 2014; Vadas et al., 1994), *Nucella* were observed for 20s every 5 minutes, over a total period of 45 mins, and their precise location noted on a diagram of the respirometer following the approach of Large et al. (2011). Care was taken not to cast a shadow over the respiration chambers to reduce any potential impacts on *Nucella* behaviour. As the exact movement of individuals between 5-minute increments could not be known, we used the shortest possible distance between two consecutive increments for our calculations. By combining the distance travelled between all increments, we were able to estimate the total distance travelled during the experiment.

### Statistical analysis

As the focus of this study was on comparing the effect of predation risk on oxygen consumption rates of vulnerable and invulnerable prey and not directly on the effects of size, analysis was conducted on whole animal rates of oxygen consumption (Packard and Boardman 1999, Hayes 2001, Dahlhoff et al. 2008). To assess the potential impact of predation risk on the oxygen consumption rates of vulnerable and invulnerable prey both in the field and in the laboratory, we used a model selection approach using Gamma distributed Generalized Linear Mixed Models (GLMMs). In both analyses, we used whole animal oxygen consumption rates as a response variable and wave-exposure (exposed and sheltered), *Nucella* vulnerability (vulnerable and invulnerable) and predation risk treatment (No predator cue and predator cue) as fully crossed fixed effects. Log transformed total length was used as a covariate to account for any potential size related differences within the size ranges selected. We considered site as being nested within wave-exposure and *Nucella* ID as a repeated measure (both treated as random effects). We also performed a GLMM with *Nucella* movement as a response variable using the same fixed, nested and repeated terms effects as in previous analyses. The movement results showed over-dispersion and were therefore analysed using a negative binomial GLMM. We used the glmm.TMB package to analyse movement results (Brooks et al. 2017).

All GLMMs were constructed and compared in R 3.5.0 (R Core Team 2013) using the *lme4* package (Bates et al. 2015). Backward model selection was achieved using the drop1 function and models with the lowest AIC were selected (Bolker et al. 2008). When interaction terms were significant at the α = 0.05 level, Tukey *post hoc* tests were carried out using the *emmeans* package (Lenth et al. 2004).

## Results

### Field results

Exposure calculations (measured as average wave fetch per site) using the Burrows et al. (2008) map-based method concurred with our assessment of exposure with sheltered and exposed shores having a mean wave fetch of 17.89 km (± 7.77 SD) and 49.55 km (± 10.09 SD) respectively. These values agree with Burrows et al. (2008) assessment of wave-sheltered and wave-exposed shores which they defined as being 20-40 km and 40-60 km respectively. There was a significant effect of wave exposure on crab numbers per trap (ANOVA: *F*1,11 = 7.42, *P* = 0.016, Table 2.1), with wave-exposed sites having an average of 0.89 ± 0.42 crabs per trap compared with wave-sheltered sites which had an average of 41.22 ± 5.81 crabs per trap (Fig. 2.2). The combination of wave exposure effects on the perception of kairomones and the stark difference in predator densities indicate that *Nucella* at each exposure were exposed to different levels of predation risk.

**Table 2.1** *Wave fetch indices for exposed and sheltered sites using the Burrows et al., 2008 map based method using 32 angular sectors and mean crab number per trap per site.*

Site Wave exposure Mean Fetch (km) Mean Crab Number (± SE)

Moelfre Sheltered 11.71 54.66 (10.7)

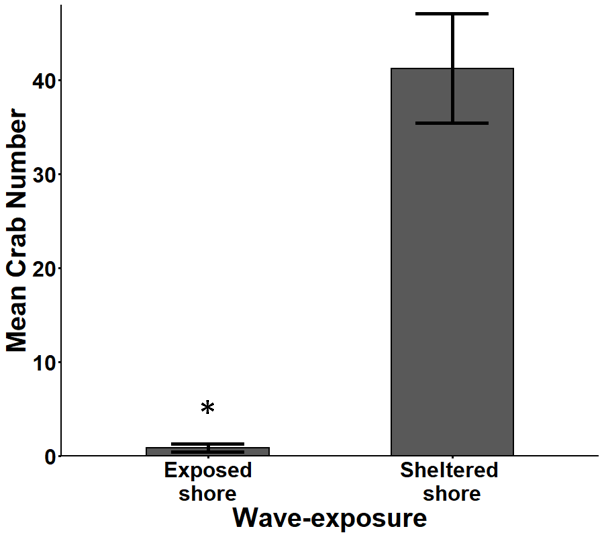
Porth Cwefan Sheltered 26.62 45.66 (4.48)

Bull Bay Sheltered 15.37 23.33 (3.17)

Point Lynas Exposed 55.10 0.00 (0.00)

Cemlyn Bay Exposed 55.64 1.33 (0.88)

Trearddur Bay Exposed 37.89 1.33 (0.88)



**Figure 2.2** *Mean number (± SE) C. maenas caught per trap for exposed and sheltered shores, t-test was used to compare means. Significant differences are indicated using (\*) at  < 0.01.*

The addition of *Nucella* shell length to all models tested had no impact on the AIC calculated and it was therefore not included in further analysis. Model selection results for our field and laboratory measurements are summarised in Table 2.2. Background respiration rates were 0.52 µmol O2/h across treatments. Oxygen consumption rates of vulnerable *Nucella* (mean 3.60 µmol O2/h, SE = 0.25) were lower than those of invulnerable individuals (mean = 16.65 µmol/l/h, SE = 0.85) across all exposure gradients and treatments (gamma distributed GLMM: *N* = 65, *P* < 0.001, Fig. 2.3). GLMM analysis showed that the two size classes of *Nucella* reacted differently when exposed to crab effluent (gamma distributed GLMM: *N* = 65,, *P* = 0.003). Further *post hoc* analysis showed that in the presence of predation risk, invulnerable *Nucella* reduced their respiration rates by 36.2%, (Table 2.3, Tukey pairwise comparison: *P* < 0.001), whereas vulnerable individuals showed a slight non-significant increase in oxygen consumption rates of 6.01% (Table 2.3, Tukey pairwise comparison: *P* = 0.914). This pattern was consistent across both exposure levels (gamma distributed GLMM: *N* = 65,, *P* = 0.49, Fig. 2.3).

**Table 2.2** *Field and laboratory model selection scores results for the potential influence of predation risk on wave exposed and wave sheltered population of dogwhelks (Nucella lapillus).*

**Terms removed df Field Laboratory Movement**

3-way factorial 11 700.52 619.47 1478.19

2-way interaction E\*T\*S 10 700.52 617.67 1476.61

T:S 9 706.07 619.47 1486.37

E:S 9 696.98 629.18 1478.99

E:T 9 696.98 615.74 **1475.10\***

Single 2-way interaction E:S + T:S 8 704.20 627.26 1485.34

E:T + T:S 8 705.28 629.54 1488.90

E:T + E:S 8 **696.58\* 616.86\*** 1478.19

Fixed terms E:T + E:S + T:S 7 703.37 627.60 1487.41

T 6 717.00 643.29 1500.16

S 6 766.94 688.82 1485.59

E 6 701.87 627.26 1490.31

Single Fixed terms T + S 5 785.58 704.94 1499.03

E + S 5 715.65 642.90 1503.99

E + T 5 765.00 687.03 1488.46

Random Factor Site 10 699.21 617.67 1476.19

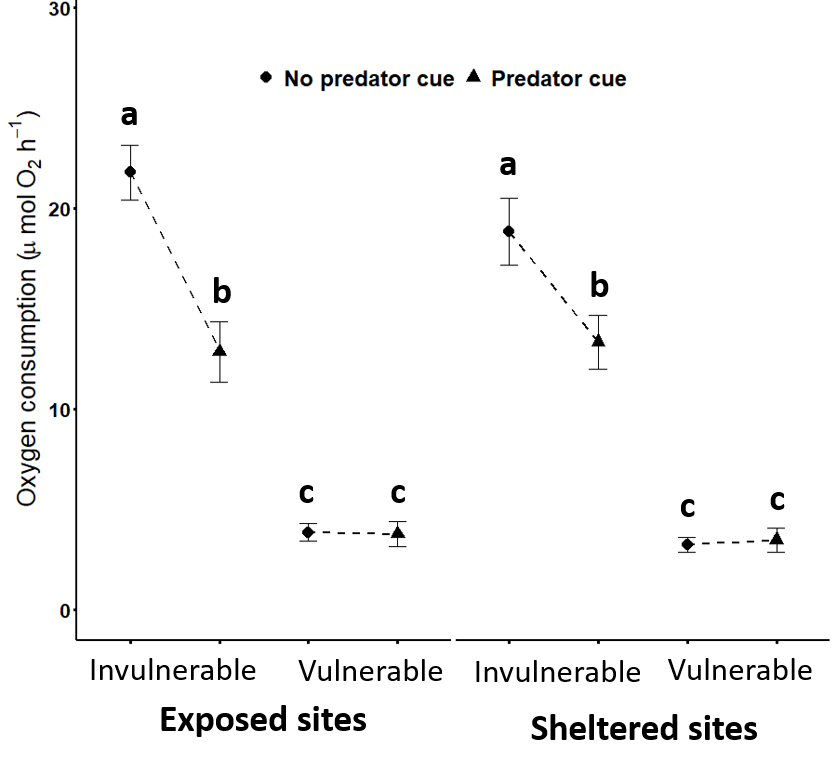
*Best Model Field = Risk Treat\*Size, Laboratory = Risk Treat\*Size,*

*Movement = Risk Treat\*Size + Wave-Exposure\* Size*

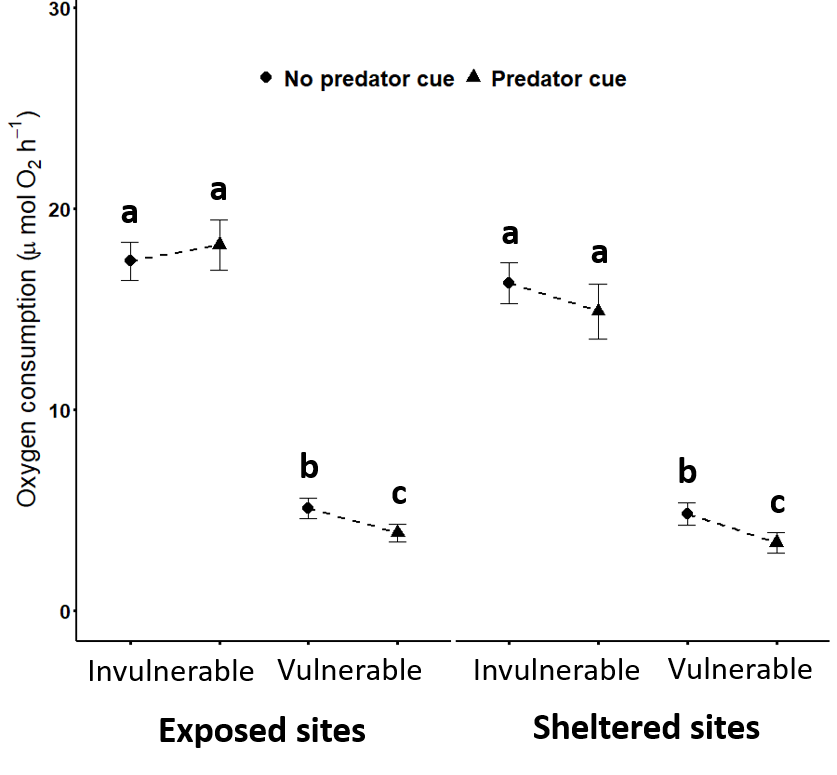
*Abbreviations: E = Wave-Exposure, T = Risk Treatment, S = Size (vulnerability). Models selected using generalised linear square models and Akaike Information Criteria (AICc) for all dependent variables. Selected models are highlighted in bold with an (\*).*

### Laboratory results

Overall laboratory background respiration rates contributed in control respirometers were 0.30 µmol/O2/h. As with the field results, in the laboratory, invulnerable *Nucella* had higher oxygen consumption rates than vulnerable individuals (gamma distributed GLMM: *N* = 65,, *P* < 0.001). However, under laboratory conditions although there was an interaction between treatment and size (gamma distributed GLMM: *N* = 65,, *P* < 0.001), it was vulnerable *Nucella* that showed a reduction in respiration rates by 26.8% under predator risk (Table 2.3, Tukey pairwise comparison: *P* < 0.001), whereas respiration of invulnerable individuals did not differ between treatments (Fig. 2.4**,** Tukey pairwise comparison: *P* = 0.64). Once again, differences between size classes were the same irrespective of exposure level (gamma distributed GLMM: *N* = 65,, *p* = 0.169).

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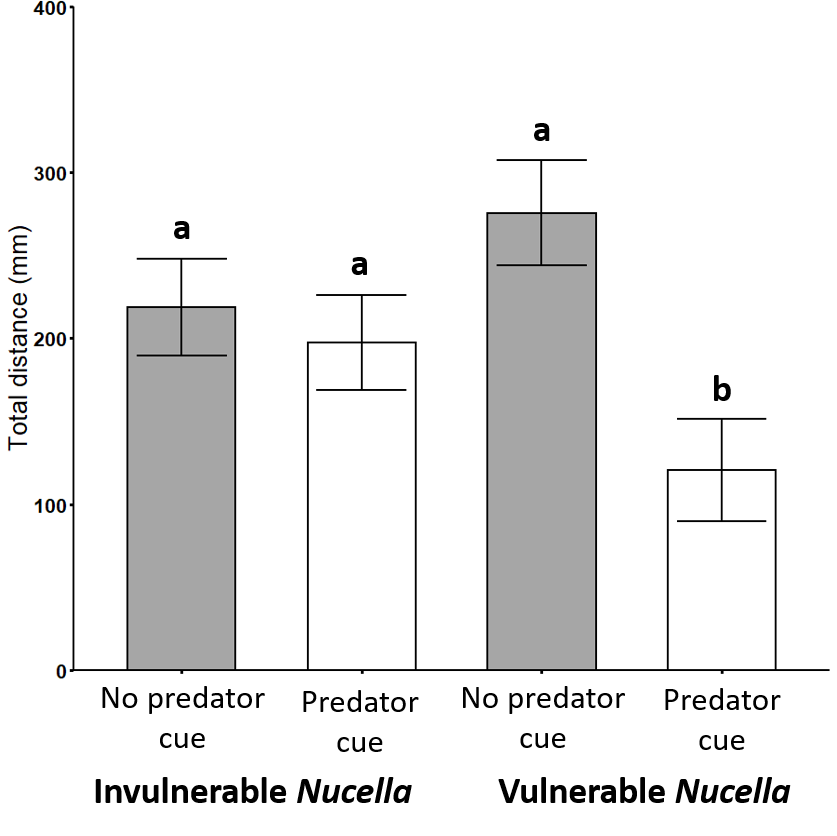
**Figure 2.3** *Field oxygen consumption rates of vulnerable and invulnerable dogwhelks (Nucella lapillus) from sheltered and exposed shores in filtered seawater (No Predator Cue) and under predation risk (Predator Cue).**Letters**indicate results from the Post hoc Tukey’s HSD and bars represent ± SE among sites.*



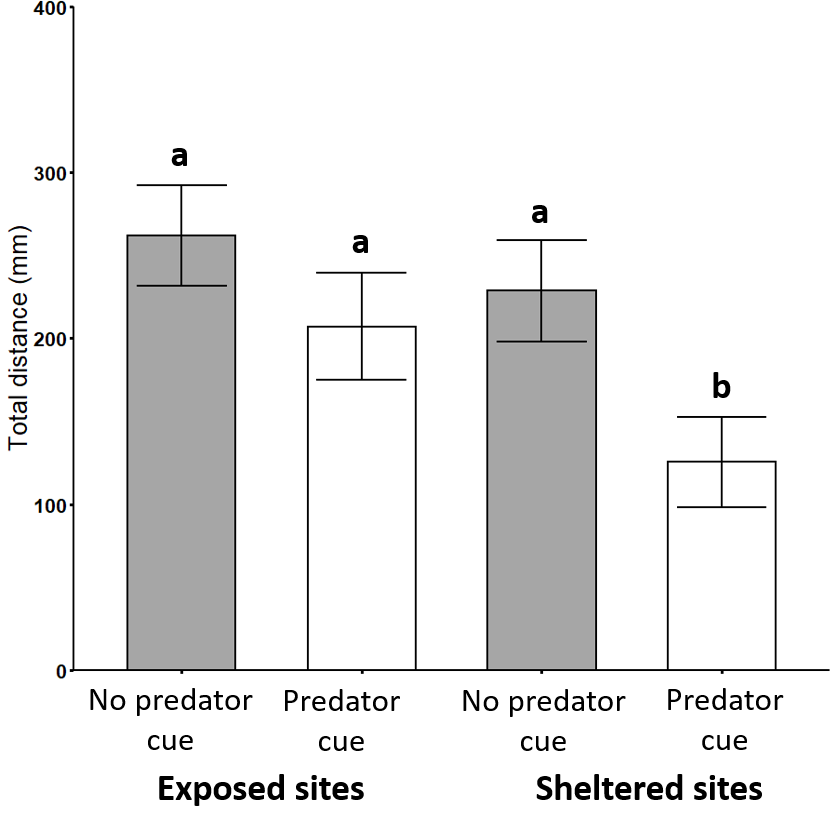
**Figure 2.4** *Laboratory oxygen consumption rates of vulnerable and invulnerable dogwhelks (Nucella lapillus) from sheltered and exposed shores in filtered seawater (No Predator Cue) and under predation risk (Predator Cue). Letters indicate results from the Post hoc Tukey’s HSD and bars represent ± SE among sites.*

### Laboratory behavioural responses

The detection of a predatory cue affected the behaviour of *Nucella*, and its impact was influenced by both prey vulnerability as well as wave-exposure level (Fig. 2.5). Overall, vulnerable *Nucella* reduced their movement in the presence of a predatory cue, whereas invulnerable *Nucella* did not show a reduction (negative binomial GLMM: *N* = 65,, *P* < 0.001). *Post hoc* testing revealed that both size classes moved similar distances in filtered sea water (Fig. 2.5) but, in crab effluent vulnerable *Nucella* reduced their movement (Table 2.3, Tukey pairwise comparison: *P* < 0.001), whereas invulnerable individuals did not (Table 2.3, Tukey pairwise comparison: *P* = 0.715). Under predation risk conditions, there was an overall effect of wave-exposure on *Nucella* behaviour (Fig. 2.6, negative binomial GLMM*:* *N* = 65,, *P* = 0.03). *Nucella* from sheltered shores, naturally exposed to higher ambient levels of predation risk reduced their movement when exposed to crab effluent (Table 2.3, Tukey pairwise comparison: *P* <0.001), whereas *Nucella* from wave-exposed shores remained active (Table 2.3, Tukey pairwise comparison: *P* = 0.157).



**Figure 2.5** *Movement of A) vulnerable and B) invulnerable dogwhelks (Nucella lapillus) from sheltered and exposed shores in filtered seawater (black bars) and under predation risk**(grey bars). Letters**indicate results from the Post hoc Tukey’s HSD. Bars represent ± SE among sites.*



**Figure 2.6** *Combined movement of all sizes classes of dogwhelks (Nucella lapillus) from sheltered and exposed shores in filtered seawater (grey bars) and under predation risk (white bars).**Letters**indicate results from the Post hoc Tukey’s HSD. Bars represent ± SE among sites.*

## Discussion

Short-term metabolic responses of invertebrate prey to predation risk and the resulting antipredator behaviour are not well understood (Canero and Hermitte 2014, Mitchell et al. 2017), and the influence of individual vulnerability even less so (DeWitt et al. 1999). Exposing *Nucella* to a predatory cue affected their routine MR as well as their antipredator behaviour, but each response was influenced differently by individual vulnerability and wave-exposure. When vulnerable and invulnerable *Nucella* were exposed to a predatory cue in the field*,* moments after being collected, it was the invulnerable size class which reduced its oxygen consumption rate, as opposed to vulnerable ones. It is important to note that *Nucella* were not tethered while in the respiration chamber as our intention was to capture the total oxygen consumption related to the detection and subsequent short-term response to predation risk. Therefore, our results do not allow us to distinguish between a potential physiological response to predation risk and the metabolic cost of the resulting behavioural response. Changes in oxygen consumption rates are therefore a combination of stress induced changes in metabolic rate as well as behavioural changes. Notwithstanding, our results clearly indicate that predation risk may still influence prey which are otherwise safe from direct predation by specific predators. In addition, through making comparisons of oxygen consumption in the field, in individuals extracted directly from natural conditions, with those maintained in the laboratory, we also show important differences in response. Our results and particularly the differences between the field and laboratory observations are explained in relation to size related risk taking as well as through potential changes in *Nucella* physiological and energetic state.

### Oxygen consumption in vulnerable *Nucella*

When vulnerable *Nucella* were exposed to a predatory cue in the field, there was no change in oxygen consumption when compared to respiration in filtered sea water alone. Previous laboratory based studies show a cessation of movement in the presence of a predatory cue (Vadas et al. 1994, Large and Smee 2010). However, our field-based results, when framed from the perspective of foraging-risk trade-off, may be explained by Clark’s Asset Protection Principle (APP, Clark, 1994). The APP asserts that foraging decisions relate to the relative amount an individual stands to lose or gain from foraging at a particular time. The APP argues that for a given amount of energy to be gained from foraging at a specific time, smaller individuals have less to lose (less already invested in growth) and proportionally more to gain, than a larger individual. In this context, smaller individuals should forage under risky conditions if the potential energy gain is high enough. In essence, it is more favourable for smaller prey to forage during risky periods compared to larger individuals. Thus, vulnerable *Nucella* under predation risk conditions are likely to continue to search for food due to the high fitness gains, resulting in similar levels of oxygen consumption between our two treatments.

In the laboratory, where *Nucella* were fed *ad libitum* and did not experience risk cues or wave action for one month, oxygen consumption responses to risk differed to those in the field. Vulnerable *Nucella* reduced their routine metabolic rate as well as their movement when exposed to a predatory cue. These seemingly contradictory patterns between field and laboratory results may be explained in the context of the Risk Allocation Hypothesis (RAH). The RAH rests on the inextricable link between current energy reserves and decision making under risky conditions, meaning that behavioural changes are not a result of momentary trade-offs, but rather as forming part of an overarching foraging strategy (Mangel and Clark 1986, Lima and Dill 1990, Burrows and Hughes 1991a, Lima and Bednekoff 1999). In essence, low energy reserves force prey to forage irrespective of risk, whereas when energy reserves are high, prey are able to wait out risky periods in order to forage during more favourable ones (Lima and Dill 1990, Lima and Bednekoff 1999). One of the important predictions of the RAH is that if prey experience prolonged periods of safety interspersed with short high risk periods, then prey should stop foraging during the high risk periods (Lima 1998b). In the laboratory, *Nucella* experienced “safe” conditions, where they were able to increase energy reserves. Thus, when faced with predatory risk they reduced activity as predicted by the RAH which in turn was reflected in a reduction in oxygen consumption. When Matassa and Trussell (2014) tested the response of starved and satiated *Nucella* in the laboratory they found patterns which corroborate our field-lab comparisons. Satiated animals did not forage during risky periods whereas starved individuals were forced to forage even under high risk conditions.

### Oxygen consumption in invulnerable *Nucella*

Although vulnerable *Nucella* reacted predictably to predation risk in terms of the RAH, the pattern seen in the invulnerable adults was less clear. Under field conditions, invulnerable *Nucella* reduced their oxygen consumption rate in response to a predatory cue. Large invulnerable *Nucella* are much more likely than small vulnerable individuals to have sufficient energy reserves to be able to reduce activity in the presence of increased risk (Feare 1970). Thus, a reduction in oxygen consumption in the field may be a consequence of a reduction in movement. Although the exact mechanism underpinning this reduction in oxygen consumption is beyond the scope of this investigation (stress response and movement), these observations are important in showing that *Carcinus* is still able to affect *Nucella* even after they have reached a size refuge. This pattern changed after *Nucella* had been housed under “safe” conditions in the laboratory. When tested again in the laboratory, where we expected a similar pattern to that seen in the field (with satiated individuals reducing their respiration as well as their movement), there was no reduction in oxygen consumption or movement under predation risk condition. Clearly the complexity of behavioural and physiological changes in laboratory housed *Nucella* at size refuge warrants further study, as these size classes have a disproportionate influence on population dynamics.

### Antipredator behaviour

Antipredator behaviour was only assessed in the laboratory, and not in the field. Our observations showed an interesting influence of wave exposure (and hence prior experience of predatory threat) on the propensity for *Nucella* to adopt an antipredator behaviour. Wave-sheltered populations (which naturally experience higher predation risk) showed higher levels of antipredator behaviour (cessation of movement) than their wave-exposed counterparts. At the population level, comparisons of the effects of sympatric and allopatric predators on the behaviour of prey have shown that the influence of local ambient predation pressure informs antipredator behaviours (Rochette et al. 1997, 1999, Aschaffenburg 2008, Large and Smee 2013). In comparisons of antipredator behaviour of the common whelk (*Buccinum undatum),* from populations naturally exposed to different suites of predators, Rochette and Himmelman (1996) found that individuals adopt more appropriate antipredator behaviour to sympatric predators than allopatric ones. In *Nucella* investigations of wave-exposure effects on behaviour are conflicting. Large and Smee (2013) found that crabs caused a reduction in *Nucella* movement in both wave-sheltered and wave-exposed populations. By contrast, and in accordance with our own work, Freeman et al. (2014) showed lower levels of antipredator behaviour in more predator naïve populations from exposed shores.

### Potential ecological importance

The changes in behavioural and physiological traits of prey can have potentially cascading effects on the rest of the biological community. A predator’s influence on community dynamics is not limited to their regulation of prey densities (Density-Mediated Indirect Interactions - DMIIs), but also through their influence on prey physiological and behavioural traits (Trait-Mediated Indirect Interactions - TMIIs). Investigations into the potential strength of DMIIs compared to TMIIs between *Carcinus* and *Nucella* have shown that direct predation and predation risk exert similar influence on the community dynamics of this rocky shore food chain (Trussell et al. 2006a). Our results indicate that this may in fact be an underestimation of the importance of TMIIs as previous studies have only used small size classes of *Nucella* that have lower foraging rates than individuals at size refuge used in this study (Dunkin and Hughes 1984). The persistence of the indirect effects of *Carcinus* on *Nucella* even after a size refuge has been reached will mean that TMIIs persist for longer than DMIIs, increasing their ecological importance. The greater impact larger individuals have on resources combined with the protracted temporal scale at which prey are influenced by predation risk, may result in a considerable underestimation of the overall impacts predators have on an ecosystem.

## Conclusions

Our results provide insight into the effects of predators on prey of differing vulnerability and the potential for antipredator behaviours to be influenced by local environmental factors (wave exposure and hence presumed influence of predation risk). Importantly we have shown that the influence of a predator may be more far reaching than originally thought, as they are able to affect oxygen consumption of prey even after they are no longer susceptible to direct predation. Our contrasting results from field and laboratory experiments show clearly that understanding of prey state (e.g. physiological/energetic condition which is likely to change dependent on handling and husbandry) is critical in generating a holistic understanding of predator prey responses (e.g. Matassa & Trussell, 2014). Finally, although not consistent across all responses, we found an effect of wave exposure on antipredator behaviour (cessation of movement) indicating an effect of prior experience of predation risk. Further studies into the potential for predators to indirectly influence prey which are not at risk of direct predation will enable us to better appreciate the overall influence predators have on the ecosystem they inhabit.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Contrast** | | | | |  | **Combination** |  |  |  |
| **Experiment** | **Model** |  | **Factor** |  | **Levels** | | |  | **Vulnerability** | **Estimate** | **Z ratio** | ***P*** |
| Field O2 Consumption | O2 con ~ Vulnerability + Risk Treatment + Wave-exposure +  Vulnerability \* Risk Treatment +  (1|Site) + (1 | *Nucella* ID) |  | Risk Treatment |  | *No*  *Predator* | *vs.* | *Predator* |  | Vulnerable | -0.0608 | -0.609 | 0.914 |
|  |  |  |
|  |  |  | Invulnerable | -0.4573 | -5.290 | **<0.001** |
|  |  |  |
| Laboratory O2 Consumption | O2 con ~ Vulnerability + Risk Treatment + Wave-exposure +  Vulnerability \* Risk Treatment +  (1|Site) + (1 | *Nucella* ID) |  | Risk Treatment |  | *No*  *Predator* | *vs.* | *Predator* |  | Vulnerable | -0.3379 | -5.883 | **<0.001** |
|
|  |  |  | Invulnerable | -0.0555 | -1.094 | 0.644 |
|
| Laboratory Movement Analysis | Movement ~ Vulnerability + Risk Treatment + Wave-exposure +  Vulnerability \* Risk Treatment +  Wave-exposure \* Risk Treatment +  (1|Site) + (1 | *Nucella* ID) |  | Risk Treatment |  | *No*  *Predator* | *vs.* | *Predator* |  | Vulnerable | -1.6137 | -5.052 | **<0.001** |
|
|  |  |  | Invulnerable | -0.2426 | -0.984 | 0.715 |
|
|  |  |
|  | Risk Treatment |  | *No Predator* | *vs.* | *Predator* |  | **Wave- Exposure** |  |  |  |
|  |  |  |  |  |  |
|  |  |  | Sheltered | -1.3559 | -4.401 | **<0.001** |
|  |  |  | Exposed | -0.5004 | -1.982 | 0.157 |

**Table 2.3** *Post hoc* Tukey’s HSD test results for significant interactions from GLMMs conducted on field and laboratory experiments. Statistically significant models (*p* = 0.001) are in bold

# **Parental effects and individual experience of predation risk informs growth and the energy budget of prey Parental effects and individual experience of**

**CHAPTER THREE**

Parental effects and individual experience of

predation risk informs growth and the energy budget of prey

## Abstract

Predation risk influences how prey acquire and use energy, impacting energy reserves and growth, with potential implications for ecosystem functions. Prey must respond to both small-scale fluctuations in predation risk as well as adapting to larger-scale differences in the predation pressure experienced by separate populations. In many cases these larger-scale environmentally driven differences in predation risk can have a profound effect on the amount of energy prey invest in antipredator defences relative to that invested in growth. Furthermore, as the predation pressure faced by prey may be closely correlated with that of their parents, many prey species can influence the antipredator responses of their offspring through parental effects. Comparing the responses of prey to risk in various contexts will help us develop a deeper understanding of the full impact of predators on prey life history and community dynamics. I reared the offspring generation of intertidal gastropods (*Nucella lapillus*), from populations naturally exposed to different predation pressure, under different temporal patterns of risk throughout development. The shell length, relative investment in defensive structures (body Vs shell tissue) and long-term energetics of offspring were then analysed to determine the influence of parental habitat and developmental experience of risk. My results showed that shell length was influenced by parental habitat and developmental experience of risk, however *Nucella* from both populations allocated more resources to shell tissue relative to soft body tissue in response to the amount of time they spent under risky conditions. Although the overall the energetic budget of *Nucella*, was not influenced by developmental risk or parental habitat, analysis revealed that they play important roles in the proportion of energy stored as protein, carbohydrate and lipid. Our results highlight the impacts of individual experience of risk on growth, allocation of resources to defensive structures and the energetic budget of *Nucella* as well as the potential influence parental experience can have through parental effects.

## Introduction

The fear of being eaten profoundly influences prey behaviour, physiology, morphology and life history (Werner et al. 1983, Lima et al. 1985, Trussell et al. 2003, Hawlena and Schmitz 2010b). Predator-induced increases in metabolic costs and reductions in foraging are common observations in a number of taxa leading to subsequent impacts on growth (Dill 1987, Lima and Dill 1990). However more recently studies have begun to show that risk can have more profound effects on prey energetics. For short-term exposure to predation risk, prey are able to rely on previously acquired energy reserves, but in some predator-prey systems extended exposure to risk has been shown to impact the body stoichiometry of prey (Van Dievel et al. 2016, 2019a, Tigreros et al. 2018). Predation risk has been shown, in some instances, to influence the amount of energy stored as protein, carbohydrate and lipids which can influence future foraging predation risk trade-offs (Tigreros et al. 2018). The long-term exposure of the damselfly *Enallagma cyathigerum*, to predatory cues results in changes to the C:N ratio of body tisues (Van Dievel et al. 2016) which can influence nutrient cycling and ultimately affect the level of production (Van Dievel et al. 2019a). As the risk posed by predators can modulate the acquisition and utilisation of energy (Trussell et al. 2006b, Thaler et al. 2012, Van Dievel et al. 2016), how prey balance their energy budgets with their experience of risk will affect their overall lifetime fitness (Lima and Bednekoff 1999, Ferrari et al. 2009, Trussell et al. 2011) as well as impacting ecosystem functions (Trussell et al. 2006a, 2017, Hawlena and Zaguri 2016, Van Dievel et al. 2016).

While the threat of predation is often examined through relatively simple experiments which consider risk as either present or absent, such studies are prone to exaggerate or neglect the indirect effects of predation risk (Ferrari and Chivers 2009, Trussell et al. 2011). In nature, environmental conditions change, predators move and prey increase in body size; therefore, predation risk is neither constant nor predictable, thus perception of risk can vary through time in a complex manner (Lima and Bednekoff 1999, Sih et al. 2000, Ferrari et al. 2008b). Initially theoretical, and increasingly empirical studies are showing that manipulations of temporal patterns of risk, provide a more realistic test of the effects of predation risk on prey (Lima and Bednekoff 1999, Ferrari et al. 2008b, 2009). Furthermore, recent investigations have also shown that individual variation in behaviour is also driven by environmental and temporal pattern of risk (Cornwell et al. 2019, Jolles et al. 2020). Specifically, time of day (Ferrari et al. 2008a), frequency (Sih and McCarthy 2002, Ferrari et al. 2008b), predictability (Trussell et al. 2011, Matassa and Trussell 2014), as well as the relative duration of exposure to predation risk (Van Dievel et al. 2016) have all been shown to elicit different responses in prey. For example, tadpoles (*Rana sylvatica*) reduce their movement under risky conditions, but the magnitude of this antipredator response is sensitive to the time of day, as well as the level of perceived threat (Ferrari et al. 2008a, Ferrari and Chivers 2009). Similarly, energy acquisition (foraging rates) and anti-predator escape behaviours in the freshwater snail, *Physa acuta,* appear to be directly influenced by fluctuating patterns of predation risk from predatory crayfish (*Procambarus* spp, Kain & McCoy, 2016). Thus, further highlighting the potential importance of experimental manipulations examining temporal patterns of risk, which may provide more reliable estimates of risk response in prey organisms.

Although individual experience of risk will undoubtably have a profound influence on the acquisition and utilisation of energy in prey, in many systems experience of risk of one generation can also influence the antipredator traits of subsequent ones (Mousseau and Fox 1998, Agrawal et al. 1999). Chronic parental exposure to predation risk can reduce the number and quality of offspring (Mcghee et al. 2012) and modify fitness through changes in behaviour (Storm and Lima 2010, Mcghee et al. 2012, O’Brien et al. 2017) and their physiological responses to predation risk (Donelan and Trussell 2018a, 2018b, 2019). For example, offspring of gravid female field crickets (*Gryllus pennsylvanicus*) previously exposed to cues from the wolf spider *Hogna helluo,* exhibit elevated antipredator responses compared with offspring of unexposed females (Storm and Lima 2010). These results revealed higher survival rates in parentally informed offspring than the corresponding uninformed juvenile crickets and were later confirmed in field populations, with local predation pressure by wolf spiders correlating with antipredator responses in field crickets. In addition, this type of parental effect can be seen in the post-consumptive processes in some prey species. Donelan and Trussell (2018b) have shown, for example, that parental experience with risk can influence the long-term growth efficiency of the intertidal snail, *Nucella lapillus*. Parental pairs of snails exposed to predation risk produce offspring with higher growth efficiencies than those of unexposed parents (Donelan and Trussell 2018b). It is, therefore, important to appreciate the potential influence that parental experience may have on the behaviour and physiology of their offspring when exposed to predators.

Here we address the question of how experience of risk by offspring may be modified by parental experience and how those experiences affect the growth and long-term energetics of offspring. Using an intertidal (dogwhelk *-* crab) predator-prey system, we combine parental experience of risk with manipulations of developmental experience (i.e. that occurring throughout the development of offspring). In considering how parental experience can influence life-history traits of distinct populations, it is necessary to consider the experiences of individuals at larger temporal and spatial scales. Variability in predation risk tends to decrease over larger spatial scales (Luttbeg and Sih 2004, Hammond et al. 2007), as differences in environmental conditions between populations begin to play a larger role in species interactions (Paine 1976, Menge 1978b, Menge et al. 1987, Menge and Olson 1990, Moody and Aronson 2007). For instance, in intertidal habitats, as abiotic factors become more extreme, mobile predators become less abundant and efficient, and hence the overall predation pressure experienced by prey is lower (Moody and Aronson 2007, Freeman and Hamer 2009). In the rocky intertidal, predation pressure by crabs (*Carcinus maenas*;hereafter *Carcinus*) on dogwhelk (*Nucella lapillus*;hereafter *Nucella*) is higher in sheltered shores as compared to those exposed to wave action (Hughes and Elner 1979, Large and Smee 2013, Karythis et al. 2020). We therefore collected adult dogwhelks from populations occurring in wave-exposed and wave-sheltered shores and allowed them to breed in the laboratory. After measuring their size of emergence, offspring were then exposed to both predictable and unpredictable patterns of predation risk throughout their development. We then analysed and compared the growth (shell length, body size and relative commitment of resources to defence) and energetic budget (energy reserves and energy consumption) of our offspring generation of *Nucella* reared under three risk levels (no risk, constant risk and variable risk). Energetic budgets were compared using the Cellular Energy Allocation (CEA) technique (i.e. a ratio of energy reserves and energy usage, De Coen and Janssen 1997) which provides a measure of the cellular energy budget of *Nucella*. It was anticipated that the size at which *Nucella* offspring emerged would differ for wave-exposed and wave-sheltered offspring (Donelan and Trussell 2018a) and that their growth will be negatively affected by predation risk experienced during development (Trussell et al. 2006b). Finally, we expected that exposure to predation risk during development would influence the energetic budget of *Nucella*, resulting in decreases in the CEA (Tigreros et al. 2018, Van Dievel et al. 2019b).

## Methods

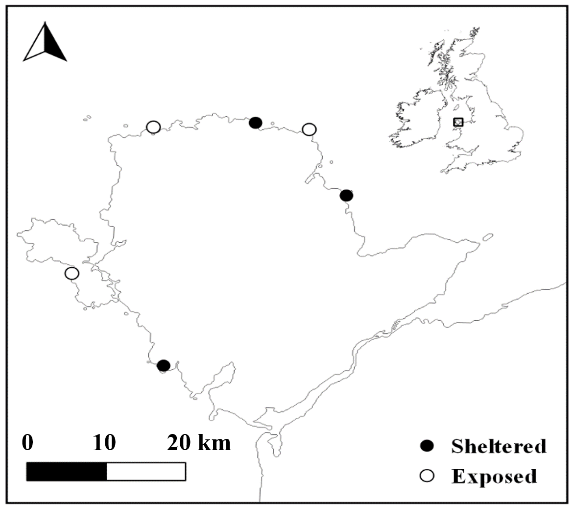
### Study System and general approach

The intertidal gastropod *Nucella lapillus* and its crab predator *Carcinus maenas* co-occur in rocky shores along a gradient of wave-exposure across northern Europe (Crothers 1985) and have been used extensively in the study of the indirect effects of predation on prey (Vadas et al. 1994, Trussell et al. 2002, 2017, Aschaffenburg 2008, Matassa and Trussell 2014). Previous findings have shown that once *Nucella* detects *Carcinus*, they reduce movement (Vadas et al. 1994), suspend foraging (Matassa and Trussell 2014) and increase their use of refuges (Matassa et al. 2016). Prolonged exposure to *Carcinus* results in predator-induced morphological changes (Hughes and Elner 1979, Appleton and Palmer 1988), as well as negatively impacting post-consumptive efficiencies as well (Trussell et al. 2006b, 2011). Moreover, *Carcinus* can have sustained influence on the behaviour and physiology of *Nucella* even after a brief exposure during their early stages of development (Pascoal et al. 2012b, Donelan and Trussell 2018a, 2019).

We used *Nucella* from two distinct intertidal environments; wave-exposed and wave-sheltered shores (Fig. 3.1), where it is expected that individuals are exposed to two different predatory regimes. It is well established that *Nucella* respond to local hydrodynamic regimes and differences in the intensity of crab predation through investment in morphological defences, mainly through changes in the rate of deposition of shell material (Appleton and Palmer 1988, Palmer 1990). Crab density and activity is reduced at sites of high wave-exposure (Hughes and Elner 1979, Menge 1983, Robinson et al. 2011, Large and Smee 2013). In addition kairomone plumes inadvertently released by *Carcinus* are homogenised throughout the water column in exposed environments reducing their reliability as predator cues (Weissburg et al. 2002a). Thus, *Nucella* are likely to perceive a reduction in ambient predation pressure as wave-exposure increases(Large and Smee 2013; Donelan and Trussell 2019; Karythis et al. 2020)*. Nucella* collected from three wave-exposed and three wave-sheltered shores were allowed to breed naturally in laboratory mesocosms under no predatory risk. We then explored how the progeny of these individuals (from the egg capsule stage) responded to different temporal patterns of risk. Offspring of wave-exposed and wave-sheltered populations were reared under three predator risk regimes; no risk, constant risk and variable risk. After eight months (just prior to reaching reproductive maturity, Crothers 1985) individuals across all treatments were measured to determine impacts on growth. They were then frozen (-80°C ) prior to analysis of cellular energy budgets.

### *Nucella* collection and breeding

Animals were collected in December 2017 from sites around the coast of Anglesey, North Wales, UK (Fig. 3.1). Previous work has demonstrated clear differences in wave exposure based on average wave fetch (Burrows et al. 2008a) between exposed and sheltered sites. This difference in exposure to wave action has been shown to result in very clear differences in crab abundance and hence predation pressure at the selected sites (Karythis et al. 2020). Observed crab abundances based on intertidal traps were 40 times lower at wave exposed (0.89 ± 1.27 SD crabs per trap) compared with wave sheltered sites (41.22 ± 17.43 SD crabs per trap).



***Figure 3.1*** *Map of study sites across the Isle of Anglesey, Wales, UK. Open circles = Exposed sites, closed circles = sheltered sites.*

*Nucella* breed throughout the year, but peaks in early spring in North Wales (Crothers 1985, Morton 2009). They are internal fertilisers and reproduce by forming breeding aggregations. Egg capsules clutches are then deposited (100s per aggregation) in crevices and on the underside of rocks, with each egg capsule containing between 5-30 fertilised embryos (Pechenik et al. 1984). Unsexed adult *Nucella* (150 – 200 individual) were collected from each site, placed in site-specific breeding tanks (transparent plastic 1 x 1 x 0.6 m tubs) and provided with barnacle covered rocks as a food source *ad libitum* for a period of 4 months (Dec 2017-March 2018). In March 2018, over a 2-week period *Nucella* from all 6 sites formed breeding aggregations and deposited egg masses on the walls of the tanks and the undersides of rocks. Egg capsules were carefully removed from the tanks using a scalpel and 8 capsules were transferred to each replicate mesocosms where they were exposed to different predation risk regimes. Egg capsules were checked every two days and upon emergence (~ six weeks), a subsample of hatchlings were used to estimate offspring emergence size of each treatment combination. Offspring were provided with food and refuge, i.e. with rocks covered in newly recruited barnacles, followed by progressively larger barnacles as they grew, and held under the various treatments for eight months. Due to the large numbers and the diminutive size of hatchlings the monitoring of mortality rates was unfeasible. Tanks were housed in a temperature-controlled room, in a flow through system reflecting ambient water temperatures and on a 12:12 light dark cycle.

### Risk Manipulation

Risk was manipulated by placing a large male crab (mean carapace length ± SD = 56.6 ± 4.8 mm) into a perforated “crab pot” (500 ml), that was then placed in each mesocosm. For the no risk regime, crab pots remained empty for the entire eight-month period. Crab pots for the constant risk treatment contained a crab for the entirety of the experiment, with crabs being used for a maximum of four days before being replaced. In the variable risk regime, *Nucella* were exposed to a predatory cue for 50% of the time with a crab being added or removed from the crab pot every three or four days. The combination of offspring from 6 shores (3 wave-exposed and 3 wave-sheltered) and 3 predator treatments resulted in 18 treatment combinations (n = 6 replicates for each treatment combination).

### Data collection

#### Growth

Within two days of hatching, images were captured and used to measure the maximum shell length (±0.01 mm) of hatchlings as an estimate of size at emergence (ImageJ image analysis, Abramoff et al. 2004). A total of 422 hatchlings were measured across all treatment combinations, although sampling between treatments was not even (wave-exposed = 165, wave-sheltered = 262, see table S1 for *n* per treatment combination). At the end of the experiment the shell lengths of individuals (total n = 830) were measured again using vernier calipers (±0.1 mm). However, although previously used as a proxy for growth in other gastropods (Trussell et al. 2017), total shell length has been shown to be a poor predictor of tissue growth in *Nucella* *lapillus* (Palmer 1990, Pascoal et al. 2012a), as shell shapes and thickness vary greatly depending on local biotic and abiotic conditions. We therefore compared the tissue and shell mass of a subsample of *Nucella* from each treatment combination. Comparisons could then be made of shell mass per unit tissue mass which provides a reliable index for the relative commitment of resources to shell defence (Palmer 1990). Wet body weight and shell weight were measured by carefully cracking and removing *Nucella* shells, blotting dry before weighing the shell and tissue separately (±0.001 g).

#### Energetic state analysis

To determine the energy budgets of *Nucella* offspring, we used the Cellular Energy Allocation (CEA) technique. This assay has been used previously to investigate seasonal and environmentally driven changes in energy reserves as well as to test the effects of long-term exposure to abiotic (Beyer et al. 2013, De Troch et al. 2013, Van Dievel et al. 2019b) and biotic factors (De Troch et al. 2013) including predation (Tigreros et al. 2018, Van Dievel et al. 2019b). CEA analysis was conducted on whole-animal samples and is defined as the ratio of energy reserves available (Ea, measured as the combined energy stored in total proteins, lipids and carbohydrates) to energy consumption (Ec, determined through the activity of the Electron Transport System in the mitochondria). Bioassays followed the protocol used by De Coen & Janssen (1997) with slight modifications described below.

Owing to the small body size of *Nucella* and the amount of tissue needed for the complete CEA analysis, samples consisted of the whole-body homogenates of two *Nucella* individuals combined. The original 6 replicate mesocosms of each treatment combination were therefore aggregated into 3 pairs at random and only *Nucella* from pairs of mesocosms were combined. Frozen snails were homogenised in liquid nitrogen using a pestle and mortar resulting in a well-mixed fine powder. Each homogenate combination was then split into a total of 12 subsamples (10-20 mg) and triplicates were used in each of the four subsequent biochemical assays.

In brief, protein content of the sub-samples was determined by using a micro-modification of the Pierce BCA Protein Assay (Thermo Scientific). Total lipid content was measured using a modified sulpho-phospho-vanillin (SPV) assay, originally described by Bligh and Dyer (1959) and modified for microplates (Torres et al. 2007) against cholesterol as a standard. Finally, total carbohydrate content was determined using a modified (Leyva et al. 2008) anthrone-sulphuric acid assay (Roe 1954) for marine invertebrates using glycogen as a standard.

To estimate the Energy consumption (Ec) or cellular respiration rates, we measured the activity of the electron transport system (ETS) using the methodology provided by Owens and King (1975) and modified by Smolders (2004). An extinction coefficient of 15,900 M-1 cm-1 was used to determine the amount of formazan produced during the ETS reaction. Formazan was subsequently converted into Ec, assuming that for every 2 μmol of formazan formed, 1 μmol of O2 was consumed (De Coen and Janssen 1997). The quantity of O2 consumed was subsequently converted into energy equivalents using the oxyenthalpic equivalents for an average mixture of protein, carbohydrate and lipids of 480 kJ/mol O2 (Gnaiger 1983). Individual energy reserve fractions were then converted to their energetic equivalents using their combustion energy values (Gnaiger, 1983): protein 24,000 mJ mg-1, lipid 39,500 mJ mg-1, and glycogen 17,500 mJ mg-1. Total available energy (Ea) was calculated by adding the three energy fractions and energy consumption (Ec) was estimated as ETS activity. Cellular respiration rate was calculated as the ratio of available energy to energy consumption (Ea/Ec), which reflects the energy status of an organism at the cellular level.

### Data Analysis

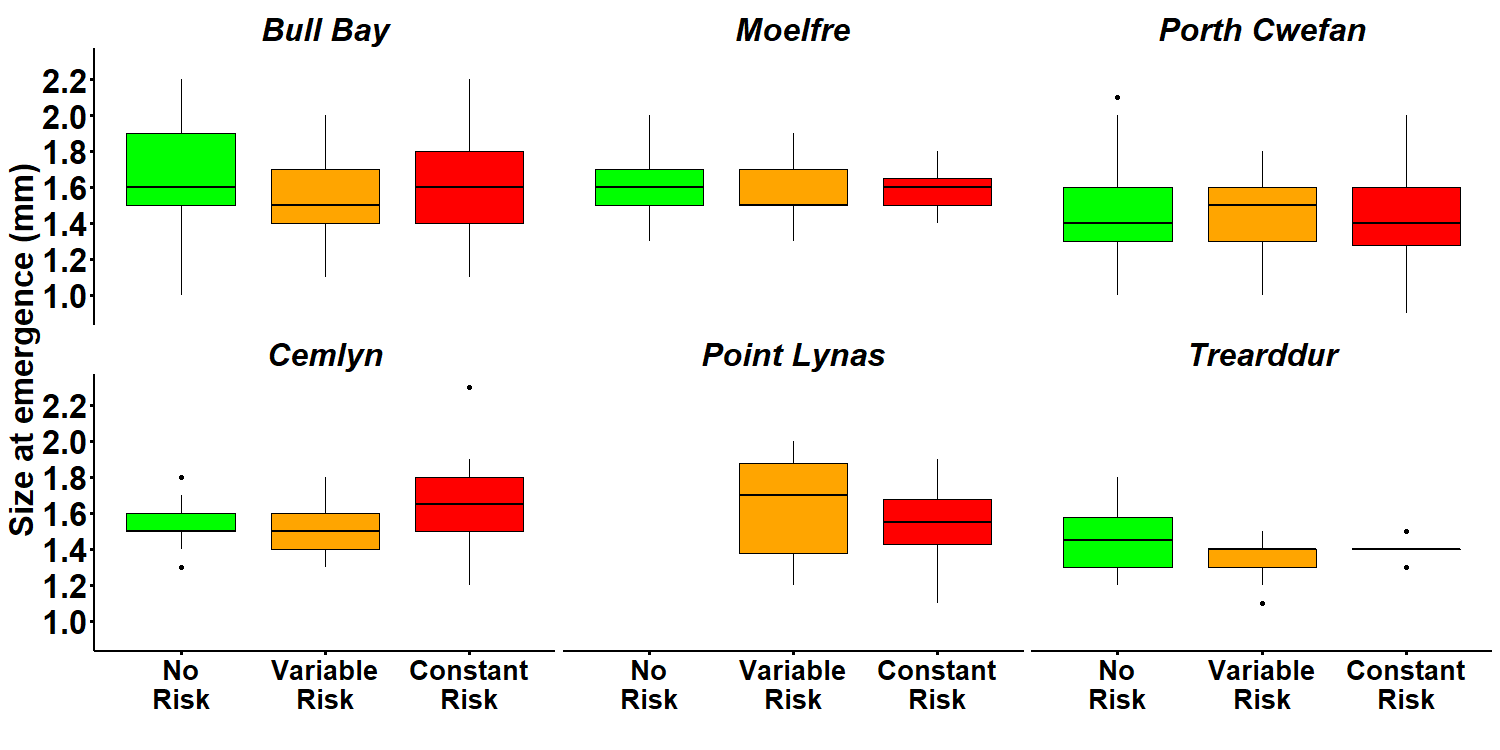
We tested the influence of developmental experience of predation risk and parental habitat (wave-exposed vs wave-sheltered) on emergence size, growth as well as the energy budget of *Nucella* after eight months. *Nucella* growth was split into three response variables: shell length, tissue mass and tissue mass per unit shell mass. The latter two variables were log transformed prior to analysis. Energy budgets were analysed by comparing the energy fractions of proteins, lipids and carbohydrates, the total energy available, energy consumption (ETS activity) and the resulting CEA of the offspring generation. Parental habitat and developmental experience of risk were fully crossed fixed factors, and site (i.e. a specific wave-exposed or wave-shelter shore) was nested within the parental habitat. To account for potential unequal variances between treatment combinations, we used the REML-weighted variance estimates developed by Zuur et al. (2009). Once the variance structure was identified, it was used throughout the rest of the model selection process. We used backward model selection, by removing terms from the full model and selecting the most parsimonious model with the lowest AIC score. All analyses were constructed in R 3.5.0 (<www.r-project.org>, Studio 2012) using the *lme4* package (Bates et al. 2013). When interaction terms were significant at the α = 0.05 level, the *lsmeans* package was used to conduct Tukey *post hoc* tests (Lenth 2016).

## Results

Analysis of the images of hatchlings revealed no influence of parental habitat or developmental experience of risk on the size at emergence of *Nucella* (*lm*, *F*= 1.75, *p* = 0.17, Fig. 3.2). However, parental habitat and developmental experience of risk influenced the final total shell length of *Nucella* (*lm, F*= 8.68, *p* <0.001, Fig. 3.3). In both wave-sheltered and wave-exposed offspring the no risk treatment resulted in greater final total shell lengths than under constant risk. However, the variable risk treatment resulted in growth rates equivalent to no risk in exposed shore offspring but equivalent to constant risk in the sheltered shore (Fig. 3.3 A). *Nucella* log-tissue mass did not vary between treatments, with only *Nucella* from wave-exposed parents reared under the constant risk treatment showing significant reductions in tissue mass (Fig. 3.3 B). Finally, the relative investment in shell and tissue (shell mass per unit tissue mass) by *Nucella* differed depending on the developmental experience of risk (*lm, F*= 8.68, *p* <0.001). As the experience of risk increased (no risk < variable risk < constant risk), log-shell mass in *Nucella* increased relative to log-tissue mass (Fig. 3.3 C), demonstrating an increase in the relative investment in shell defence.

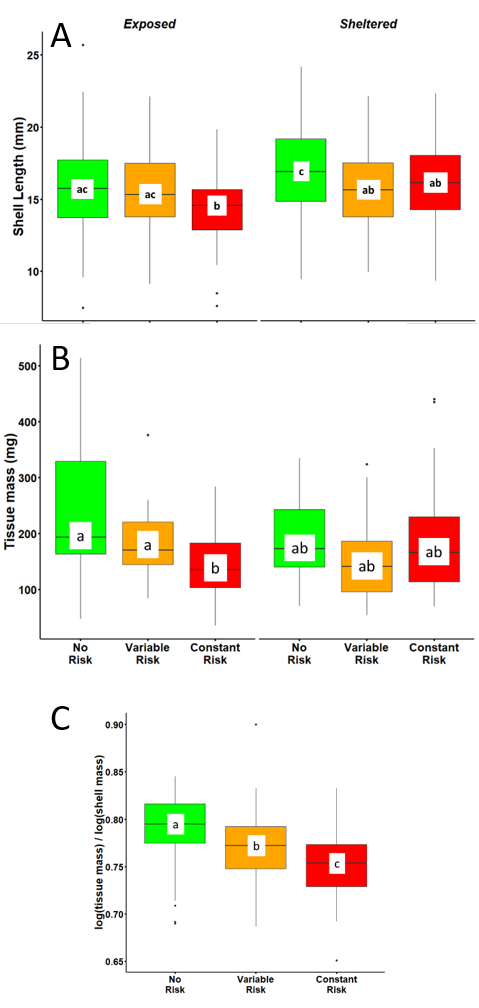
Sheltered shore

Exposed shore



N.D.

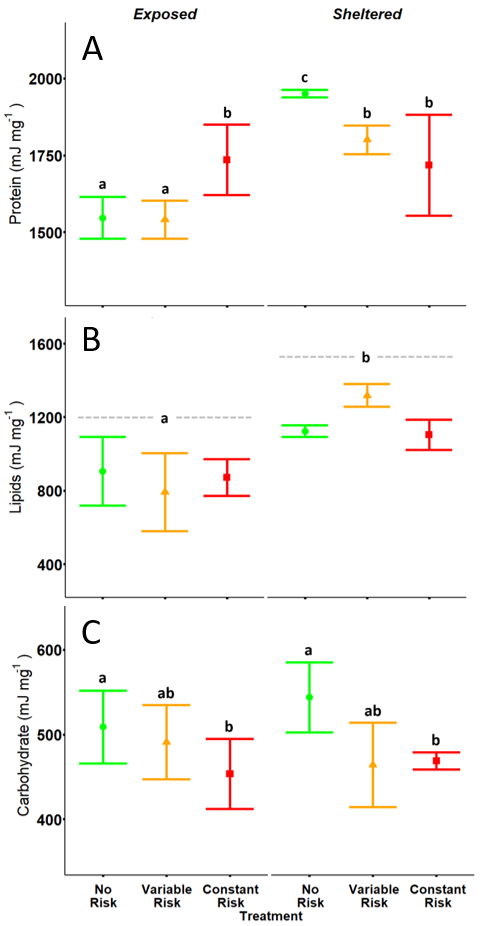
***Figure 3.2*** *Emergence size (maximum shell length) of Nucella hatchlings from wave-exposed and wave-protected sites reared no risk (green), variable risk (orange) and constant risk (red) treatments.**Boxplots represent medians with 95% confidence intervals. ND = No Data*



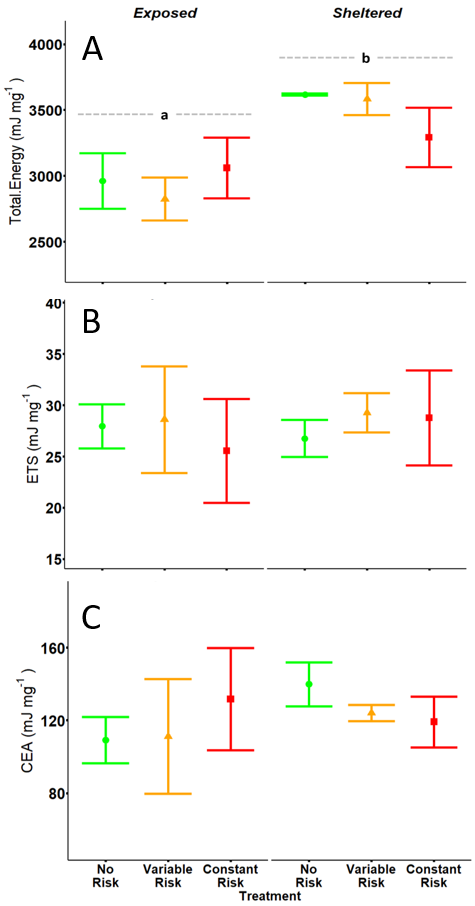
***Figure 3.3*** *a) Shell length b) tissue mass and c) shell mass per unit tissue mass of the offspring generation of dogwhelks (Nucella lapillus) from wave-exposed and wave-protected sites reared no risk (green), variable risk (orange) and constant risk (red) treatments.**Values are means ±SE. Different letters**indicate significant differences (Tukey’s HSD, a=0.05).*

Analysis of the effects of parental habitat and developmental experience of risk revealed different responses for each of the three energy reserve fractions: protein, lipid and carbohydrate. For protein content there was a significant interaction between parental habitat and developmental experience of risk (Table 3.1, Fig. 3.4 A). Total protein content was 12% higher in the constant risk treatment for wave-exposed offspring than in the other two treatments, but wave-sheltered offspring showed a different pattern, with total protein energy reserves highest in the no risk treatment (Fig. 3.4 A). Lipid energy reserves were not affected by developmental experience of risk but there was a significant effect of parental habitat (*F*= 23.37, *p <* 0.01); across all developmental risk treatments wave-exposed *Nucella* had 29% less energy stored as lipids compared to wave-sheltered offspring (Fig. 3.4 B). Finally, carbohydrate energy reserves showed a significant main effect of developmental risk, which was consistent across wave-exposed and wave-sheltered populations (Table 3.1). The highest carbohydrate energy reserves were found in *Nucella* reared under the no risk treatment, with those in the variable risk and constant risk treatments recording 12% and 14% less energy stored as carbohydrate, respectively (Fig. 3.4 C).

Across all treatments, the highest contribution to total energy availability of *Nucella* came from proteins (53%)*,* followed by lipids (31%) and finally carbohydrates (16%). When all the energy fractions were combined into total energy reserves i.e. Ea, there was no effect of risk treatment (Table 1), but a clear significant effect of parental population (*lm, F*= 21.39, *p =* 0.009, Fig 5 A). *Nucella* from wave-exposed populations had 12.5% lower overall energy availability than offspring from wave-sheltered populations. Analysis of the electron transport system revealed no difference in the energy consumption (Ec) of *Nucella* across all treatment combinations (Table 1, Fig 5 B). Despite differences in *Nucella* Ea between treatments, CEA analysis showed no differences in the cellular energy budgets of *Nucella* between treatments, although wave-exposed parents tended (*lm, F*= 6.25, *p =* 0.066, Fig 5 A) to produce offspring with a lower CEA than their wave-sheltered counterparts (Fig 5 C). On closer inspection, results showed that both *Nucella* ETS and CEA were affected by developmental experience of risk, but responses differed at the site level (Fig.6). Contradictory patterns of ETS and CEA between sites nested within each parental habitat resulted in no overall effect of developmental risk treatment.



***Figure 3.4*** *The energy equivalents of total (A) Protein, (B) Lipids and (C) Carbohydrate of dogwhelk (Nucella lapillus) offspring from wave-exposed and wave-protected parents reared under no risk (green circles), variable risk (orange triangles) and constant risk (red squares) treatments.**Values are means ±SE. Different letters**indicate significant differences (Tukey’s HSD, a=0.053).*



***Figure 3.5***  *Cellular energy budgets including the components: (A) total energy available (Ea); (B) total energy consumption (Ec); and (C) CEA of dogwhelk offspring (Nucella lapillus) from wave-exposed and wave-protected parents reared under no risk (green circles), variable risk (orange* *triangles) and constant risk (red squares) treatments.**Values are means ±SE. Different letters**indicate significant differences (Tukey’s HSD, a=0.05).*

***Table 3.1*** *Summary of full model results from GLMs of protein reserves, lipid reserves, carbohydrate reserves, total energy, electron transport system (ETS) activity, the cellular energy allocation (CEA), shell length (SL), tissue mass (TM) and SL:TM of wave-exposed and wave-sheltered offspring of Nucella lapillus reared under different risk treatments.*

Response variable Effect *Df* *F P*

Size at emergence Parental habitat 1 0.59 0.484

Develop risk 2 1.87 0.156

Ph x Dr 2 1.95 0.144

Error 421 2352.51

Protein reserves Parental habitat 1 11.47 **0.028**

Develop risk 2 1.70 0.190

Ph x Dr 2 5.83  **0.005**

Error 62 6479.45

Lipid Reserves Parental habitat 1 24.13 **0.008**

Develop risk 2 0.017 0.983

Ph x Dr 2 1.84 0.169

Error 62 806.35

Carbohydrate Parental habitat 1 0.030 0.871

reserves Develop risk 2 3.2963  **0.045**

Ph x Dr 2 0.471 0.627

Error 62 1520.175

Total Energy Parental habitat 1 21.39 **0.009**

Develop risk 2 0.776 0.465

Ph x Dr 2 3.100 0.054

Error 62 4481.10

ETS Parental habitat 1 0.0277 0.876

Develop risk 2 0.138 0.871

Ph x Dr 2 0.3814 0.685

Error 62 995.53

CEA Parental habitat 1 0.0154 0.307

Develop risk 2 0.065 0.938

Ph x Dr 2 1.356 0.267

Error 62 860.53

Shell length Parental habitat 1 3.89 0.119

Develop risk 2 16.37 **<0.001**

Ph x Dr 2 8.68 **<0.001**

Error 819 2601.03

Tissue mass Parental habitat 1 0.151 0.717

Develop risk 2 4.458 **<0.013**

Ph x Dr 2 5.35 **<0.006**

Error 153 4286.55

Tissue: Shell Parental habitat 1 2.04 0.227

Develop risk 2 20.88 **<0.001**

Ph x Dr 2 2.61 <0.077

Error 153 42706.9

## *Results may vary slightly after model selection, but the significance of any factors or factor combinations remains unchanged. Statistically significant effects (a = 0.05) are in bold.* Discussion

As expected, predation risk influenced the growth and energetic budget of the offspring generation of *Nucella,* but its effect was strongly influenced by parental habitat. Contrary to our expectations, *Nucella* offspring from both wave-exposed habitats emerged from their egg capsules at the same size. This indicated that any differences in shell growth recorded at the end of the eight-month exposures are not attributed to initial differences in size of offspring. *Nucella* shell length at the end of the investigation was affected by both parental habitat and developmental risk, although differences in *Nucella* tissue were only present in wave-exposed *Nucella* reared under constant risk. Interestingly, however, our results clearly show that *Nucella* allocated resources to tissue growth and shell deposition in accordance with the developmental risk they experienced. The ratio of tissue mass to shell mass of *Nucella* was highest in the no risk treatment, lowest in the constant risk treatment and intermediate ratios were recorded in the variable risk treatment, indicating an increase in commitment of resources to defence (shell deposition, Palmer 1990; Pascoal et al. 2012b) potentially related the amount of time *Nucella* are exposed to predatory cues. Energy consumption (ETS activity) and overall energy budget (CEA) remained the same across all treatment combinations, with only wave-sheltered offspring showing a trend (*p* = 0.066) in having higher CEA than that of wave-exposed *Nucella*; mainly driven by differences in the total energy availability in *Nucella* from each parental habitat. Predation risk did however, impact individual energetic fractions of offspring. Proteins levels for wave-exposed and wave-sheltered offspring were influenced by developmental risk, although this was not reflected in total energy availability, despite proteins accounting for half the energy reserves in *Nucella*. Possible differences in carbohydrate and lipid levels between treatments may have obscured the influence of developmental risk in *Nucella* total energy availability. Overall, our results demonstrate that parental effects can combine with individual experience of risk, to influence energy budgets and define important growth metrics of offspring from wave-exposed and wave-protected populations of *Nucella.*

All *Nucella* hatchlings emerged at the same size, irrespective of parental habitat or the risk they were exposed to while still in the egg capsule. Previous findings have shown that egg capsules collected from wave-exposed shores contain smaller and more numerous hatchlings than their wave-sheltered counterparts (Etter 1989, Donelan and Trussell 2019). These conflicting results may highlight the important role played by predation risk just prior to breeding, as in the current study *Nucella* reproduced under no risk and in relatively benign laboratory conditions. Despite no differences in initial shell size (size at emergence), *Nucella* final shell length was influenced by the pattern of predation risk they experience during development, with offspring from the two parental habitats responding differently. Although as with previous studies (Palmer 1990, Bourdeau 2010, Pascoal et al. 2012a) there was a negative effect of predation risk on shell length of both wave-exposed and wave-sheltered populations, shell lengths in the variable treatment responded differently for each parental habitat. Offspring of low predation pressure wave-exposed parents reared under the variable risk treatment had shell lengths similar to those exposed to no risk. On the other hand, offspring of high predation pressure populations in the variable risk treatment reach shell lengths comparable to those reared under constant risk. In each case it would seem that when *Nucella* are exposed to variable (and potentially unpredictable) predation risk, they attain shell lengths that are influenced by parental experience of local predation pressure levels. Furthermore, although it is unclear whether these threat-sensitive adaptive responses are achieved through behavioural or physiological compensation, or a combination of both, results show that growth in *Nucella* may be influenced by parental effects (Etter 1996, Beckerman et al. 2007, Thaler et al. 2012).

The influence of predation risk on the comparative rate of shell deposition and tissue growth is well-documented in *Nucella lapillus* (Palmer 1990, Pascoal et al. 2012a). In the current study, *Nucella* tissue growth remained relatively constant for all treatments, with only significant reductions in tissue mass recorded for wave-exposed *Nucella* reared under constant risk. However, comparisons of the shell mass per unit tissue mass clearly show that relative shell deposition in *Nucella* may be threat sensitive. Relative shell deposition of both parental habitats was higher when *Nucella* were exposed to risk than for predator naïve offspring. While these results confirm previous findings (Palmer 1990, Trussell and Nicklin 2002, Bourdeau 2010), results of our variable risk treatment also reveal new insights into the influence of predation risk on the commitment of resources to defence in *Nucella*. Offspring exposed to variable risk allocated an intermediate amount of resources into shell deposition compared to those in the no risk and constant risk treatments. It is not yet clear whether increases in shell deposition in gastropods are a direct result of the chemical cues released by crabs or as a result of reductions in foraging under elevated predation risk conditions (Trussell and Nicklin 2002, Trussell et al. 2003, Brookes and Rochette 2007, Bourdeau 2010, Pascoal et al. 2012a). Although our results are unable to shed more light on this issue, they do indicate that there is a temporal link between the amount of time spent under risky conditions and the relative amount invested in shell defences.

Predation risk influenced energetic budget of *Nucella*, with responses of offspring effected by parental habitat. Energy consumption (Ec) results showed that ETS activity was the same for all *Nucella* offspring, indicating that the energy demands at the cellular level did not differ for offspring irrespective of treatment combination. This finding contrasts with those of previous studies showing that predation risk is able to alter the energy consumption of prey (Huuskonen et al. 1997, Steiner and Van Buskirk 2009, Hawlena and Schmitz 2010b, Barry and Syal 2013), including that of *Nucella* (Karythis et al. 2020). However, despite no significant overall impact on Ec, predation risk did influence the Ec of offspring, but responses were different at the site level. Contradictory patterns between sites nested in the same habitat may have obscured the treatment effects. The metabolism (energy consumption) of intertidal invertebrates can be influenced by food availability (Shick et al. 1988), thermal stress (Tomanek and Somero 1999, Sanford 2002) as well as wave driven mechanical stress (Donovan and Taylor 2008), therefore, different metabolic responses to predation risk at site level may not be so remarkable. Despite results indicating an influence of parental habitat on the total energy available (Ea) to *Nucella*, contradictory patterns in Ec also resulted in similar overall CEA results of *Nucella* offspring. Therefore, despite the impacts on Ea, cellular energy budgets were unaffected by offspring experiences of risk or parental habitat. Ea was lower for wave-exposed offspring than for sheltered-shore *Nucella* and was mainly driven by differences in protein and lipid reserves. Further examination of the energy fractions of Ea, however, did reveal some interesting differences, with potential implications for individual fitness discussed below. Our results indicate that *Nucella* from both wave-exposed and sheltered populations respond and adapt their growth and cellular energy budgets to different temporal patterns of predation risk, but responses may vary between sites.

The predatory risk regime *Nucella* offspring were exposed throughout their development influenced how *Nucella* utilised individual energetic fractions, but its effect differed depending on parental habitat. The two most energetically important fractions, protein and lipids, were both influenced by parental habitat. Previous studies have shown that these two energy reserves play important roles in the life history of *Nucella* (Stickle 1975, Etter 1989) and this may help explain the adaptive potential for parental effects to play a role in their utilisation. For instance, *Nucella* *spp.* utilise protein catabolism to respond to their energetic demands (Stickle 1975), with a closely related species *Nucella lamellosa* using energy stored as proteins to provide up to 85% of the energy they require during periods of fasting (Stickle and Bayne 1982). Lipids on the other hand play a pivotal role in the reproductive strategy of *Nucella*, with most of the energy invested in egg production coming from lipid reserves (Etter 1989). These habitat differences in lipid reserves may reflect the ability of parental experience to influence the different reproductive strategies of populations at each wave exposure (Crothers 1974, Etter 1989). As wave-exposed *Nucella* produce four times more offspring than individuals on wave sheltered shores (Etter 1989), parental effects on the utilisation of lipids will ultimately affect the reproductive success of their progeny. Only carbohydrate energetic reserves were not influenced by parental habitat, with levels responding negatively to the amount of time *Nucella* spent under risk during development (Fig. 3C). As carbohydrates accounted for just 16% of energy reserves in *Nucella* and generally serve as a dynamic energetic reserve used in many prey, the lack of a parental habitat effect is to be expected. Although necessarily speculative, due to the complex nature of energy use in *Nucella* (Stickle and Bayne 1982, 1987, Chu et al. 2014), our results indicate that parental experience in *Nucella* is able to inform offspring of how best to utilise different energetic reserves in relation to the probable conditions they may encounter.

# **Parental and individual experience of predation risk informs short-term antipredator responses in the** dogwhelk, *Nucella lapillus*.

**CHAPTER FOUR**

Parental and individual experience of predation risk informs short-term antipredator responses in the dogwhelk, *Nucella lapillus*.

## Abstract

How prey respond to the detection of a predator will ultimately affect their chances of survival. Many behavioural antipredator responses of prey are plastic, enabling prey to learn from previous experience increasing their chance of surviving future encounters. Furthermore, when the risk experienced by parents is predictive of that faced by their offspring, many prey can influence the antipredator traits of their offspring, through parental effects. The dogwhelk *Nucella lapillus* inhabits rocky intertidal sites along a wave-exposure gradient which in turn controls local abundances of the crab predator, *Carcinus maenas*, resulting in populations being subject to different predation pressure. In this study I exposed the offspring generation of wave-exposed and wave-sheltered dogwhelks to different patterns of risk throughout development and then investigated their short-term responses to crab effluent. As expected, dogwhelks were less likely to move when exposed to a predatory cue, although the risk experienced throughout development and parental habitat did not influence this response. We found no impacts of parental habitat or development risk on the distance dogwhelks travelled or on the likelihood of choosing a refuge. However, the pattern of risk experienced throughout development did influence the frequency with which *Nucella* crawled above the water line rather than hiding under a hard substrate refuge. The overall similarity in the response of *Nucella* offspring from wave-exposed and wave-sheltered parents, may reflect habitat specific behavioural constraints brought about by contrasting biotic and abiotic factors. These findings suggest that it is important to appreciate the dynamic nature of antipredator responses if we are to understand the impacts predators have on prey and their ecosystem.

## Introduction

The responses of prey to the threat of predation have a profound implications for prey fitness. Along with morphological defences, prey utilise avoidance and escape behaviours to evade predators, which can typically include shifts in speed and direction, as well as changes of microhabitats and the use of refuges (Chivers and Smith 1998, Wirsing et al. 2010, Hawlena et al. 2011, Donelan et al. 2017). In marine gastropods both morphological and behavioural responses to the risk of predation are common. Apart from the ubiquitous development of more robust shells to better withstand crushing by predators (Hughes and Elner 1979, Appleton and Palmer 1988, Trussell and Nicklin 2002, Edgell and Neufeld 2008), many gastropods also respond behaviourally to the detection of predators, by reducing activity and seeking the safety of a refuge (Fairweather 1988, Donelan et al. 2017). To enable prey to respond to changes in predation risk many antipredator responses are plastic, allowing for previous experiences of predation risk to inform future behaviour (Mery and Burns 2010). For example, when juvenile whelks (*Buccinum undatum*) were initially exposed to cues from the predatory asteroid *Leptasterias polaris* they showed weak antipredator responses, with no whelks adopting foot contortion, the strongest antipredator response to asteroids (Rochette et al. 1998). However, after a short exposure (30 minutes) to predatory cues over four consecutive days, an increase in the overall intensity of antipredator response was observed with foot contortions seen in 25% of responses*.* Slight changes in individual behaviours can have significant impacts on predator-prey dynamics (e.g. vertical distribution of gastropods in response to predation risk, Phillips, 1976) and hence have important effects on community-level processes.

The intertidal dogwhelk *Nucella lapillus (hereafter Nucella),* has been used extensively to investigate the influence of predation risk on the antipredator traits of prey (Trussell et al. 2006b, Johnston et al. 2012, Large and Smee 2013). *Nucella* detect chemical cues from the green shore crab *Carcinus maenas* which enhance both morphological (Hughes and Elner 1979) and behavioural (Vadas et al. 1994) antipredator traits in response. In addition to developing thicker shells when exposed to crab kairomones several studies have shown, that *Nucella* also alter their activity under risky conditions (Large and Smee 2010, 2013, Large et al. 2011, 2012). For example, when Large and Smee (2010) exposed *Nucella* to crab effluent their activity (mean number of movements) was reduced by up to 40% when compared with controls. As with other gastropods, in addition to reducing activity, *Nucella* also change their use of microhabitats to hide from predators. In response to risk, *Nucella* are not only more likely to seek refuge out of the water (Vadas et al. 1994), but will also choose the safety of a rock refuge more often as well (Feare 1971, Burrows and Hughes 1989, Matassa et al. 2016). However, reductions in activity and the increased use of refugia by *Nucella* in response to risk can also be affected by the risk experienced by their parents. Donelan and Trussell (2015) exposed parental couples of *Nucella* to predatory crab cues for 10 weeks and then tested the antipredator responses of the resulting offspring. They showed that exposure of parental couples to risk reduced the sensitivity of offspring to predation risk. By increasing the amount of time, the offspring spent in risky habitats, they increased the number of barnacles consumed which resulted in increased growth from predator exposed parents. Moreover, in a later study the same authors exposed *Nucella* to predation risk while they were still in the egg capsule, resulting in hatchlings from some populations emerging at smaller sizes and with higher growth efficiencies than for predator-naïve hatchlings (Donelan and Trussell 2019). These studies indicate that understanding the drivers of how and why prey respond to predation risk differently in different contexts can be complex.

Predictable environmental differences have a major impact on the interaction between predators and prey (Menge 1976, 2000). This has been demonstrated in *Nucella* across gradients of wave action. As wave action increases on rocky shores, predatory crab abundance declines (Hughes and Elner 1979, Large and Smee 2013, Karythis et al. 2020) and hunting and handling efficiency is reduced (Robinson et al. 2011). The combined effect is a reduction in the levels of predation pressure on wave-exposed populations (Menge et al. 1987, Large and Smee 2013). Such differences in localised predation pressure, apart from being reflected in the shape and thickness of *Nucella* shells (Pascoal et al. 2012a, 2012b), have implications for their antipredator behaviour as well. For example, Large and Smee (2013) tested the short-term responses of *Nucella* from both wave-exposed and wave-protected sites, and observed that although all *Nucella* showed behavioural responses (reduction in movements) to the presence of predatory crab cues, the response was more intense in individuals from sheltered shores where predator abundances are higher. This difference in the behavioural response of *Nucella* from exposed and sheltered populations are not universal however, with several studies indicating that the two populations respond equally to the presence of a predatory crab cue (Palmer 1990, Freeman et al. 2014, Dernbach and Freeman 2015). A greater understanding of how prey from high and low predation pressure habitats respond to predation risk is necessary in order to fully appreciate the ecological importance of predation risk.

In this study we investigated the influence of parental experience of risk (through the use of adults sourced from populations differing in predation pressure) and developmental experience of risk (through rearing offspring under different risk regimes) on the short-term behavioural response of *Nucella*. We first reared the offspring generation of *Nucella* from different parental habitats (wave-exposed and wave-sheltered populations) under three developmental risk regimes and then conducted a short-term behavioural assay to see how they responded to a predatory cue from the crab *Carcinus maenas*. *Nucella* were reared under no risk, constant risk and variable risk conditions. Despite the predictable differences in predation pressure between wave-exposed and wave-sheltered sites, the predation risk faced by prey in each habitat is not constant but instead varies spatially and temporally (Feare 1970, Brown et al. 1999, Lima and Bednekoff 1999, Gallagher et al. 2017). Therefore, rearing *Nucella* under variable risk should provide a more reasonable insight into the development of antipredator traits. Short-term behavioural assays were conducted by adding crab effluent or filtered seawater to an experimental mesocosm and then assessing the propensity of offspring to express antipredator behaviours. We initially recorded if *Nucella* moved or remained still in response to the addition of a predatory cue, and for those that moved, measured the distance covered. Finally, we recorded if *Nucella* chose to seek refuge in response to a predatory cue as well as determining the type of refuge selected.

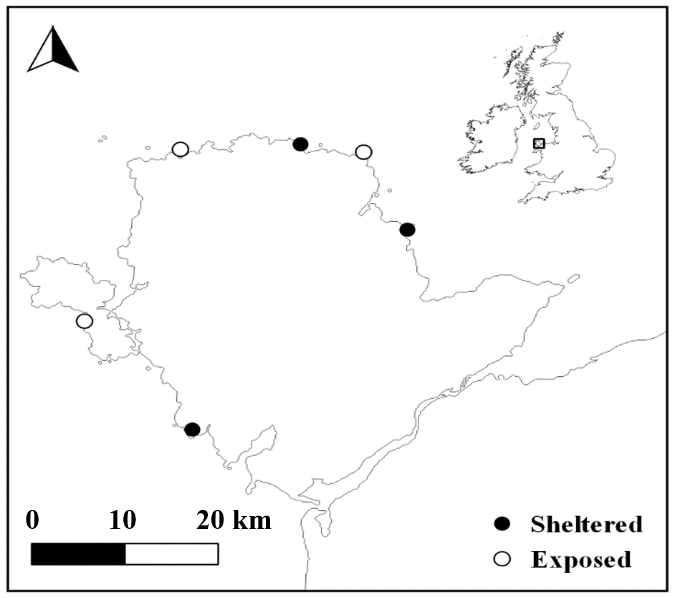
## Methods

### *Nucella* collection and breeding

Adult *Nucella* were collected in December 2017 from three wave-exposed and three wave-sheltered sites, located around the coast of Anglesey, North Wales, UK (Fig.4.1). The selected sheltered and exposed sites show clear differences in their wave exposure level (Karythis et al. 2020) based on their average wave fetch (Burrows, Harvey, & Robb, 2008). In addition, previous work has shown that these sites also differ in their crab abundances (Karythis et al. 2020), with the selected wave-sheltered sites recording crab abundances as much as 40 times higher (41.22 ± 17.43 SD crabs per trap) than those recorded at exposed sites (0.89 ± 1.27 SD crabs per trap). Once collected, *Nucella* (n=200) were placed into site-specific tanks, on a 12:12 light dark cycle. Water temperatures reflected local ambient seawater temperatures and *Nucella* were provided with barnacle-covered rocks as food *ad libitum*. *Nucella* breeding peaks in March around the coast of North Wales (Crothers 1985, Morton 2009), when snails form large reproductive aggregations (100s of individuals) and lay egg capsules in crevices and under rocks. Egg capsules contain an average of 30 fertilized embryos which are accompanied by hundreds of nurse eggs (Pechenik et al. 1984). Under laboratory conditions, *Nucella* formed breeding aggregations in February and egg capsules were deposited by *Nucella* from all 6 sites within a 2-week period in mid-March.

### Risk Regimes

Egg capsules from each of the six sites were randomly assigned to a no risk, a variable risk or a constant risk regime resulting in a total of 18 treatment combinations (n = 6 mesocosm replicates per treatment combination). Using a scalpel blade, egg capsules were carefully removed, and 8 egg capsules were randomly assigned to one of the three risk regimes. Hatchlings were initially provided with rocks, covered in newly-recruited barnacles, and then subsequently with larger barnacles as they grew in size. In addition to food, the rocks provided a refuge throughout the rearing period. Behavioural assays were conducted after an 8 month rearing period, when snails were not yet sexually mature (Hughes et al. 1992) but still remaining vulnerable to crab predation (Hughes and Elner 1979), as these factors may have influenced behavioural responses (Clark 1994, Karythis et al. 2020). Risk exposurewas manipulated by the addition, to individual mesocosms, of a perforated crab pot which housed a large male crab (mean carapace length ± SD, 56.6 ± 4.8 mm). Housing crabs in such pots allowed the free dispersion of kairomones without allowing predators direct access to *Nucella*. Under the no risk regime crab pots remained empty, whereas under the constant risk regime crabs were present throughout the experiment. For the variable risk regime, crabs were added and removed from the crab pot every three or four days, resulting in *Nucella* in this regime being exposed to risk for 50% of the time. In the variable and constant risk treatments, crabs were held in the crab pots for a maximum of 4 days and only used once before being released.



***Figure 4.1*** *Map of study sites across the Isle of Anglesey, Wales, UK. Open circles = Exposed sites, closed circles = sheltered sites.*

### Behavioural assay

To assess the influence of parental habitat and developmental experience of risk on the short-term antipredator behavioural responses of *Nucella*, we conducted a series of behavioural assays in the presence and absence of a predatory cue. A total of 108 *Nucella* (6 for each treatment combination) from wave-exposed and sheltered sites, reared under the three risk regimes were starved for two days prior to beginning the behavioural assays. Individual *Nucella* were placed in the centre of an experimental mesocosm (30 x 20 x 10 cm) with a rock refuge at one end and allowed to acclimatise for five minutes. After acclimatisation, the predator treatment was introduced to the experimental mesocosm by adding 500 ml of filtered seawater (control) or crab effluent. The predator treatment was introduced to the same area of the mesocosm each time and was non-directional. Crab effluent was prepared by adding 8-10 large male crabs to 20 l of aerated seawater for one hour.

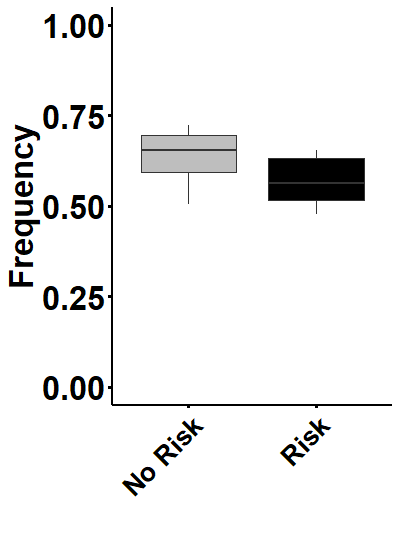
Following introduction of the predator treatment, an initial observation was taken to record whether *Nucella* chose to move or to remain still. The exact position of *Nucella* within the mesocosm was then recorded every three minutes for a total of 21 minutes. This was then used to calculate a proxy for the total distance moved, by measuring the distance between each successive position. *Nucella* were then left for a further 30 minutes before their final position was recorded. Final position was recorded as: in the water (unsafe), in the rock refuge (safe) and out of the water (Air refuge, safe). After each assay, animals were returned to their original mesocosms where exposure to the long-term risk regime (developmental risk) continued. Assays were repeated on the same individual a total of ten times over consecutive days: 5 in filtered seawater and 5 in crab effluence. The order in which individuals were exposed to crab effluent or controls was selected at random. *Nucella* were not fed throughout the experimental period and therefore we included trial day in our analysis to account for the effects of increases in hunger.

### Data Analysis

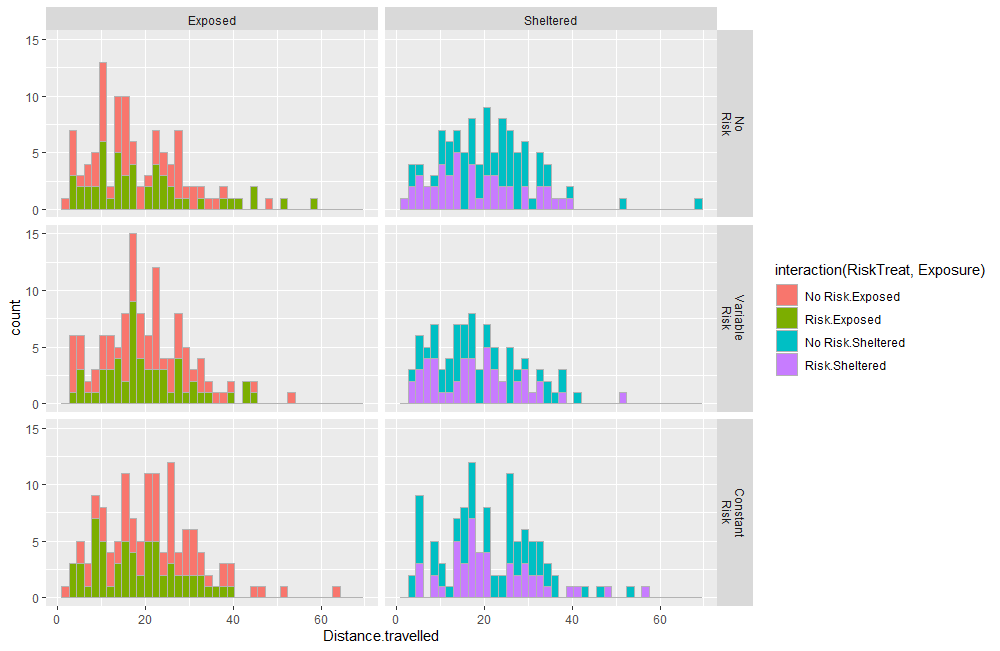
Data was analysed using R 3.5.0 (Studio 2012) using the lme4 package (Bates et al. 2013). Using the backward selection of GLM models, we tested the influence of parental habitat (wave-exposed and wave-sheltered), developmental experience of risk, predator treatment and their interaction on the short-term decision making of *Nucella*. Behaviour was assessed by considering four response variables: i) initial response (moved or not moved), ii) distance travelled, iii) final position (safe or unsafe) and iv) refuge type (rock or air). In addition to the four response variables, we used *Nucella* ID, trial day and experimental mesocosm as random factors, and site as a nested term within parental habitat. Backwards model selection was employed to determine the most parsimonious model with the lowest AIC, by removing terms from the full model. Any interactions which were found to be significant were then analysed using the Tukey *post hoc* test (*lsmeans* package Lenth, 2016).

## Results

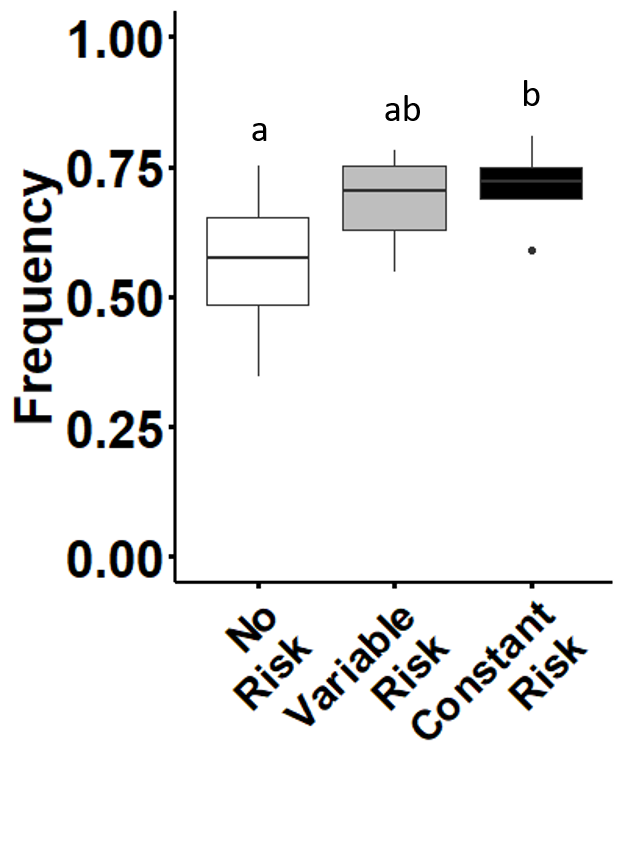
In total 1080 behavioural assays were conducted, and they showed that exposure to a predatory cue in our short-term experiment affected *Nucella* responses. *Nucella* were less likely to move when exposed to crab effluent than when exposed to filtered seawater (Fig. 4.2, binomial GLM, *F* = 7.08, *p* < 0.001). Model selection revealed that the likelihood of moving was not affected by parental habitat or by developmental experience of risk (Table 4.1). Despite predator treatment influencing the decision of *Nucella* to move, once the decision was taken the total distance travelled was the same across all treatment combinations (Table 1, Fig. 4.3).



***Figure. 4.2*** *Frequency with which individual Nucella lapillus moved in response to the addition of filtered seawater (No risk) and effluent from the green shore crab, Carcinus maenas (Risk). Boxes indicate the 25th and 75th percentiles (whiskers 1.5 x interquartile range) and the median (horizontal line within each box. All individual (n=108) were exposed to control and predator conditions 5 times each.*



***Figure. 4.3*** *Distance travelled by Nucella lapillus in response to the addition of filtered seawater (No risk) and effluent from the green shore crab (Risk). All individual (n=108) were exposed to control and predator conditions 5 times each.*



***Figure. 4.4*** *Frequency with which individual Nucella lapillus chose to move above the waterline depending on the developmental exposure to risk. . Boxes indicate the 25th and 75th percentiles (whiskers 1.5 x interquartile range) and the median (horizontal line within each box. All individual (n=108) were exposed to control and predator conditions 5 times each. Letters**indicate results from the Post hoc Tukey’s HSD.*

Of all the instances (60.67 % of all trials) in which *Nucella* moved from their initial position, in 88 % of cases, snails chose the safety of a refuge as their final position rather than remaining in the open and exposed to a potential attack. None of the factors tested here influenced the choice of *Nucella* to seek safety (Table 4.1). However, further analysis into the types of refuge selected by *Nucella*, revealed that the risk regime they were exposed to throughout development impacted their choice of refuge (Fig. 4.4). *Nucella* reared under constant risk were more likely to choose to move out of the water (73 % frequency), than those reared under no risk conditions (54% frequency, Tukey’s pairwise comparison: *p* = 0.019). *Nucella* reared in the variable risk treatment selected to crawl above the waterline at a frequency intermediate between no risk and constant risk (Fig. 4.4).

***Table 4.1*** *Behavioural assay model selection scores for wave-exposed and wave-sheltered population of dogwhelks (Nucella lapillus), reared under no risk, variable risk and constant risk conditions exposed to crab effluent from the green shore crab or filtered seawater.*

**Terms removed df Moved Distance Safe Refuge**

**Not Moved Travelled Not Safe Out**

3-way 16 1322.077 4969.775 1320.536 911.6078

2-way A\*B\*C 14 1320.906 4972.473 1316.714 908.7896

A:B 13 1320.239 4970.555 1314.722 911.0364

B:C 12 1318.472 4970.182 1315.020 905.6681

A:C 12 1319.170 4969.707 1313.826 909.0082

Single 2-way A:B + B:C 11 1317.755 4968.280 1313.026 908.1236

A:C + A:B 11 1318.462 4967.796 1311.836 911.2840

A:C + B:C 10 1316.815 4967.357 1312.190 905.8400

Fixed A:B + A:C + B:C 9 1316.059 4965.466 1310.199 908.1842

C 7 **1313.033\*** 4963.112 1309.788 912.1668

B 8 1320.540 4965.311 1308.345 906.6639

A 8 1317.384 4963.467 1310.699 907.5606

Single Fixed B + C 6 1317.608 4962.989 1307.978910.5472

A + C 6 1314.378 4961.117 1310.305 911.6489

A + B 7 1321.784 4963.312 1308.836 **906.0211\***

Null 5 1318.876 **4960.997\*** **1308.486**\* 910.0149

Best model A + B Null Null C

*Abbreviations: A = Parental habitat, B = Predator Treatment, C = Developmental exposure to risk. Models selected using generalised linear square models and Akaike Information Criteria (AICc) for all dependent variables. Selected models are highlighted in bold.*

## Discussion

Our results show that *Nucella* respond behaviourally to the presence of a predatory cue from *C. maenas*, but the risk experienced by parents (parental habitat) and of offspring throughout development had little impact on prey response. When compared to filtered seawater, *Nucella* offspring from both wave-exposed and wave-sheltered populations moved less when exposed to crab effluent, irrespective of the risk experienced during development. Overall, our results reveal no effect of parental habitat or development risk on the distance travelled or the likelihood of choosing the safety of a refuge. These findings agree with those of previous studies indicating that embryonic exposure to risk (while in the egg capsule) has no effect on whether *Nucella* chose to seek a refuge (Donelan and Trussell 2018b). However, analysis of the choice of refuge selected by *Nucella* offspring revealed that developmental experience of risk did have an effect on the type of refuge theychose. *Nucella* were more likely to select to crawl above the waterline if they had been exposed to continuous risk throughout development than when reared under the no risk or variable risk treatments. These findings indicate that the behavioural antipredator responses of *Nucella* to predator cues from *Carcinus*, are influenced by current risk and that experienced throughout development, with parental habitat having little influence on the behavioural responses tested here.

*Nucella* offspring from both parental habitats were less likely to move when they were exposed to predation risk than when under control conditions and this was not influenced by developmental experience of risk. These results broadly agree with previous behavioural assays conducted on *Nucella* that have also shown a decrease in activity levels in response to predation risk (Vadas et al. 1994, Donelan et al. 2017). For example, Vadas et al. (1994) exposed *Nucella* to cues from *Carcinus* and found similar reduction in the proportion of *Nucella* that moved from their initial position, as in the current study. Since our experiment was designed to encourage *Nucella* to move in order to shelter from predation risk, rather than starting from within a refuge (as was the case in Vadas et al., 1994), the decision to remain in their original position or move to a refuge is likely to be a crucial one (Burrows and Hughes 1989, Sih and Kats 1991, Sih and McCarthy 2002). One potential explanation may be that as *Nucella* spend 50 – 80% of their time in refuges and only leave them in order to forage before returning to digest their food in safety (Burrows and Hughes 1991b, Donelan et al. 2017), remaining inactive after the detection of a predator will most likely result in remaining in the safety of a refuge. The decision to remain inactive may therefore be adaptive, as in most cases remaining inactive will decrease their exposure to predation and increases their chance of survival (Burrows and Hughes 1991b, Sih and Kats 1991, Sih and McCarthy 2002). However, as the introduction of predation risk to the experimental tanks in the behavioural assays represented a pulse of risk, our results may be more representative of *Nucella’s* adaptive response to such pulses of risk. The adaptive response of prey to pulses of predation risk depends on the availability and proximity of refuges, as a lack of suitable refuges may mean that increased inactivity may reduce prey conspicuousness and therefore the chances of being attacked (Sih and Kats 1991, Sih and McCarthy 2002). As has been previously demonstrated with salamander larvae (*Ambystoma barbourin*, Sih & Kats, 1991)*,* *Nucella* may be “freezing” as an adaptive response to a lack of (or at least a lack of awareness of) suitable refuges. Although distinguishing between these two possible explanations is beyond the scope of this study, results indicate that the initial positions (inside or outside of refuge) as well as the availability of refuges could potentially impact the antipredator responses of *Nucella*.

Across all treatments, where *Nucella* makes the initial decision to move, in the vast majority of cases (88%) individuals choose the safety of a refuge (air and rock) over remaining in a vulnerable area of the tank. Previous investigators have argued that due to the difference in the speed of *Carcinus* and *Nucella*, the snails have “relatively little behavioural capacity to escape green crab predators once detected” (Donelan and Trussell 2019). The inherent difference in speed between predator and prey in this context, would place greater pressure on *Nucella* to adopt avoidance behaviours to evade a predator before they are detected themselves. As avoiding detection or reducing vulnerability to direct predation through the increased use of a refuge is highly context dependent (i.e. distance to suitable refuge), therefore *Nucella* are forced to continue seeking a refuge until they are safe. Intriguingly, we found that the distance travelled once *Nucella* chose to move and seek a refuge was the same regardless of parental habitat or the predation risk experienced during development. *Nucella* naturally face a range of adverse biological and environmental factors which may compel them to seek a refuge (Freeman and Hamer 2009). As remaining out of a refuge increases the chance of predation (Vadas et al. 1994, Donelan et al. 2017), desiccation (Etter, 1988) or dislodgement (Gosselin and Bourget 1989, Hughes and Taylor 1997), for individuals in both wave-exposed and wave-sheltered populations finding a safe refuge is of vital importance. Indeed, previous studies have argued that each habitat has its own dominant constraint on *Nucella* behaviour, with predation risk and wave-action being the primary mortality forces on wave-sheltered and wave-exposed shores respectively (Burrows and Hughes 1989, Freeman and Hamer 2009).

Perception of risk through development had an impact on the type of refuge selected by *Nucella*. Overall, the more time spend under predation risk throughout development, resulted in *Nucella* increasingly choosing to escape to the air refuge rather than selecting to hide in the rock refuge. *Nucella* reared under no risk conditions selected the air and rock refuge at approximately the same frequency, with individuals reared under constant risk selecting the air refuge almost three quarters of the time. The reasons for this distinction in refuge use based on the developmental experience of risk are unclear. One possible explanation is the potential for *Nucella* to have become habituated to *Carcinus* kairomones during exposure during development and thus produce an enhanced response. Such differences in the kairomone threshold required to induce antipredator traits have been previously highlighted in the closely related species *Nucella lamellosa* (Edgell 2010). *N. lamellosa* exposed to crab kairomones for 30 days, produce thicker shells when they are later exposed to dilute (50%) crab effluent, whereas unconditioned *Nucella* showed no response to such low concentrations of kairomones. Despite the reasons remaining elusive, our results show that *Nucella*’s choice of refuge can be influenced by individual long-term experience of risk. As the behaviour of individuals combine to have community level impacts, understanding the factors which affect the short-term behavioural responses of prey to the detection of a predator remains a key focus of community ecology.

# **Parental effect and individual experience of predation risk informs the foraging behaviour of a wave-exposed and a wave-sheltered population of *Nucella lapillus*.**

**CHAPTER FIVE**

Parental effect and individual experience of predation risk informs the foraging behaviour of a wave-exposed and a wave-sheltered population of

*Nucella lapillus*.

## Abstract

The impact of predation risk on the foraging behaviour of prey is profound. With prey relying on their energy reserves while remaining in the safety of a refuge between foraging bouts, the decision to forage in one instance will impact future foraging/predation risk trade-offs by influencing the energy reserves of prey. As these trade-offs form part of a larger foraging strategy, prey are also able to learn from previous experience and therefore the overall foraging strategy of prey can be influenced by past experience of risk. Apart from predation risk, wave-action places constraints on the foraging behaviour of intertidal gastropods, with many only able to forage under favourable conditions. I investigated the influence of the pattern of risk experienced by a wave-exposed and a wave-sheltered population of the intertidal dogwhelk, *Nucella lapillus*. I first reared offspring from these two populations under constant and unpredictable risk using effluent from the crab, *Carcinus maenas*, and then analysed their foraging behaviour under different predator treatments. My results showed that the foraging behaviour of *Nucella* offspring from each of the two sites was affected differently depending on their developmental experience of risk. Overall, the foraging behaviour of the sheltered shore population was unaffected by either developmental risk or predator treatment. In contrast offspring of the wave-exposed population changed their foraging behaviour as well as their likelihood to utilise refuges, although no changes were recorded in the number or the average size of the barnacles consumed. As only represented by a single wave-exposed and a single wave-sheltered population, no firm conclusion as to the influence of parental habitat can be drawn based on these results. Despite this, my results indicate that the pattern of risk experienced throughout development may impact the foraging strategy of *Nucella* offspring, although this may be dependent on physical/biotic conditions as indicated by the clear differences between the populations drawn from different wave exposures.

## Introduction

Predation risk has been identified as a strong driver of the foraging behaviour of many intertidal invertebrates (Menge, 1978; Trussell, Ewanchuk, & Bertness, 2003; Trussell, Matassa, & Ewanchuk, 2017). In most cases finding and consuming resources comes at the expense of reductions in vigilance and antipredator behaviour, resulting in trade-offs between resource acquisition and predator avoidance (Dill, 1987; Lima & Dill, 1990). For intertidal gastropods, such as the common dogwhelk *Nucella lapillus* (hereafter *Nucella*), for example, foraging generally requires individuals to leave a refuge in search of food in more exposed areas (Donelan et al. 2017), where they are more likely to encounter the predatory crab, *Carcinus maenas* (hereafter *Carcinus*, Burrows & Hughes, 1989). However, permanently remaining in a refuge is not an option irrespective of the levels of risk, as animals need to acquire a minimum amount of energy to survive (Lima and Bednekoff 1999). Prey are therefore forced to forage as their energy reserves begin to decline. For example, starved *Nucella* are 2.6 times more likely to forage than satiated individuals (Matassa & Trussell, 2014) when under risky conditions. Furthermore, since the choice of whether or not to forage is influenced by an individuals’ digestive and energetic state (Burrows and Hughes 1991a, Lima and Bednekoff 1999), the decision to forage will impact current energy reserves, and in turn affect future foraging decisions. As a result, prey must assess and respond to current levels of risk based on their previous experience, and the likelihood of starvation if a foraging opportunity is overlooked (Lima and Bednekoff 1999).

As with most intertidal organisms, *Nucella* have adapted to a range of environmental conditions, which can influence predator/prey interactions, and ultimately shape their foraging behaviour (Feare 1970, 1971, Crothers 1985). In the case of *Nucella*, as crabs are detected via chemical cues transported in the water column, changes in flow speeds and turbulence influence their transport and ultimately affect how *Nucella* perceive risk (Weissburg et al. 2002b, Large et al. 2011). For instance, when the antipredator responses of *Nucella* exposed to crab kairomones were tested under different flow velocities, *Nucella* only responded at intermediate flow rates, with no responses recorded in either low or high flow conditions (Large, Smee, & Trussell, 2011). Although on small temporal and spatial scales abiotic factors are likely to fluctuate, when such factors are viewed over larger spatial and temporal scales they become more predictable (Ballantine 1961, Underwood and Chapman 1996, Burrows et al. 2008a). With wave exposure being a function of the average wave fetch of each site, wave-action differences between sites are one of the most predictable and influential abiotic factors faced by intertidal species (Burrows, Harvey, & Robb, 2008). In addition to affecting predator detection, wave action also influences the abundance of predators (Hughes and Elner 1979). As well as increasing the likelihood of being dislodged, increases in flow speed have been shown to decrease the foraging time and overall hunting efficiency of crab predators (Robinson, Smee, & Trussell, 2011; Hughes & Elner, 1979; Yamada & Boulding, 1996). Therefore persistent differences in wave exposure between sites result in differences in both predation pressure as well as its influences on community dynamics (Ballantine 1961, Menge 1976). At wave-sheltered sites where wave action is consistently low, predator numbers are high and predation pressure plays an important role in community dynamics (Menge and Olson 1990). On the other hand, in wave-exposed sites it is the physiological stress placed on animals by the harsher environmental conditions that drives ecological processes (Hughes and Taylor 1997, Freeman and Hamer 2009).

These longer-term differences between wave-exposed and wave-sheltered sites have been shown to influence the foraging behaviour of *Nucella*. In one instance, Freeman and Hamer, (2009) showed that *Nucella* from wave-sheltered sites almost halved the number of mussels they consumed under predation risk, whereas exposed shore individuals showed no difference in foraging rates between risk treatment and controls. The authors argue that any differences seen in the foraging rates of *Nucella*, would likely relate to interpopulation differences in their experience of predation risk. Further support for the important influence of prior experience on how *Nucella* respond to predation risk come from a study by Large and Smee (2013) in the Gulf of Maine, where both *Nucella* and *Carcinus* are an invasive species. The study compared the foraging behaviour of *Nucella* from sites where they had an established history with *Carcinus*, with that of *Nucella* from areas that had yet to be invaded. They found that only *Nucella* from wave-sheltered sites that had an established history with *Carcinus* reduced their foraging rates in the presence of risk. These investigations highlight potential differences in the predation risk responses of *Nucella* according to the likelihood of encountering or detecting *Carcinus* in their natural environments.

Adaptive antipredator responses, however, are not necessarily restricted to a single generation, but in some cases, parents are able to influence their offspring’s antipredator traits, through adaptive parental effects (Marshall and Uller 2007). These transgenerational effects were first recorded in *Nucella* in relation to the heritability of shell morphology, between exposed and sheltered shores. Pascoal et al., (2012) showed that laboratory reared *Nucella* retained their parental shell morphologies, but that differences diminished with subsequent generations. Other studies have shown the ability of *Nucella* to inform the behaviour of their offspring (Donelan and Trussell 2018b, 2019). When parental couples of *Nucella* were exposed to predation risk, they produced offspring that were 30% more likely to utilise refuges when exposed to predatory cues, compared to responses of offspring from risk naïve parents (Donelan and Trussell 2018b). In this way, *Nucella* (as is the case with many other prey species) are able to prepare the subsequent generation for the potential biotic and abiotic conditions they may face.

The aim of this chapter is to investigate the foraging activity of offspring of *Nucella* from two populations naturally exposed to different predation pressure, reared under different predation risk. First, a wave-exposed and a wave-sheltered population of *Nucella* were bred in the laboratory under a no risk, a constant risk and a variable risk regime throughout their development. After approx. 8 months *Nucella* were then used in a 17-day foraging experiment, where they were assigned to one of three predator treatments: no predator, constant predator and variable predator. Firstly, I investigated the behavioural impacts of different temporal patterns of predation risk on the likelihood of *Nucella* to forage or to seek the safety of a refuge. I also counted the number of barnacles consumed throughout the experience and calculated the average size of barnacle consumed.

## Methods

### Site selection and *Nucella* Breeding

Approximately 200 adult *Nucella* were collected from two rocky intertidal sites on the coast of Anglesey in December 2018. These sites differed in their exposure to wave action, as described in detail in chapter 1. The first, Cemlyn bay is a wave-exposed site, with an average wave fetch index of 55.64 and as with many wave-exposed sites, is dominated by a barnacle zone. The second site, Porth Cwyfan is a wave-sheltered site dominated by macro algal cover (mainly fucus species) and has an average wave fetch index of 26.62. In addition to their wave fetch, both these sites also differ in crab abundances, with the wave-sheltered shore having 45.66 (± 4.48 SE) crabs per trap compared to the wave-exposed site where crab abundance is 1.33 (± 0.88 SE) crabs per trap. See chapter 1 for a detailed description of the methods used to estimate crab abundances at these sites. The combination of the differences in wave-action (mixing) and crab abundances results in *Nucella* at these sites being subjected to different levels of predation pressure (Leonard et al. 1998, Smee et al. 2010, Large et al. 2011, Large and Smee 2013).

After *Nucella* were collected from each site they were transported to the Nuffield laboratories, Bangor University, Menai Bridge, where breeding and the foraging study took place. Animals from each site were held in separate site-specific breeding tanks, where they were provided with barnacle covered rocks as food *ad libitum,* in water mirroring local ambient sea temperature on a 12:12h light:dark cycle. *Nucella* are internal fertilisers and produce egg capsules (10s per female) which they attach to moist rocky overhangs and crevices (Crothers 1985). Each egg capsule contained 10-30 fertilised embryos and 100s of nurse eggs used to nourish the developing embryos (Pechenik et al. 1984). Around the coast of Anglesey, *Nucella* normally form breeding aggregations in January and February each year and then deposit their egg capsules in spring (March – April, Crothers, 1985; Morton, 2009). In the laboratory, *Nucella* from both the exposed and the sheltered site formed breeding aggregations in February and deposited their egg capsules within a two-week period in mid-March. Once deposited, egg capsules were carefully removed with the aid of a scalpel blade and 8 randomly selected egg capsules were transferred into separate mesocosms where they were exposed to different risk regimes throughout their development (See risk regimes below). Exposure to the different risk regimes began while *Nucella* were still in the embryonic stage, as previous work has shown that exposure to cues from *Carcinus* while still in the egg capsule influenced several pre- and post-consumptive processes in *Nucella* (Donelan and Trussell 2019). Once hatched, juvenile *Nucella* were provided with barnacles covered rocks, providing simultaneous food and refuge. Juvenile *Nucella* were fed newly recruited barnacles followed by adult barnacles once large enough to consume them.

### Risk Regimes

In order to test the effects of individual experience of risk on the foraging activity of *Nucella*, offspring of field collected animals were reared under three developmental risk regimes. Firstly, offspring assigned to the no risk regime, were not exposed to a predator at any point of their development. *Nucella* reared in the constant risk regime, however, were exposed to a predatory cue throughout their development. Finally, animals reared in the variable risk regime, were exposed to crab effluent 50% of the time. The 50% treatment in the variable risk regime was achieved by adding or removing predatory cues every 3 or 4 days. Crab cues were introduced to each mesocosm by adding and removing a large male crab (mean carapace length ± SD, 56.6 ± 4.8 mm), housed in a perforated “crab pot”. These perforated crab pots allowed kairomones to diffuse into the mesocosm but did not allow crabs to attack dogwhelks directly. *Nucella* were exposed to the risk regimes for a total of 8 months prior to the foraging experiment described below.

### Foraging experiment

Offspring of *Nucella* from a wave-exposed and a wave-sheltered site reared under the three long-term risk regimes were used to assess how exposure to risk during development influenced the foraging behaviour and growth. Prior to beginning the foraging experiment all *Nucella* were starved for seven days to standardise their digestive state (Matassa and Trussell 2014). After the starvation period, tagged pairs of *Nucella* reared under the same risk regime were transferred to foraging mesocosms (300 mm x 200 mm x 100 mm) where they were subsequently exposed to one of three short-term predator treatments. Predator treatment manipulation was once again achieved by adding and removing crabs from crab pots as described in the *Risk Regime* section above. Over the 17-day experiment I exposed *Nucella* to a no predator treatment, a constant predator treatment or a variable predator treatment and their foraging behaviour was monitored (Table 5.1). Once again, *Nucella* in the no predator treatment were not exposed to a predatory cue throughout the foraging experiment, whereas animals in the constant risk treatment were continually exposed to a predatory cue for the entirety of the experiment. Finally, in the variable predator treatment animals were exposed to crab effluent 75% of the time, by manipulating the risk as shown in the schematic below (Table 1). It was decided to set the variable predator treatment at 75% exposure to predation risk, as more time spent under risky conditions would force prey to decide to forage during risky periods, rather than simply foraging during safe periods (Matassa and Trussell 2014). The experiment could not be monitored on day 14 (25th December 2018) as the university buildings were inaccessible, and therefore it was omitted from the final analysis. Overall, offspring from each of the two sites were reared under the three risk regimes and then exposed to three predator treatments resulting in 18 treatment combinations. Table 5.2 provides a breakdown of the level of replication of each treatment combination, as this was defined by the numbers of offspring produced during the breeding.

***Table 5.1*** *Schematic of the pattern of exposure of Nucella to the three different short-term predator treatments. Red indicates the presence of crab in the crab pot and white identifies predator free days.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| No  Predator |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Variable Predator |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Constant Predator |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

***Table 5.2*** *Breakdown of the level of replication (number of mesocosms) for the 18 treatment combinations used in the foraging experiment.*

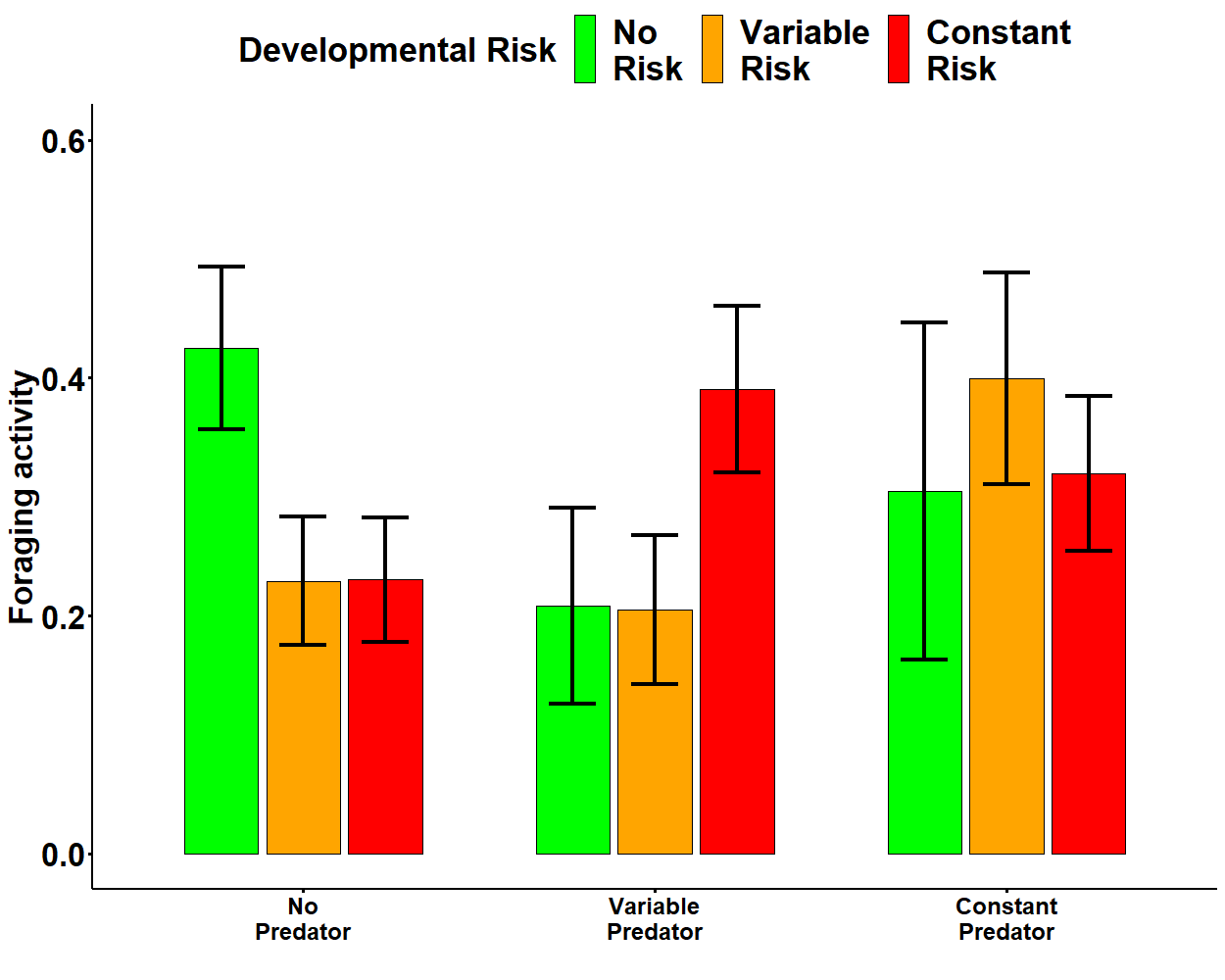
|  |  |  |  |
| --- | --- | --- | --- |
| Risk Regime | Predator treatment | Wave-exposed site | Wave-sheltered site |
| No Risk | No Predator | **5** | **5** |
| No Risk | Variable Predator | **4** | **4** |
| No Risk | Constant Predator | **5** | **4** |
| Variable Risk | No Predator | **5** | **6** |
| Variable Risk | Variable Predator | **5** | **5** |
| Variable Risk | Constant Predator | **6** | **6** |
| Constant Risk | No Predator | **6** | **6** |
| Constant Risk | Variable Predator | **6** | **5** |
| Constant Risk | Constant Predator | **6** | **6** |

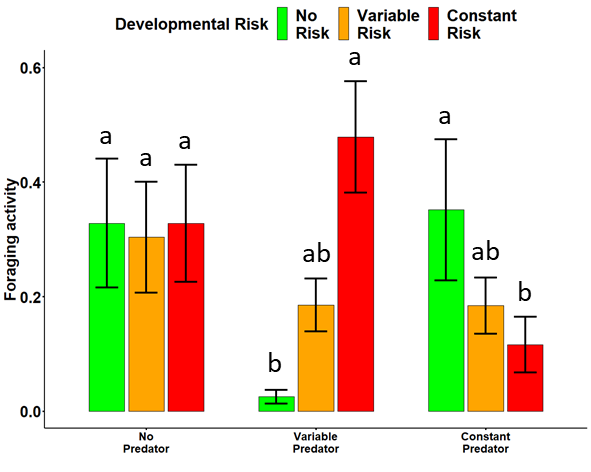
Barnacle covered rocks were added to each of the foraging mesocosms to provide pairs of *Nucella* with a foraging opportunity as well as a refuge to hide in. Barnacles were scraped off the undersides of rocks to ensure only the tops of the rocks had barnacles attached to them, as determining if an individual is foraging or not while in a refuge is challenging (Matassa and Trussell 2014). Foraging activity was recorded every 24 hours for the 17-day duration of the experiment (except on day 14) before any changes to risk in the variable predator treatment were conducted. *Nucella* were classified as “foraging”, if they were on top of a barnacle for 20s or “Safe” if they were in a “rock refuge” or an “air refuge” (crawled above the water line). Although our definition of foraging is somewhat crude, it is impossible to determine if *Nucella* are actively foraging without disturbing them (Matassa and Trussell 2014). Rocks were photographed before and after the experiment, to enable an estimate of the numbers of barnacles consumed as well as the average size of the barnacle consumed in each mesocosm. Control rocks (no predatory cue) were placed in empty mesocosms (n= 6) and did not experience any mortality over 17-day experiment. Average barnacle size for each mesocosm was calculated by measuring the opercular opening of 5 – 10 consumed barnacles, depending on how many barnacles were consumed in each mesocosm.

### Data Analysis

All data was analysed using the *lme4* package (Bates et al. 2013) in R 3.5.0 (<www.r-project.org>, R-Studio, 2012). As only a single wave-exposed and wave-sheltered site was used in this experiment, all variables tested were analysed separately for each site. For each site, I analysed the potential influence which developmental exposure to risk and the short-term predator treatment had on the (i) foraging behaviour, (ii) refuge use, (iii) the number of barnacles consumed and (iv) the average size of the barnacle consumed by the offspring generation of *Nucella*. To analyse both foraging behaviour and refuge use, I divided the number of observations during which *Nucella* were foraging or utilising a refuge, by the total number of observations. The resulting frequencies were then used in the analysis. The total number of barnacles consumed in each mesocosm was divided by two to calculate the per capita number of barnacles consumed before being analysed. I used a Levene’s test to test for homogeneity of variance utilising the *car* package (Fox and Weisberg 2019). As all variables had equal variances, they were then analysed using linear mixed-effects models with developmental exposure to risk and predator treatment as fully crossed fixed factors and mesocosm as a random factor. Where interactions were found to be significant, Tukey’s post hoc tests were used, utilising the *lsmeans* package (Lenth 2016).

## Results

**Sheltered Shore**

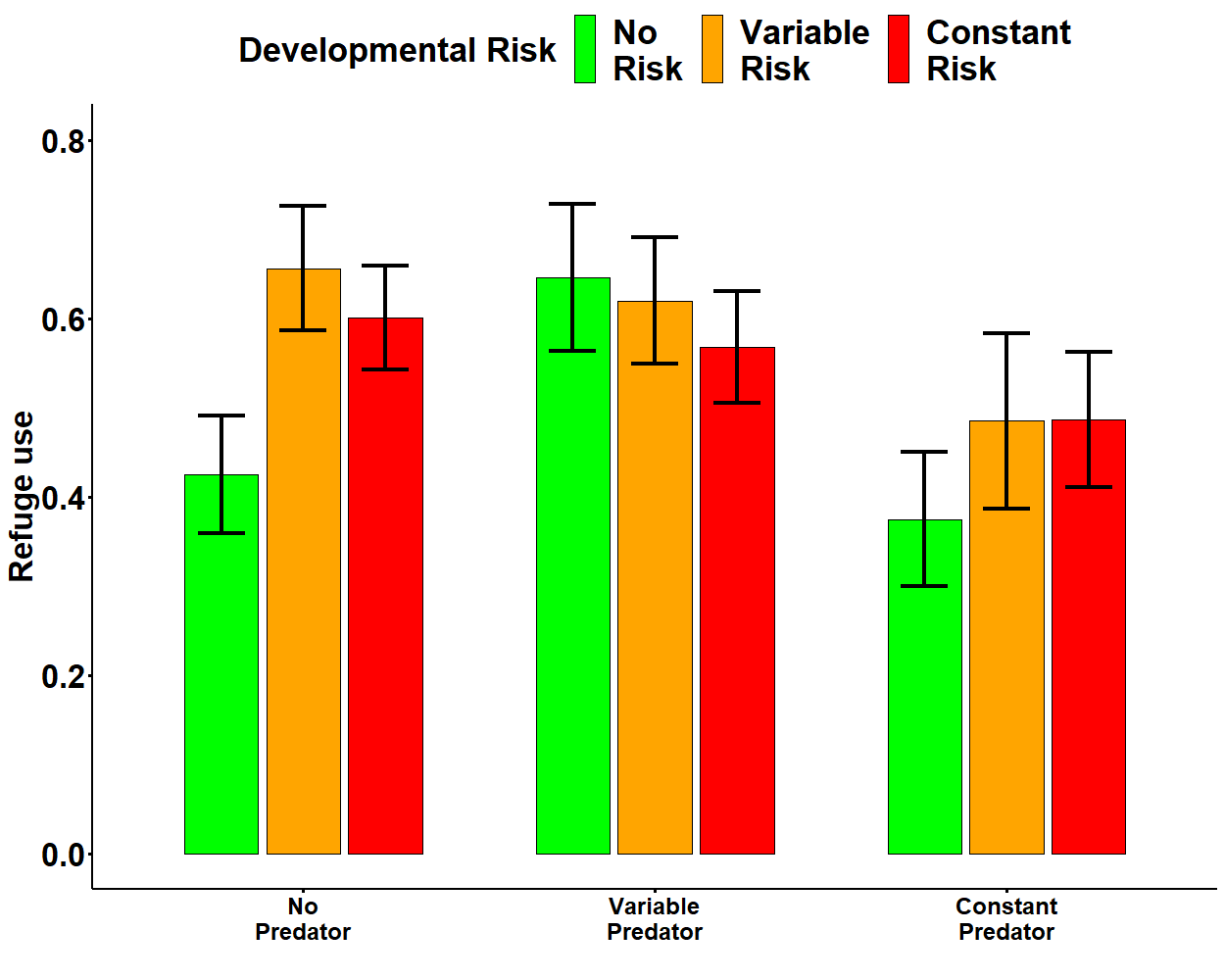
**Exposed Shore**

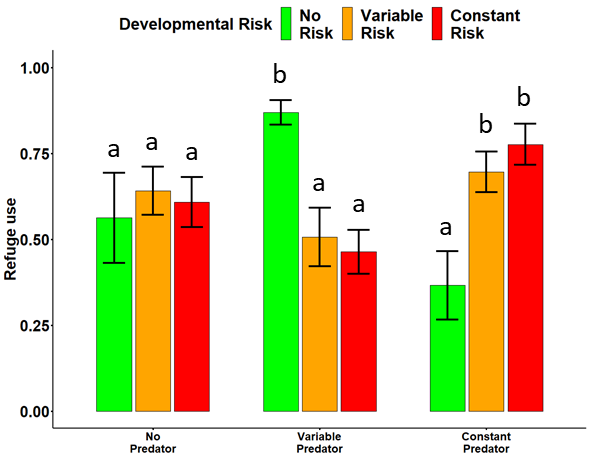
***Figure 5.1*** *The average foraging activity (proportion of snails foraging) of the offspring generation of a) wave-sheltered b) wave-exposed Nucella reared under no risk (green), variable risk (orange) and constant risk (red) treatments. Values are means ±SE. Different letters**indicate significant differences (Tukey’s HSD,* *=0.05).*

There was a distinct difference in how threat of predation affected the behaviour of *Nucella* from sheltered and exposed shores. Overall, the foraging behaviour of offspring of the wave-sheltered population was not influenced by either the risk experienced throughout development or the short-term predator treatments they were exposed to during the foraging experiment (Fig. 5.1 A, Table 5.3). In contrast both of these types of predatory threat affected offspring from the wave-exposed population. Analysis of the foraging activity of offspring of the wave-exposed population, revealed an interaction between the developmental experience of risk and predator treatment (*lm*, F1,32 = 4.93, p = 0.003). *Nucella* reared under the three risk regimes had similar foraging frequencies when not exposed to risk (Fig. 5.1 B) during the foraging experiment. Tukey post-hoc testing revealed *Nucella* in the variable predator treatment that were reared under no risk conditions foraged less (Tukey pairwise comparison: *p* = 0.003), than those reared under a variable and constant risk treatment. Moreover, *Nucella* in the constant predator treatment, foraged less frequently (Tukey pairwise comparison: *p* = 0.05), if they were reared under constant risk than if they experienced no risk or variable risk during development (Fig. 5.1 B).

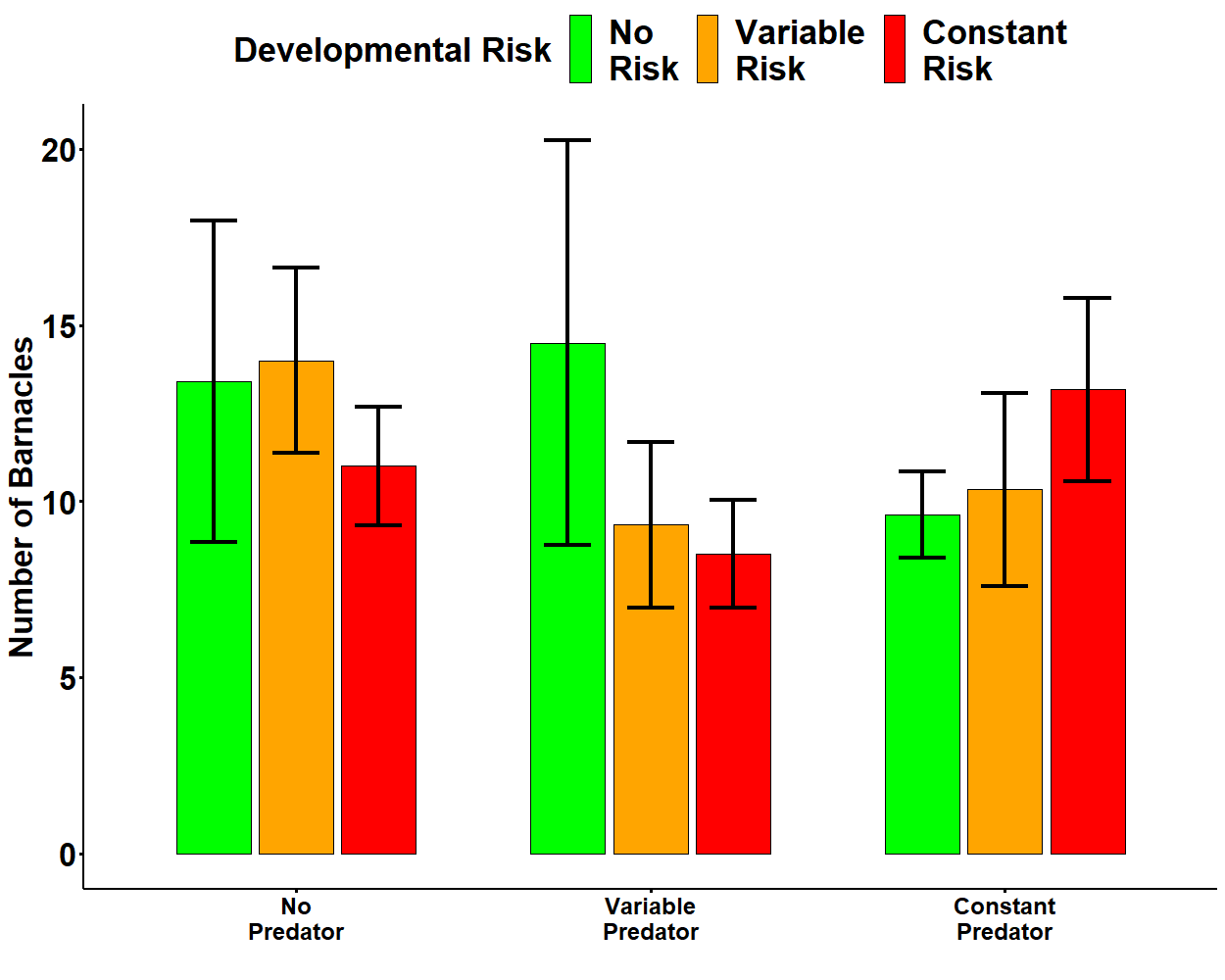
Similarly, to *Nucella* foraging behaviour, my analysis revealed that refuge use in wave-sheltered offspring was unaffected by any of the treatment combinations (Fig 5.2 A, Table 5.3). Refuge use in wave-exposed *Nucella* was influenced by the combined effect of both developmental risk and predator treatment (*lm*, F1,32 = 5.89, p > 0.001). *Nucella* reared under no risk conditions throughout development, selected the safety of a refuge more frequently when exposed to variable risk than when there was no risk (Tukey pairwise comparison: *p* = 0.003) or when the risk was constant (Tukey pairwise comparison: *p* = 0.001).

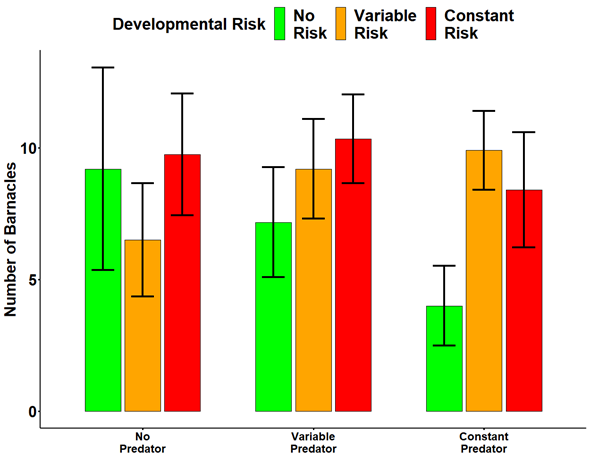
*Nucella* offspring of both populations consumed the same number of barnacles per mesocosm as well as consuming the same average size barnacle across all treatment combinations (Fig. 5.3 and 5.4). Overall, wave-exposed offspring consumed 26% fewer barnacles (mean 8.55 ± 4.90 SD), that were on average 16% smaller (mean 2.175 mm ± 0.373 SD) compared to those consumed by wave-sheltered counterparts (mean number of barnacles consumed 11.68 ± 6.55 SD, average barnacle size 2.59 mm ± 0.378 SD). However, although not significant, analysis revealed that the average size of barnacle consumed by wave-sheltered offspring tended to be affected by the predator treatment they were exposed to (*lm*, F1,32 = 3.08, p = 0.058), with *Nucella* consuming the largest barnacles when exposed to the variable treatment, followed by no predator treatment and then finally the smallest barnacles were consumed in the constant predator treatment.

**Sheltered Shore**

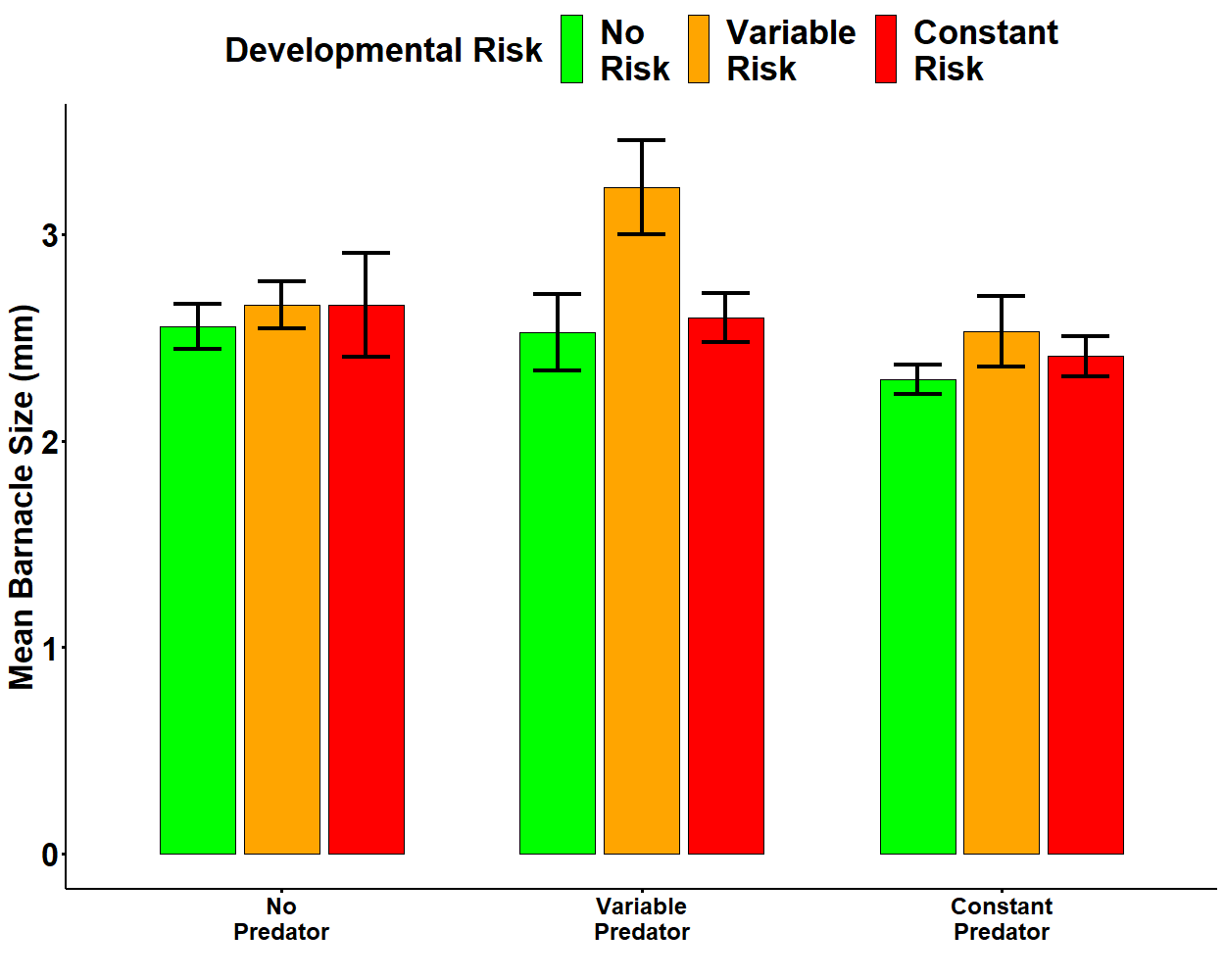
**Exposed Shore**

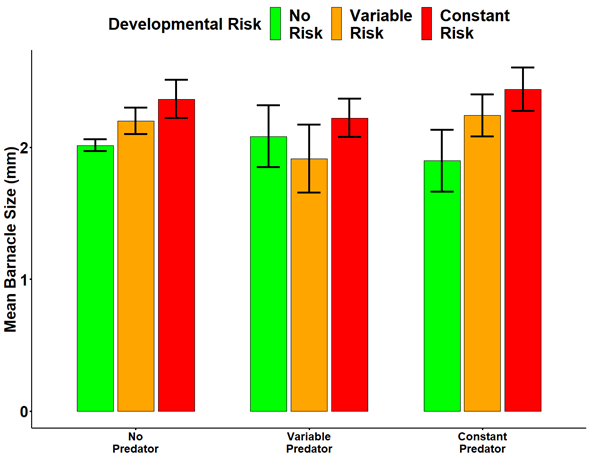
**Figure 5.2** The average foraging activity (proportion of snails foraging) of the offspring generation of a) wave-sheltered b) wave-exposed *Nucella* reared under different long-term risk regimes. Values are means ±SE. Different lettersindicate significant differences (Tukey’s HSD, =*0.05).*

**Sheltered Shore**

**Exposed Shore**

***Figure 5.3*** *Average number of barnacles consumed by the offspring generation of a) wave-sheltered b) wave-exposed Nucella reared under different long-term risk regimes. Values are means ±SE. Different letters**indicate significant differences (Tukey’s HSD,* =*0.05).*

**Sheltered Shore**

**Exposed Shore**

**Figure 5.4** Mean barnacle size consumed by offspring generation of a) wave-sheltered b) wave-exposed *Nucella* reared under different long-term risk regimes. Values are means ±SE. Different lettersindicate significant differences (Tukey’s HSD, =*0.05).*

***Table 5.3*** *Summary of full linear mixed model results of foraging activity, refuge use, number of barnacles consumed, average size of barnacle consumed, and total amount of energy consumed by offspring from a wave-exposed and a wave-sheltered population of Nucella lapillus reared under different risk treatments.*

Response variable Effect *Df* *F P*

**Sheltered shore *Nucella***

Foraging Activity Develop risk 2 0.281 0.756

Predator Treatment 2 1.032 0.365

Pt x Dr 4 1.468 0.229

Error 49 105.9

Refuge use Develop risk 2 0.85 0.433

Predator Treatment 2 2.43 0.101

Pt x Dr 4 1.00 0.418

Error 49 325.04

Barnacles consumed Develop risk 2 0.19 0.829

Predator Treatment 2 0.39 0.681

Pt x Dr 4 0.744 0.568

Error 38 325.04

Av. Barnacle size Develop risk 2 2.02 0.146

Predator Treatment 2 3.08 0.058\*

Pt x Dr 4 0.65 0.634

Error 38 4.73

**Exposed shore *Nucella***

Foraging Activity Develop risk 2 0.584 0.562

Predator Treatment 2 2.06 0.141

Pt x Dr 4 4.93  **0.003**

Error 46 77.55

Refuge use Develop risk 2 0.05 0.953

Predator Treatment 2 0.45 0.642

Pt x Dr 4 5.89 **> 0.001**

Error 46 452.82

Barnacles consumed Develop risk 2 0.94 0.400

Predator Treatment 2 0.29 0.749

Pt x Dr 4 0.824 0.519

Error 34 864.38

Av. Barnacle size Develop risk 2 1.19 0.317

Predator Treatment 2 0.46 0.637

Pt x Dr 4 0.913 0.913

Error 34 560.34

*Statistically significant models* *( = 0.05) are in bold and marginally significant (> 0.06) results are indicated with a (\*).*

## Discussion

Overall, my results revealed that the foraging behaviour of *Nucella* offspring from the wave-sheltered populations was not affected by developmental experience of risk or predator treatment, whereas foraging in offspring of the wave-exposed population was influenced by a combination of current and previous exposure to risk. It is important to acknowledge, that although my results show clear differences between the offspring of the two populations tested here, as only a single population of each wave exposure was used in this study, conclusions cannot be ascribed to differences in wave-exposure. Offspring of the wave-exposed population reduced their foraging activity under risky conditions as well as selecting the safety of a refuge more frequently when exposed to a predatory cue. In both of these cases responses were different depending on the developmental experience of risk *Nucella* were reared in. The foraging activity of offspring of the wave-exposed population when no predatory cue was present were the same irrespective of developmental experience of risk, indicating that developmental exposure of risk did not impact foraging activity during safe periods. However, when *Nucella* were exposed to either variable or constant risk, they responded differently. When foraging under the variable predator treatment, *Nucella* offspring foraged less frequently if they were predator naïve (reared under no risk during development) and more frequently when constantly exposed to predation risk during development. However, when foraging under constant predator treatment, predator naïve offspring (those reared in the no risk regime) foraged more frequently than those exposed to a constant risk throughout development. The differences in foraging activity between each predator treatment can also be seen in the refuge use of offspring of the wave-exposed population. As analysis of the frequency of refuge use revealed the opposite pattern to that seen in foraging, it indicates that when *Nucella* were not foraging they were generally utilising refuges. Finally, in contrast to what I expected based on previous studies (Vadas et al. 1994, Trussell et al. 2011, Matassa and Trussell 2014) the number of barnacles consumed by *Nucella* offspring was not influenced by exposure to risk while foraging, although our results showed a trend for wave-sheltered *Nucella* to choose smaller barnacles on average when under constant risk.

In contrast with previous results (Freeman and Hamer 2009, Freeman et al. 2014, Dernbach and Freeman 2015), only *Nucella* offspring from the wave-exposed population altered their foraging behaviour in response to predation risk, whereas the foraging behaviour of offspring of the wave-sheltered population was unaffected by risk. This result is surprising as previous experience of predation risk has been shown to be an important driver in the foraging behaviour of *Nucella* as well as many other prey species (Freeman et al. 2014). Similarly, results of the use of refuges by *Nucella* followed the opposite pattern of that seen in my foraging results, but once again were counter to what I expected based on previous findings (Donelan and Trussell 2015). My analysis did reveal that foraging and refuge uses behaviour of wave-exposed offspring was different according to the risk they experienced throughout development. Firstly, offspring of wave-exposed *Nucella* reared under the three risk regimes foraged and utilised refuges at the same frequency when exposed to no risk. This result indicates that when under no risk, the foraging and refuge use behaviour of *Nucella* from this population was unaffected by the risk they were exposed to throughout development (Donelan and Trussell 2018b). However, both these behaviours differed when *Nucella* were exposed to a level of risk. When risk was unpredictable (variable predator treatment), predator naïve offspring foraged less frequently and utilised refuges more often than offspring that had previous experience of predatory cues (variable risk and constant development exposure to risk). However, when forced to make foraging and refuge use decisions under constant risk, predator naïve *Nucella* foraged more frequently and used a refuge less frequently than did offspring exposed to a predator (both variable and constant risk treatments). These results seem to suggest that previous experience of predation risk during development influences how *Nucella* respond to current levels of risk. When risk is unpredictable, predator naïve *Nucella* respond strongly, by reducing their foraging activity and increasing their use of refuges, whereas when risk is constant, the same predator naïve *Nucella* are seemingly able to overcome the influence of the predatory cues and forage at levels similar to those under seen under the no predator treatment.

*Nucella* offspring from the two populations consumed the same number of barnacles irrespective of developmental exposure to risk or predator treatment. Several previous studies have shown that *Nucella* consume fewer barnacles when exposed to predatory cues (Trussell et al. 2006b, Matassa and Trussell 2011, Matassa et al. 2016) a pattern that was not seem in my results. However, my results showed that, overall wave-exposed *Nucella* consumed fewer and smaller barnacles than those consumed by the wave-sheltered population. These results do confirm earlier studies which have shown that exposed shore *Nucella* tend to consume fewer and smaller barnacles than do *Nucella* from more wave-protected sites in the field (Menge 1978b) and under laboratory conditions (Trussell et al. 2006b, Dernbach and Freeman 2015). In one instance, Palmer (1990) showed that *Nucella* exposed to crab kairomones reduced the number of barnacles they consumed by up to 75% depending on the level of risk they were exposed to. The lack of a difference in the number of barnacles consumed under risk in the current study when compared to results from previous investigations may be due to *Nucella* in the current study being reared under laboratory condition, whereas previous studies have been conducted on field collected animals (Trussell et al. 2006b, Matassa and Trussell 2014).

Analysis of the size of barnacle consumed by *Nucella* in this experiment did not reveal any significant interaction between developmental experience of risk and predator treatment, although some interesting patterns did emerge. Firstly, as stated earlier, offspring of the wave-exposed population of *Nucella* selected smaller barnacles across all treatment combinations than wave-sheltered offspring. Secondly, my results showed a strong trend (*p* = 0.056), that although not significant, does follow similar trends as other predators of reducing the size of prey being consumed when under risky conditions. However, this trend was only present for wave-sheltered offspring. It is important to restate that *Nucella* generally have to leave the safety of a refuge in order to forage and are therefore naturally more exposed to abiotic and biotic factors. In the case of wave-exposed *Nucella* consuming smaller barnacles than wave-sheltered snails, previous authors have argued that the reduction in handling time gained by choosing smaller prey, also reduced the amount of time they are exposed to wave-action (Burrows and Hughes 1991b). Moreover, *Nucella* on wave-sheltered shores tend to be more exposed to predation risk while foraging (Menge 1978a), especially as resources are depleted near to refuges quickly and they are therefore forced to venture into more risky areas to forage (Donelan et al. 2017). As *Nucella* from wave-sheltered populations are subjected to higher levels of predation pressure, potential changes to the handling times of prey will reduce the amount of time they are out of a refuge and increase their chances of survival. This trend should, however, be viewed cautiously, as previous studies have shown that *Nucella* prey selection remains constant irrespective of the level of risk perceived, although these results refer to the selection of mussel prey rather than barnacles (Quinn et al. 2012).

# **General Discussion**

**CHAPTER SIX**

General Discussion

## General overview

The impacts that predators have on prey and the ecological systems they inhabit are indisputable, but appreciation of the indirect effects of predation risk is only recently becoming apparent (Lima 1998b, Lima and Bednekoff 1999, Duffy 2003, Bednekoff and Lima 2011). Recent advances in the investigation of these indirect effects have shown them to be context dependant in many instances, with both biotic and abiotic interactions adding to their complexity (Bartley et al. 2019). With the influence of past experience, prey state, differences in the temporal pattern of risk experienced, refuge quality as well as the potential for parental effects to guide antipredator traits in prey, there have been increased calls for investigations to test the expression of different antipredator responses under different contexts (Lima and Bednekoff 1999, Ferrari and Chivers 2009, Matassa and Trussell 2014, Ferrari et al. 2015). This thesis therefore aimed to test the antipredator responses of an intertidal dogwhelk under different predation risk scenarios, focusing on the risk experienced throughout development and the potential for parental effects to affect responses. The results presented above, demonstrate the potential predator-induced changes in metabolism, in the long-term energy budget and relative investment in somatic growth and defensive morphologies, the impacts on escape and avoidance behaviour and finally on the foraging strategy of *Nucella*. Although the results presented in this thesis undoubtably illustrate the impacts predation risk plays in the life history of *Nucella*, it also adds to the growing literature highlighting the profound role predators play in the lives of prey more generally. Changes to the accumulation of energy, its short- and long-term storage and utilisation as well as the relative investments in growth and defensive morphologies highlighted here can impact prey demography and cause wider reaching ecological impacts through cascade effects (Trussell et al. 2003, 2006b, 2017). Although not directly tested in this thesis, the mechanisms which underpin the antipredator response in prey highlighted here, may also be useful in understanding the importance that predators play on community dynamics and ecosystem functioning (Stoks et al. 2005, Hawlena and Schmitz 2010b). As future global climate change increases the pressure on already depleted populations of predators, it is important to understand the mechanisms that underpin species interactions, if we are to accurately predict and respond to such changes (Bartley et al. 2019).

### Pervasive impacts of predation risk

Despite the recognition of an organism’s body-size as one of the most important driving forces in ecological interactions (De Roos et al. 2003), few studies have attempted to incorporate differences in body size into investigations of the indirect effects of predation risk (Rochette and Himmelman 1996, Rochette et al. 1999). Moreover, as increases in body size may constitute an animals natural defence against direct predations (Hughes and Elner 1979, Chase 1999), it is surprising that more studies have not tested the indirect effects of predation on prey at a size refuge. In Chapter 2, I demonstrated that *Nucella* which had grown to a size refuge, reduced the amount of oxygen they consumed when exposed to predation risk in the field. These results provide the first insight into the pervasive nature of the indirect effect of risk in this predator-prey system, and their potential ability to persist even after pressure from direct predation has dissipated. Studies into the responses of prey with differing vulnerabilities to direct predation, although rare, have highlighted both behavioural (Rochette et al. 1996) and foraging (Rochette and Himmelman 1996) differences between the different size classes. Therefore, further investigations into other aspects of antipredator responses in *Nucella*, may help us better appreciate the impacts of risk on these larger size classes.

Investigations into the influence of predation risk on prey at a size refuge will also be important for explaining the large-scale impacts of predation risk, as these size classes disproportionately influence ecological level processes (De Roos et al. 2003). For example, large individuals have higher foraging rates (Hughes and Dunkin 1984, Matassa et al. 2016), and hence any impacts on the foraging of these larger size classes will have implications for prey demography and community dynamics. Without testing whether the indirect effect of risk persists into these size classes, animals at a size refuge are considered to have escaped the influence of predation. However, results such as the ones presented here show that more attention should be paid to the potential of the indirect effects of predation risk to continue to affect these ecologically important size classes.

In many systems, the relative importance of indirect effects as a result of direct predation (DMIIs) or brought through changes to the traits of prey (TMIIs), are still debated. For *Nucella*, previous studies have shown that despite their seemingly transitory nature, TMIIs have an equal or larger impact on ecological systems than DMIIs (Trussell et al. 2004, 2006a, 2017). Importantly, insights into the relative strength of DMIIs and TMIIs come from manipulative experiments that alter predator and prey densities and measure the resulting changes in the abundance of prey resources. In all cases, only prey that remained vulnerable to direct predation were used, presumably to guarantee that the indirect effects of predation risk would influence their expression of antipredator behaviours. As results presented here show, individuals at size refuge remain affected by predation risk and combined with the increases seen in barnacle consumption with larger body sizes (Matassa et al. 2016), indicates that the relative importance of TMIIs in this system may have been underestimated. Considerations of this type are important as they help us better understand how predators influence community dynamics through manipulations of their abundances or modifications of individual antipredator traits.

### The fluctuating nature of predation risk

The risk posed by predators is neither constant nor predictable, with daily changes in abiotic factors and the movements of both predators and prey, influencing the risk perceived by prey. As such, investigations into the indirect effects of predation risk should attempt to incorporate an element of unpredictability in their design in order to create treatments that best represent the risk experienced by prey in nature. Such studies have shown, for example, that predator-induced chances in growth efficiencies are not linear, but instead may be lower when risk is unpredictable rather than when it is constantly present (Trussell et al. 2011). They have also highlighted the importance that pattern of risk have on prey foraging strategy (Matassa and Trussell 2014), predator avoidance (Ferrari and Chivers 2009). However, short-term investigations into the antipredator responses of prey, where prey are exposed to risk for a matter of hours or days may not allow enough time for the expression of compensatory mechanisms (Thaler et al. 2012, Van Dievel et al. 2016). Prey are able to develop behavioural and physiological compensatory mechanism to help them ameliorate some of the negative impacts of predation risk in one instance and defer them to when the risk has subsided. Examples such as that provided by the tobacco hornworm caterpillar, *Manduca sexta*, which is able to postpone predator-induced inefficiencies in the assimilation of consumed energy to a period when the risk of being consumed subsides (Thaler et al. 2012). Results from Chapters 3, 4 and 5 have further highlighted the importance of incorporating an unpredictable risk treatment in long-term investigations into the antipredator responses of prey. For example, in previous investigations which consider risk as either present or absent *Nucella* attain smaller shell lengths under constant risk than when exposed to no risk conditions, a pattern that was repeated in the current study. However, the incorporation of a variable risk treatment revealed that when risk was unpredictable *Nucella*, grew differently depending on parental habitat.

### Potential climate driven changes

The impacts of climate changes are undoubtedly the most pressing challenge facing ecologists today. The potential for these accelerated changes to affect individual life histories, inter- and intraspecific interactions as well as potential catastrophic effects on ecosystem functioning must be identified and evaluated. Our ability to predict the effects of climate change on ecological communities, rests on our understanding of how environmental conditions influence the interaction of physical processes and species interactions. The net impact of large-scale environmental change on community structure, will therefore depend on how prey respond to both abiotic changes as well as how these influence the interaction of one species with another. Large scale changes such as the increased spread of invasive species, ocean acidification, increases in metabolic demands of ectotherms are predicted to follow global increases in temperature.

More specifically, with regard to intertidal invertebrate interactions, increases in ocean acidification will impact both predators and prey. It could impede the formation and robustness of gastropod shells (Coleman et al. 2014), as well as potentially weakening the attacking capabilities of some predators for example, by reducing the strength of crab claws (Landes and Zimmer 2012). Moreover potential increases in the metabolism of prey brought about by higher temperatures may outpace the increases in foraging speeds, resulting in overall reductions in growth (Iles 2014). Finally, the nature of predator-prey interactions may be affected through changes to the chemical cues themselves as well as changes to the medium through which they are transmitted. However, even closely related species may respond differently to large-scale environmental changes (Coleman et al. 2014), highlighting the importance of understanding the mechanisms which underpin the responses on organism. We must therefore aim to better understand the forces that drive community structure and influence ecosystems function if we are to best respond to environmental change.

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