THE UNIVERSITY OF HULL

Effects of multiple stressors on the stability of functional traits: examining individual variability in the green shore crab, *Carcinus maenas*, exposed to plastic odour and ocean acidification.

A Thesis submitted for the Degree of

MSc in Biological Sciences

at the University of Hull

Bethany Rose Howard, BSc Marine Biology

July 2022

Acknowledgements

I would like to thank my supervisors Jorg D Hardege and Helga D Bartels-Hardege for their constant support, encouragement, and feedback. I would also like to thank Hannah Ohnstad for being a great friend and research partner. Lastly, I would like to thank my family and friends for their unconditional love and support.

Abstract

The development of anthropogenic activities during recent centuries has led to an increase in environmental pollutants such as greenhouse gases causing atmospheric changes, resulting in elevated atmospheric heat retention, triggering global and ocean warming. The excess atmospheric carbon dioxide (CO_2) dissolved into the Earth’s oceans, reacts with water molecules in seawater, resulting in the ocean becoming more acidic and pH decreasing. This process, termed ocean acidification, threatens marine organisms alongside a multitude of other anthropogenic stressors, one of which being plastic pollution. Plastics enter the ocean at a rate of 8 to 10 million tonnes per year. The lethal and sub-lethal impacts of plastic on organisms have been described including ingestion and entanglement, through to wider population effects such as bioaccumulation of toxic compounds and info-disruption.

While global climate change and plastic pollution are acknowledged threats to marine life, the prospective interactions between changing environmental parameters and plastics remains unclear. There remain significant gaps in our knowledge as to how these two stressors interact and on their combined effects on ecosystems. Crustaceans serve a crucial part in the aquatic food chains and are fundamental within their ecosystems. In order to assess their future in an altered environment and assist management decisions, reliable knowledge and dependable data are critical.

This research builds on previous knowledge, identifying the effects of low pH on olfactory abilities on *Carcinus maenas* (Linnaeus, 1758), and explores the effects of plastic odour in tandem with ocean acidification. This thesis evaluates the effect of three levels of pH (8.2, 7.6, 7.2) on chemical communication and fitness in the green shore crab *C. maenas* when

also exposed to plastic odour cues. Individuality factors were also investigated, relating to body size and weight, sex, injury, and colour of the crabs.

The effects of synthetic and natural chemical cues were investigated at different test pH treatments for *C. maenas* for a range of behavioural responses including time taken for initial response (antennular flicking), time taken to reach the location of an odour cue, the cue that was contacted and behaviours elicited at the cue location.

The results of this study show that *C. maenas* experienced significant olfactory disruption in response to both feeding, and reproductive cues when in a reduced pH environment. Overall initial reaction times in crabs tested in low pH treatments (7.6 and 7.2) were significantly slower than the initial reaction times for crabs tested at pH 8.2. When individuals were tested with the plastic derived odour cues, there was an overall reduced response to cues with slower initial reaction time displayed. Additionally, when polyethylene (PE) plastic odour was present and tested in low pH treatments, reaction times to food and pheromone cues were significantly slower than treatments with plastic odour tested at normal pH 8.2. The data also demonstrated that *C. maenas* exhibited significant variation in response to cues. With initial reaction times showing significant variation between crabs of different size and colour. Additionally, it was found that individual variation relating to factors size, sex and weight had a significant impact on the choice of odour cue. These results may potentially consider long-term adaptation via selection for less sensitive individuals as a plausible outcome.

The observed response to chemical cues under different pH treatments suggests that pH is likely to impact the behavioural response to *C. maenas.* However, it is unclear as to whether this response is exacerbated by the presence of plastic odour and the relationship between these two stressors and how they interact is still unclear. The effect of pH on decision-making in *C. maenas* is a recognised concern and further study is essential to investigate the impacts of such multiple stressors. There is potential for plastic odour to become a more potent attractant under low pH conditions as a result of altered chemical signalling, which could have detrimental impacts on this species longevity.

This thesis examines the behavioural changes of crustacean species *C. maenas* during exposure to acidified seawater, plastic odour, and the combination effects of exposure to these stressors simultaneously. Following an introductory chapter- Chapter 1- Chapter 2 describes the methodology used for the present study, results, discussion, and conclusions.

Contents

1. Chapter 1: Introduction

1.1 Anthropogenic Impact on the Environment

Human impact on ecosystems is now irrefutable (Millenium ecosystem assessment, 2005). Within the last 150 years changes to the Earth's climate have increased exponentially with current global atmospheric CO_2 concentrations exceeding those reported for the last 800 000 years (IPCC, 2019). The human population continues to increase with advancements in medicine, technology and industrial farming resulting in lower mortality rates. However, overpopulation has become an epidemic with severe environmental impacts. A higher demand for fossil fuels, food sources and economic development results in severely damaged ecosystems due to deforestation and huge global CO_2 emissions. Deforestation reduces global carbon fixation rates to such an extent that the effect of increasing CO_2 is amplified (Houghton et al., 1991; Pan et al., 2011). Overharvesting organisms for food production is arguably the most direct impact humans have on ecosystems. Exploitation through fishing pressure increases extinction risks for many marine species that are harvested for consumption by human populations, such as Pacific salmon, genus Oncorhynchus, (Walters et al*.,* 2019) and tuna, genus Thunnini (Myers & Worm 2003). Overfishing can trigger ecosystems to shift beyond their natural equilibrium, meaning pressures upon species fitness are dramatically increased (Dulvy et al*.,* 2021). Interactions between humans and the environment can have profound effects on ecological communities, which can also consequently impact fundamental human requirements provided by ecosystem services, such as food security, economic stability, and climate regulation (Richardson, 2010; Smith et al*.,* 2013; Tai et al., 2014; Bommarco et al*.,* 2018; Hasan et al., 2020).

Coupled with humans' direct impact on ecosystems through activities such as hunting and destruction of natural habitats (Burney & Flannery 2005; Ellis et al*.,* 2013; Ayompe et al*.,* 2021), there are indirect impacts upon ecological systems that have cascading consequences on biodiversity that may not be immediately evident. Changing the physical environment that organisms depend on can also shift the structure of an ecosystem (Vitousek et al*.,* 1997; Western, 2001; Eriksson et al*.,* 2010; Murphy & Romanuk 2014; Peñuelas et al., 2013). As a result of industrialisation in the late 18th and early 19th century, anthropogenic carbon emissions have risen exponentially (Hung et al., 2020; Adamo et al., 2021; Crippa et al., 2021). The abundance of readily available fossil fuels like coal and crude oil derivatives were the drivers behind many factories and modern technologies and as a result of combustion increased quantities of gaseous carbon (Guy & Levine, 2001).

Approximately 90% of anthropogenic CO_2 emissions originate from the burning of fossil fuels and industry (Allwood et al*.,* 2010; Jackson et al*.,* 2016; Jackson et al*.,* 2017; Browman, H.I., 2017; Gao et al*.,* 2021). Aproximately 30% of the excess CO_2 emitted into the atmosphere is dissolved into the Earth’s oceans, buffering the planet's climate but having adverse effects on marine life (Broecker et al*.,* 1971; Bacastow & Keeling, 1973; Gattuso et al*.,* 1999; Kleypas et al*.,* 1999; Riebesell et al*.,* 2000; Seibel & Walsh, 2001; Langdon et al*.,* 2003; Kurihara et al*.,* 2004; Pörtner, et al*.,* 2004; Caldeira & Wickett, 2005; Reid et al*.,* 2009; Levin & Le Bris, 2015; Quéré et al., 2015; Roggatz et al., 2019). CO_2 emissions have continued to rise in line with the most extreme model predictions (Figure 1) (Kirtman, 2013; Le Quéré et al*.,* 2016), as fossil fuel demands have continued to increase into the 21st century to meet requirements needed to support the human population (Archer et al*.,* 2009). Without serious mitigation attempts, atmospheric CO_2 is anticipated to continue rising, with the upper threshold of prediction models estimating concentrations of 1200ppm by the end of the century (IPCC, 2021).

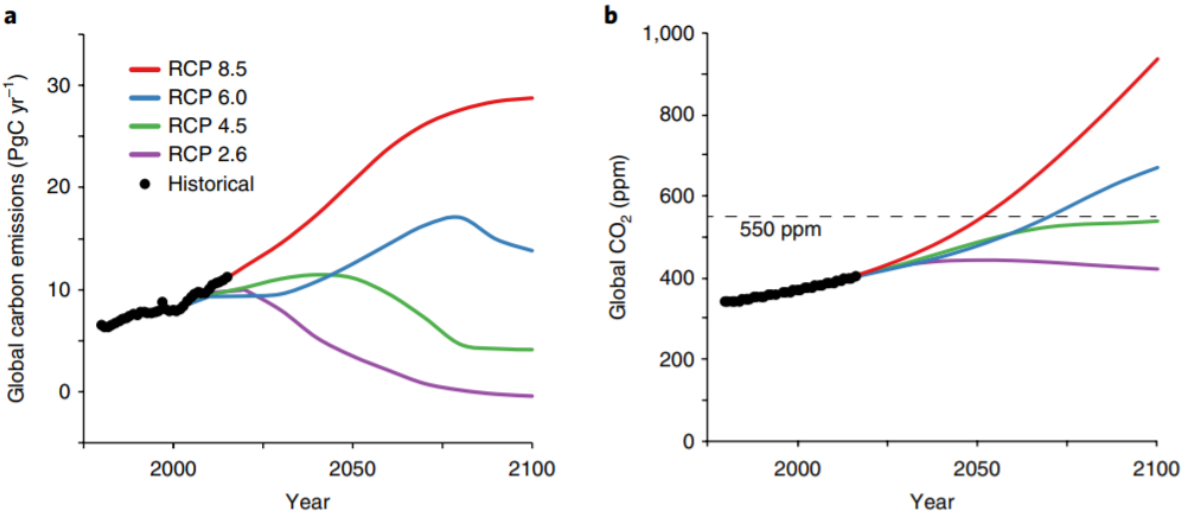


Figure 1: From Smith and Myers (2018). Recorded CO_2 emissions and atmospheric concentrations compared with model predictions of the year 2100. (a) shows CO_2 emissions since the year 1980 and predicted estimate models of carbon emissions until 2100. The current global level of emissions aligns with the most alarming model calculations. (b) shows annual surface global CO_2 concentrations. Representative Concentration Pathway (RCP) estimates in a and b from Kirtman (2013).

Human activities lead to numerous stressors in the marine environment from temperature increase to pollution (Nogales et al*.,* 2011; Häder & Gao, 2015; Fischer & Knutti, 2015). Two phenomena of major concern are plastic pollution and ocean acidification (Hönisch et al*.,* 2012; Doney et al*.,* 2020; Thushari & Senevirathna, 2020; Welden, 2020). Plastic pollution is ubiquitous due to the use of single use plastics, mismanagement of plastic waste and subsequent breakdown into micro-plastics (Boucher & Friot, 2017; Mishra et al*.,* 2019;

Booth, & Sørensen, 2020). The increased uptake of CO_2 by the ocean alters its chemical composition through the process of ocean acidification, which results in an increase in acidity and the decrease of pH (Feely et al*.,* 2001; Raven et al*.,* 2005; Albright & Langdon, 2011; Bates et al., 2012; Doney et al*.,* 2020).

These issues have been extensively studied due to the increasing significance of anthropogenic climate change and effects of marine pollution. Independently, both reduced pH and plastic pollution, have been shown to have detrimental impacts on marine organism fitness, and survival (Kroeker et al*.,* 2013, Wright et al*.,* 2013) and have been identified as 'stressors' (Schram et al*.,* 2014; Ferreira et al*.,* 2016).

Stressors very rarely act in isolation, currently 97.7% of the ocean is affected by more than one stressor (Halpern et al*.,* 2015; Carrier-Belleau et al*.,* 2021). Harvey et al. (2013) found the combined effects of ocean warming, and ocean acidification had a negative effect on calcification (the production and deposition of $CaCO_3$), survival, and reproduction, with a greater magnitude of response than either stressor alone. Complex relationships between stressors such as synergistic and antagonistic interactions take place. Synergistic interactions, also known as additive effects, occur when the combined effects of two or more stressors is greater than their individual impact (Ellis et al*.,* 2019; Bouraï et al*.,* 2020). Contrary to this, antagonistic interactions exist when multiple stressors in combination elicit a smaller response than in isolation. The interpretation of an interaction however can become more complex if multiple stressors exert opposite effects on a biological response. With combined responses of two stressors being deemed positive or negative effects if the response is more positive or more negative than the combined expectation (Carrier-Belleau et al*.,* 2021).

In order to fully comprehend the mechanisms affecting biological organisation, it is crucial to examine how multiple stressors affect the responses of whole populations as well as individuals (Crain et al., 2008; Calosi et al., 2013; Schmolke et al., 2017; Galic et al., 2018).

In this study, I aim to recapitulate the phenomenon of ocean acidification and plastic pollution and ascertain how they interact with marine organisms, with a focus on the effects upon the sensory system (chemical signalling) and how the combined stress may alter the behaviour of marine crustaceans; in this case the shore crab, *C. maenas*.

1.2 Ocean Acidification

Alterations to the physical and chemical properties of the ocean present grave repercussions for marine environments (Doney et al., 2012; Constable et al., 2014; Levin & Le Bris, 2015). Numerous factors contribute to global climate change, though the major anthropogenic influences stem from atmospheric CO_2 (Ramanathan & Feng, 2009; Seinfeld & Pandis, 1998; Jorgenson et al., 2019). Excess atmospheric CO_2 levels are increasing at an unprecedented rate and have been well established as an outcome of human activities linked to fossil fuel and industrial emissions (IPCC, 2019; Doney et al., 2020).

Oceans play a substantial role in global carbon cycles (Falkowski et al., 2000). It is known that the ocean is a sink for anthropogenic CO_2 and absorbs an estimated 25-40% of total anthropogenic CO_2 emissions (Feely et al., 2004; Canadell et al., 2007; Doney et al., 2020). The chemical reaction of CO_2 with water creates carbonic acid and H^+ ions. Increased H^+ ions result in more acidic, lower pH conditions. Due to increased uptake of atmospheric CO_2 by the ocean, the phenomenon by which this reaction accumulates H^+ ions is referred to as ocean acidification, which results in an overall shift in seawater chemistry (Doney et al., 2020) (Figure 2).

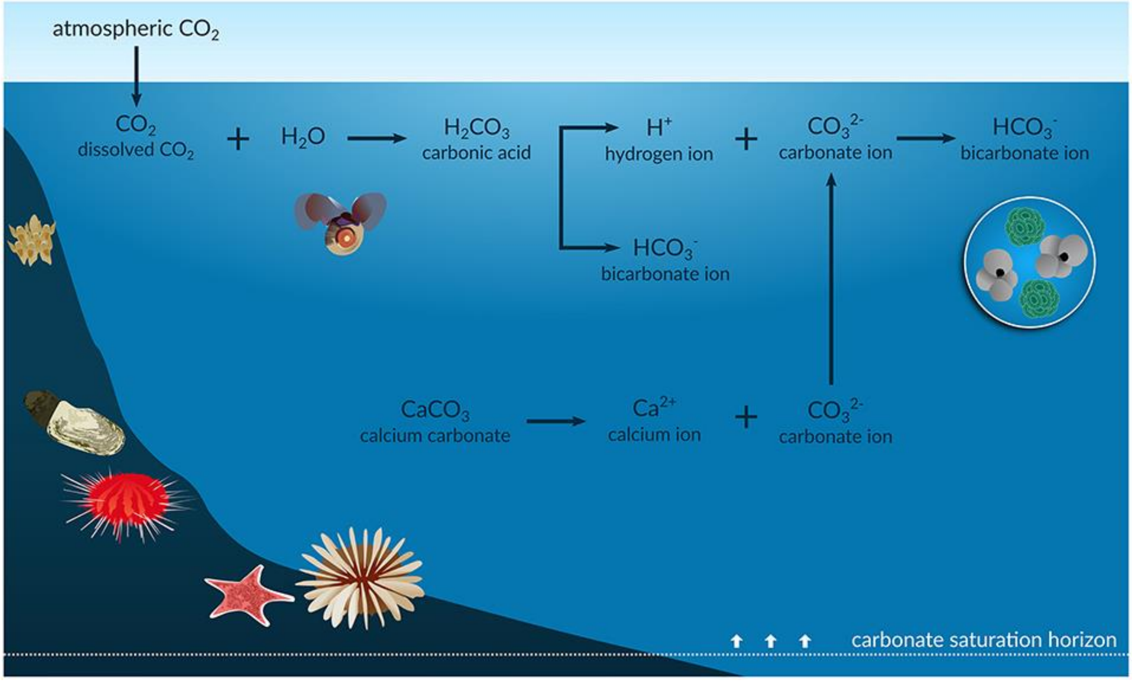


Figure 2: The process of ocean acidification illustrated as an Infographic. The atmospheric CO_2 absorbed by the ocean reacts with water (H_2O) forming carbonic acid (H_2CO_3), which disassociates further increasing the concentration of hydrogen ions (H^+) and bicarbonate ions ($HCO^{-\,3}$) and a decrease in carbonate ions ($CO_2^{-\,3}$). The reduction in $CO_2^{-\,3}$ limits the availability of carbonate, resulting in impacts on marine calcifying species such as foraminifera, corals, echinoderms, molluscs, and bryozoans (Figuerola et al*.,* 2020).

Since the start of the industrialisation period in the 1850s, global oceanic pH has decreased by an average of 0.1, a 26% increase of H^+ ions (IPCC, 2014; Jewett & Romanou, 2017; Garcia-Soto et al*.,* 2021). Oceanic pH is currently averaged at 8.2 however open ocean surface pH has declined by 0.017–0.027 pH units per decade since the late 1980s with the last two decades showing the worst acidification rates (IPCC, 2019). Prolonged oceanic absorption of atmospheric CO_2 is expected to exacerbate the effects of ocean acidification with predictions suggesting that seawater chemistry could be shifted to pH 7.8 by the year

2100 (Feely et al*.,* 2009; IPCC, 2014, 2019; Findlay & Turley, 2021). Globally, oceanic surface water pH fluctuates over a range of 0.3 pH units (Schellnhuber et al*.,* 2006), however climate change is expected to increase the range of pH fluctuation significantly (Turley et al*.,* 2006; Landschützer et al*.,* 2018). Current levels of pH are unseen in the last 20 million years with projected pH levels undeniably distinct from the pre-industrial period (Figure 3 as seen in Turley et al*.,* 2006).

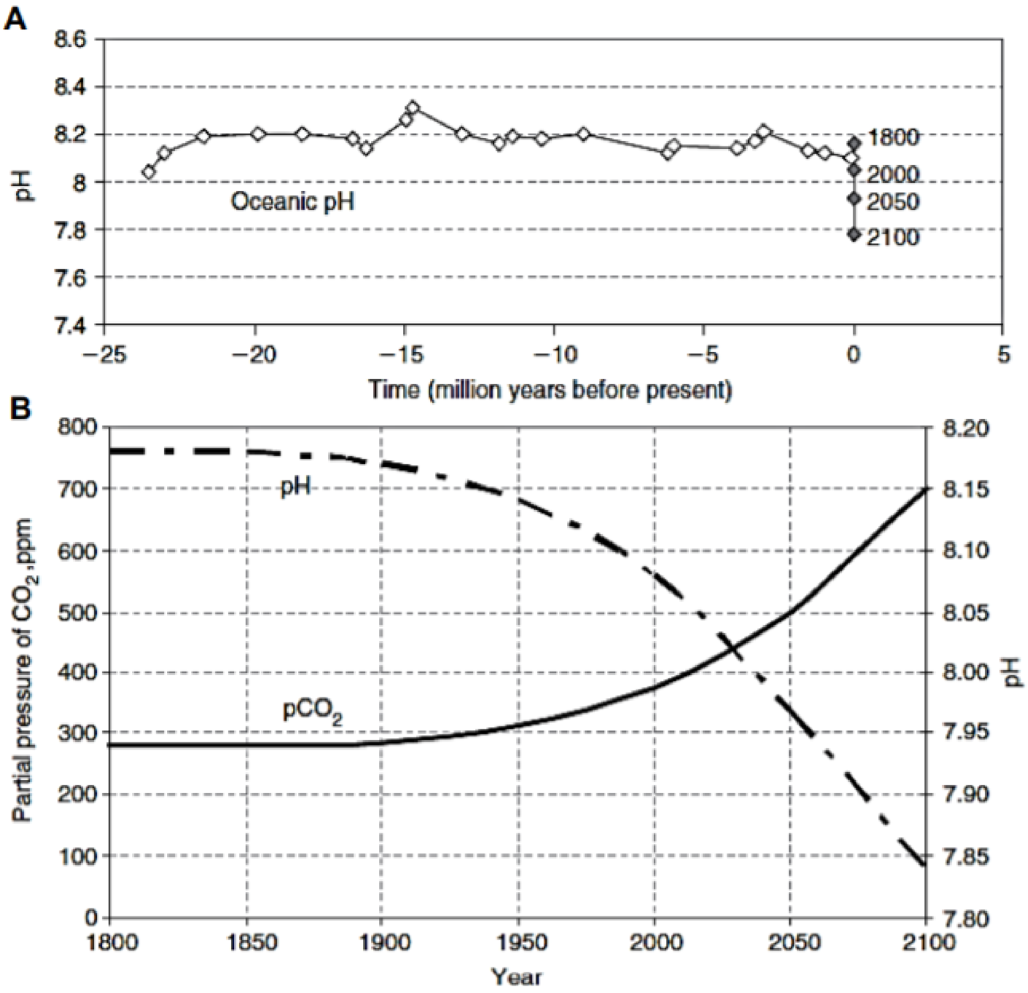


Figure 3: (A) Past and present-day variability of ocean pH. White diamonds showing past pH levels based on data collected from Pearson and Palmer (2000). Grey diamonds show model derived predictions of future pH with associated dates based on IPCC mean scenarios. (B) Past and predicted change in atmospheric CO_2 and seawater pH based on estimates of anthropogenic emissions (B) (As seen in Turley et al*.,* 2006).

1.3 Effects of Ocean Acidification on Marine Organisms

The change of carbonate chemistry of the ocean has been long recognised as a threat to marine organisms and many studies have been performed in the past decades with the aim of understanding the extent of its impact (Hofmann & Todgham 2010; Harvey et al., 2013; Kroeker et al., 2013; Waldbusser et al., 2015, 2016; Arnberg, 2016; Cyronak et al., 2016a, 2016b; Cattano et al., 2018; Cominassi, 2019).

Natural CO_2 leaks from the seafloor through hydrothermal and cold vents (upwellings) can cause severe localised gradient effects on sea water chemistry (Hall-Spencer et al., 2008; Fabricius et al., 2011). Studies conducted at sites of marine volcanic seeps give an insight into potential ecological responses to increased CO_2 while keeping within natural pH variability (Foo et al., 2018; Gao et al., 2019). A variety of organisms have been evaluated for the long-term effects of acidified waters and the consequences of frequent low pH excursions (Agostini et al., 2018). Sites examined at CO_2 seeps in the Mediterranean were characterized by an abundance of invasive macroalgal species, *Sargassum*, *Caulerpa*, and *Asparagopsis*, which thrive in acidic conditions (Gao et al., 2019) (Figure 4). At projected acidification levels from the IPCC, a loss in species diversity of around 5% is anticipated (Gao et al., 2019). However, even though many macroalgae species are resilient to fluctuations in pH, changes in algal community composition can result in an overwhelming loss of biodiversity in marine habitats (McGowan et al., 2005; Porzio et al., 2011; Enochs et al., 2015). Observations include a higher dominance of fleshy algal species, and less calcareous macroalgae, which are vulnerable to acidic conditions (Hall-Spencer et al., 2008).

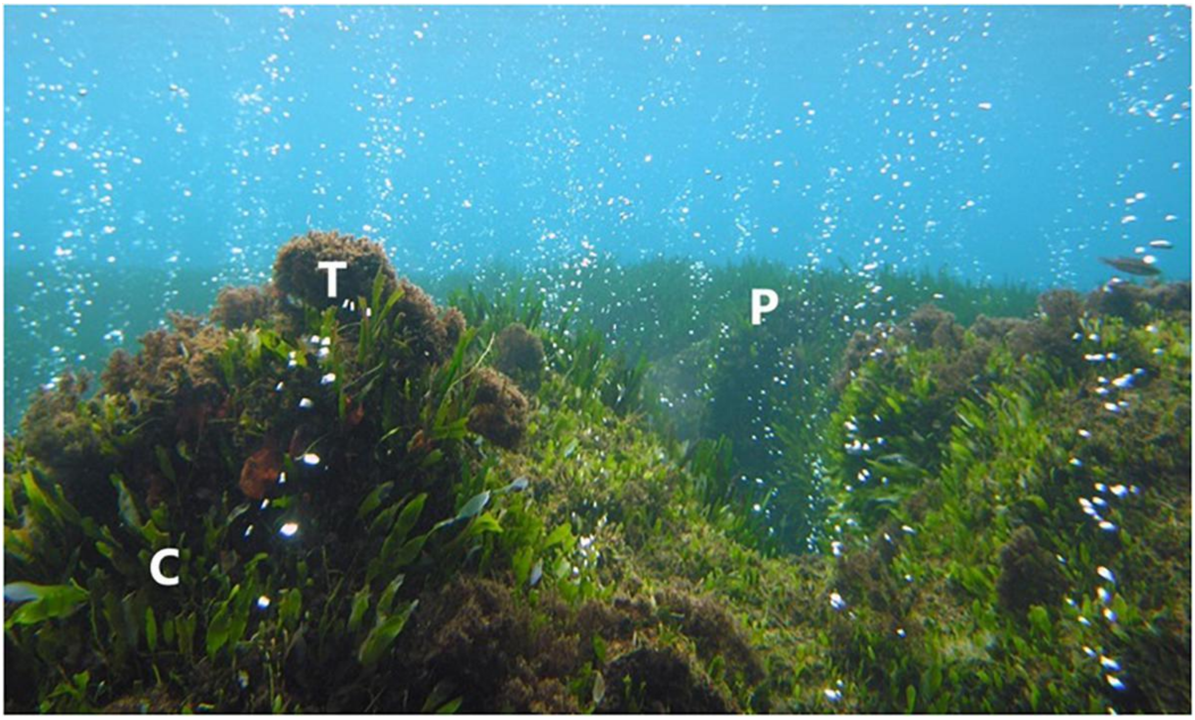


Figure 4: Photograph of a volcanic seep off Ishia Island, Italy. Photograph was taken at a depth of 1m and mean pH was recorded at pH 7.8 where CO_2 bubbles can be seen rising from the seafloor. Also pictured are a number of algae species: green seaweed *Caulerpa prolifera* (C), turf algae (T) and seagrass *Posidonia oceanica* (P). An observation was made that species of epiphytic and epilithic coralline algae were not present around the area of the CO_2 seep, however, were abundant 300 m away where seawater was measured at pH 8.1. (Photo by J. Hall-Spencer).

As a result of ocean acidification, a variety of marine species and ecosystems may suffer widespread negative consequences, which would have cascading effects throughout the ocean. Complex interconnected food webs of marine animals may be disrupted by ocean acidification due to the loss of key species (Browman, 2017).

Calcifying species such as corals, molluscs, crustaceans, and echinoderms are one major group most at risk as calcium carbonate shells become less viable in more acidic conditions

(Orr et al., 2005; Hofmann et al., 2010; Rodolfo-Metalpa et al., 2011; Melatunan, 2012; Hoegh-Guldberg et al., 2017). Species such as calcifying plankton form a key component of marine food chains and are relied upon by many larger organisms as an essential source of food (Hays et al., 2005; Azani et al., 2021). Calcification rates are estimated to drop by 30 to 40% by mid-century with ocean acidification being the major driver of these changes (Kleypas et al., 1999; Andersson & Gledhill, 2013). Population crashes or changes in species distribution would have serious implications for some of the most abundant marine ecosystems (Ullah et al., 2018; Jin & Gao, 2020).

The Southern Ocean region experiences low interannual variability in surface ocean carbonate ions. Organisms are predicted to face rapid changes, which may affect biomineralization and formation of skeletons since adaptation is unlikely within such a short timeframe (Orr et al., 2005; Andersson et al., 2008; Fabry, 2008; Conrad & Lovenduski, 2015). However, there are anticipated species-specific responses to ocean acidification, so the effects may not be synonymous across all species (Price et al., 2011; Byrne, 2012; Vargas et al., 2017; Barh et al., 2018; Bove et al., 2020).

Calcium carbonate sediments support vital habitats for a vast number of marine calcifying species and assist in global biogeochemical cycling (Roberts et al., 2017). Globally, more than 70% of cold-water corals will be subject to environments that are undersaturated with necessary calcium carbonate minerals by 2100 (Guinotte et al., 2006; Turley et al., 2007; Figuerola et al., 2020).

Echinoiderms such as starfish and sea urchins are grazers essential for protecting coral reefs from algae encroachment. When raised in acidic conditions, young sea urchins grow slower with thinner, smaller, deformed shells (Harrould-Kolieb et al., 2010). Slower growth rates and weaker shells result in higher vulnerability to predators and reduced survival (Kroeker et al., 2014). Furthermore, reproductive processes are stunted under acidified conditions reducing the creation and persistence of new larvae (Byrne et al., 2013; Duquette, 2016).

Squid and octopus require high levels of oxygen for rapid movement and speed. Increasing acidity of the oceans has been shown to interfere with acidity levels in squid's blood and consequently reduces oxygen levels (Pörtner et al., 2004, 2005). Squid are vital food sources for many marine mammals, including beaked and sperm whales, as well as a profitable fishing industry in many areas (Würsig, 2017).

1.4 Chemical Communication

Sensory abilities are crucial for organisms to interpret their surrounding environment (Derby & Sorensen, 2008; Wang & Wang, 2020). Olfactory-mediated behaviour is essential to species fitness and survival activities, including foraging, reproduction, and predator avoidance (Leduc et al., 2013). In marine environments olfactory senses are largely enhanced to compensate for limited visibility (de la Haye, 2012; Wisenden, 2000). Chemical signalling is a method of communication and perception through chemically mediated interactions. Chemoreceptors located on external antennae detect chemical signals, or 'cues', from various sources in the surrounding environment. Locations of prey or food are first identified from their odour and trigger foraging responses (Hay, 2009; Tran, 2015). Intraspecific signals such as pheromones indicate the presence of possible mating partners and social status of conspecifics (Hay, 2009; Derby & Schmidt, 2017). Interspecies alarm cues enable species to recognise and differentiate between predators and non-predators, develop antipredator responses towards unknown cues (Brown, 2003; Kelley & Magurran, 2003; Jacobsen & Stabell, 2004; Ferrari et al., 2011) and directly link to individual survival and species population dynamics (Wang et al., 2010; Ferrari et al., 2010).

Chemical cues are omnipresent in marine environments and are fundamental to community organization and ecosystem function as well as critical aspects of evolutionary behaviour and relationships (Hay & Kubanek 2002; Pohnert et al., 2007; Hay, 2009; Roggatz et al., 2016). The recognition of chemical cues and signals by olfactory receptors controls the decision-making process (Derby & Sorensen, 2008; Leduc et al., 2013), particularly in harsh environments and conditions (Brown et al., 1997; Chivers & Smith, 1998; Ferrari et al., 2010; Leduc et al., 2013).

Low pH conditions have been identified as a known cause of sensory impairment in several marine invertebrates (Gibson et al., 2011; Ashur et al., 2017; McCormick et al., 2019), compromising biological interactions and reducing survival ability (Clements & Hunt 2015; Draper & Weissburg, 2019; Velez et al., 2019; Porteus et al., 2021). Evidence indicates that low ocean pH impacts behavioural processes as a direct result of sensory impairment. These behaviours are crucial for marine invertebrate growth and survival such as: feeding behaviour, settlement site, habitat selection, anti-predatory behaviour, movement, and locomotion (Wang & Wang, 2020). Info-disruption and chemosensory impairment affects behavioural responses towards essential chemical signals such as feeding and reproductive cues (Krång & Rosenqvist, 2006; Lürling & Scheffer, 2007; Olsén, 2010; Lürling, 2012).

1.5 Olfactory Disruption by Ocean Acidification

Studies conducted in aquatic environments have confirmed significant impacts of ocean acidification on olfactory functions of fish, crustaceans, molluscs, and other macroinvertebrates, resulting in obscure behavioural responses, with potential widespread effects to community and population dynamics (Hay, 2009; Leduc et al., 2013). Studies have documented the link between reduced pH environments and changes in behaviour in a range of marine species; from polychaete (Bhuiyan et al., 2021) to mollusc (Watson et al., 2014) to crustacea (Briffa et al., 2012; de la Haye et al., 2011, 2012; Conradi et al., 2019) and chordata (Munday et al., 2009; Swain et al., 2020).

Info-disruption is caused by anthropogenic impacts that can alter the information that an animal receives (Cothran et al., 2021). For example, pH fluctuations affect organic compounds in the marine environment, such as chemical signalling molecules (Hardege et al., 2011) which are essential to biological systems. Successful chemoreception depends on both an organism's ability to receive and interpret information from the source, as well as the composition of the chemical cue (Wang & Wang, 2020). Sensory organs of organisms can become altered chemically or morphologically as a result of charge distribution on the protein receptor shifting under reduced pH conditions (Tierney & Atema, 1988; Hardege et al., 2011; de la Haye et al., 2012). Additionally, reduced pH may alter the chemical structure of a cue as found in a study on chemosensory behaviour in *C. maenas (*Roggatz et al., 2016, 2019*)*, whereby reduced pH altered the protonation state of three peptide signalling molecules presenting a concept which may explain altered behaviour in low pH conditions (Roggatz et al., 2016; Porteus et al., 2021). Due to change of charge, structure and function, the signal molecule had reduced bioavailability in lowered pH and consequently a higher concentration of the cue was required to produce a behavioural response in *C. maenas* (Roggatz et al., 2016; Velez et al., 2019). Different mechanisms have been discussed by which reduced pH can trigger an altered response to cues in crustaceans summarised into 4 pathways by Schirrmacher et al. (2021) (Figure 5). The effect of low pH on the signal source (pathway 1), the direct effects of pH on the signalling molecule and its interaction with the receptor (pathway 2 & 3) and potential interference of ocean acidification with signal transduction (pathway 4) (Schirrmacher et al., 2021).

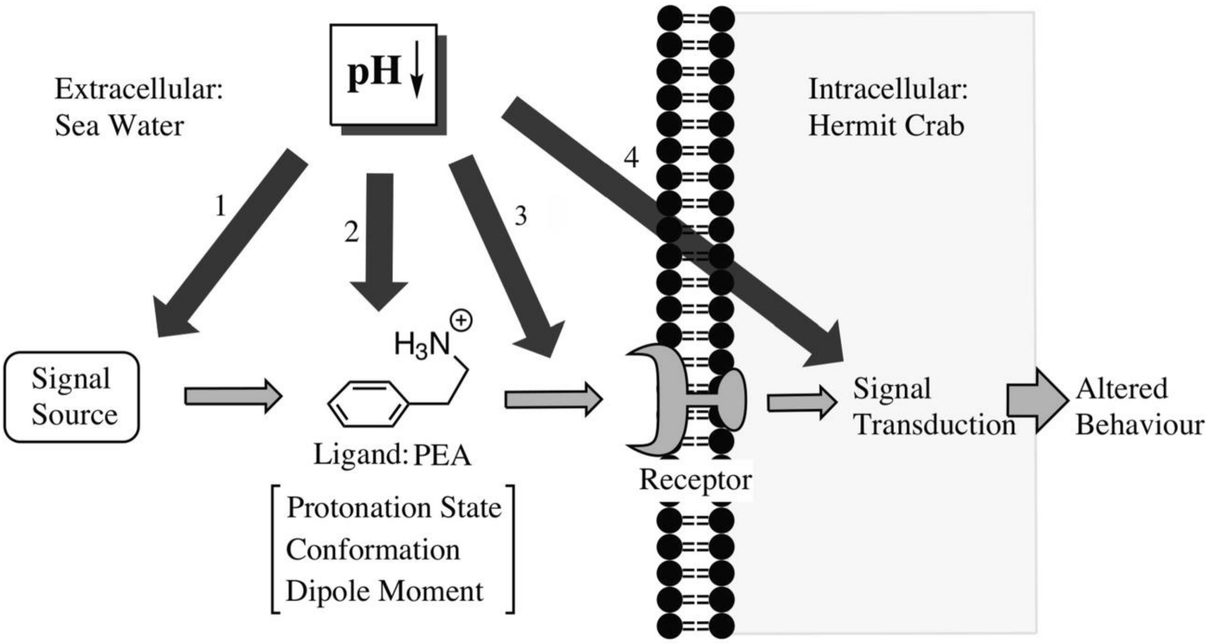


Figure 5: From Schirrmacher et al. (2021). Diagram of potential mechanisms by which ocean acidification or low pH can lead to an altered behavioural response in hermit crabs. Signal transmission from source to response is highlighted by light grey arrows. Potential mechanisms of low pH obstructing the pathway is highlighted by the dark grey arrows with numbers linking to the different mechanisms. Low pH affecting the source of the signal (1), the signalling cue (2), the receptor or its interaction with the ligand (3) and the signal transduction (4).

Munday et al. (2009) examined orange clownfish larvae (*Amphiprion percula*) for their ability to discriminate between settlement selection olfactory cues under predicted oceanic acidification scenarios. Clownfish larvae reared in current pH 8.15 seawater could discriminate between suitable and unsuitable settlement sites according to cues, and between kinships and non-kinships. This discriminatory ability, however, was reduced for larvae raised in future ocean acidification conditions pH 7.8 and 1050 ppm CO_2 and larvae showed attraction to olfactory stimuli which was typically avoided. In a similar study, cardinalfish (*Cheilodipterus quinquelineatus*) recorded in acidified conditions exhibited significantly decreased abilities to differentiate between odours from their home settlement site and an unfamiliar site (Devine et al*.,* 2012). As well as these findings, larval clownfish reared in aquariums under acidified conditions were observed to show attraction to predator odour and had difficulty recognising olfactory cues of predators and non-predators (Dixson et al*.,* 2010).

Additional experiments showed that clownfish (*Amphiprion percula*) and damselfish (*Pomacentrus wardi*) raised in low pH conditions also displayed impaired olfactory-mediated behaviour, in which low pH cultured fish initially evaded predator odours as did control fish, but eventually became attracted to these odours (Munday et al*.,* 2010). Similarly, to the failure of alarm responses in freshwater fishes, a study revealed that juvenile damselfish displayed significantly reduced response to conspecific chemical alarm cues when exposed to CO_2-acidified conditions after 4 days (Ferrari et al*.,* 2011). Predators' responses to prey alarm cues have also shown to be altered in the brown dottyback (*Pseudochromis fuscus*), a natural predator of juvenile damselfishes, spending around 20% less time in a stream containing prey odour after exposure to acidification conditions (Cripps et al*.,* 2011; Ferrari et al*.,* 2011).

Young fish eggs and larvae have been shown to have complications at early stages in development (Munday et al*.,* 2009; 2011). While adult fish may be relatively insensitive to ocean acidification, their larvae have shown a reduced olfactory ability in acidified conditions as seen in Clownfish and damselfish (Munday et al*.,* 2009; 2011; 2013; Ferrari et al*.,* 2011; Branch et al*.,* 2013; Leis, 2018). However, studies have linked increased CO_2 with fish actively swimming away from safe shelters and to not responding to predators as quickly as those not living in acidic conditions. Studies have shown that up to 9 times more fish did not survive due to these associated risky behaviours as compared to those living in a nonacidified environment (Munday et al*.,* 2010; Ferrari et al*.,* 2011).

Navigation and homing of reef fish is seen to have reduced success due to impaired cognition and olfactory abilities under elevated CO_2 conditions (Domenici et al*.,* 2012; Ferrari et al*.,* 2012; Nilsson et al*.,* 2012). Cardinalfish are one of many species which use olfactory cues and visual landmarks to assist in navigation and locating suitable resting sites after foraging, a 22– 31% reduction in homing success was displayed by individuals kept under elevated CO_2 treatments compared with control fish kept under normal CO_2 conditions (Devine et al*.,* 2012). The survival of coastal fish species largely depends on larvae successfully locating safe settlement sites where they can evade predators. The effects of ocean acidification on behaviours such as habitat selection and predator avoidance may have significant consequences on the success of fish population replenishment (Weissburg et al*.,* 2010). Sensitivity to acidification is likely to vary among prey species potentially causing instability in community structure through shifts in predator prey preference (Ferrari et al., 2011a, 2011b).

Reproductive behaviours and mate choice also heavily rely on olfactory cues. For instance, it is thought females may use scent to perceive major histocompatibility complex alleles to select suitable males (Reusch et al., 2001; Aeschlimann et al., 2003). Attraction and mating success has been studied under various pH conditions, including more alkaline pH levels. Female stickleback (*Gasterosteus aculeatus*) showed more attraction to male olfactory cues when pH was increased from 8.0 to 9.5 (Heuschele & Candolin, 2007). However, a similar pH increase reduced the number of pregnant males in pipefish (*Syngnathus typhle*) (Sundin et al., 2012). While these studies demonstrate the effects of seawater alkalinisation rather than acidification, they emphasize that variability in seawater pH levels may alter critical processes in sexual selection.

1.6 Crustaceans and Ocean Acidification

Increased acidity in the marine environment has been shown to profoundly affect crustacean behaviour. Hermit crabs (*Pagurus bernhardus*) showed impaired shell assessment and selection (De La Haye et al., 2011, 2012), survival rate of juvenile red king crabs (*Paralithodes camtschaticus*) decreased with low pH (Long et al., 2013), mud crabs (*Panopeus herbstii*) showed reduced feeding behaviour (Dodd et al., 2015), as well as compromised cue detection associated with food, reproductive and predator cues in the green shore crab (*Carcinus maenas*) (Roggatz et al., 2016; Richardson et al., 2021).

Hermit crabs (*Pagurus bernhardus*) select specific shells for protection against predation and harsh environments. Shells are physically, visually, and chemically assessed before habitation as selecting an optimal shell is critical for survival. Low seawater pH was shown to cause disruption to assessment and decision-making processes of hermit crabs (De la Haye, 2012). At low pH 6.8, crabs were more reluctant to move from a suboptimal shell to an optimal one compared to individuals kept in current condition seawater, the time taken for individuals to change shells also increased significantly in low pH (De La Haye et al., 2011; Leduc et al., 2013). Additionally, in low pH hermit crabs had reduced olfactory success and displayed less locomotory activity compared with those in current condition seawater (De La Haye et al., 2012; Leduc et al., 2013).

The effects of low pH on consumption and behavioural interactions of mud crabs (*Panopeus herbstii*) were examined at three *p*CO^{2} levels over a period of 71 days. Under acidified ocean conditions mud crabs showed reduced prey consumption of juvenile oysters, and significantly impacted foraging behaviour (Dodd et al., 2015). Long et al. (2013) assessed survival, growth rates, calcification, and condition of two juvenile crustacean species, the red king crab and tanner crab, reared under low pH conditions pH 7.5 and pH 7.8. In both species, survival decreased with pH. Although neither species had any apparent morphological damage from acidification, both species reared in low pH water grew slower than those in control pH 8.0. On assessment of calcium content post-experiment, it was suggested that juvenile red king crabs may be able to sustain calcification rates, but at a high energetic cost to the individual.

C. maenas inhabit coastal habitats and are known to experience extreme fluctuations in pH (Carstensen & Duarte, 2019) for example when isolated in tidal pools (Briffa et al., 2012). Richardson et al. (2021) found *C. maenas* individuals tested in 7.6 pH seawater took significantly longer to detect a natural prey odour cue than those acclimated to current pH conditions of 8.2. However, another finding showed that although prey detection was impacted by reduced pH, the response to a predator cue was not. Additional research into predator–prey interactions observed similar results in Blue Crabs (*Callinectes sapidus)* (Glaspie et al*.,* 2017) and Mud Crabs (*Panopeus herbstii)* (Dodd et al*.,* 2015) exhibiting reduced success in detecting an odour source and reduced foraging behaviours in predicted low pH conditions.

1.7 Plastic pollution

Marine plastic pollution has rapidly become one of the world's leading environmental challenges. Since the 1950s, 9 billion tons of plastic have been generated (Ritchie & Roser, 2018; Alabi et al*.,* 2019). Currently 400 million tons of plastic is processed annually with 8 million tons entering the ocean every year (Danso et al*.,* 2019; Maraveas, 2020). Plastic pollution endangers ecosystems and fauna as well as creating severe economic repercussions (Brems et al*.,* 2013; Wang et al*.,* 2019; Landrigan et al*.,* 2020; Zapata, 2021; Ford et al*.,* 2022). Plastics are seen to wash up on beaches, accumulate in ocean gyres, causes destruction to marine ecosystems and impact coastal communities (Cheng et al*.,* 2013; Jayasiri et al*.,* 2013; Cózar et al*.,* 2015; Baudrimont et al*.,* 2020; Ryan, 2020). Marine plastic pollution originates from terrestrial human activities through accidental or illegal disposal, which accounts for 80% of marine debris (Sheavly & Register, 2007; da Costa et al*.,* 2016; Coyle et al*.,* 2020). Negative effects of plastic pollution are commonly associated with entanglement in plastic fishing nets and wires or ingestion of plastic fragments, which has been reported in several species including mammals, birds, turtles, down to organisms such as fish and benthic invertebrates (Laist, 1997; Andrady, 2011; Wegner et al*.,* 2012; Besseling et al*.,* 2013; Foekema et al*.,* 2013). It is now estimated that there are approximately 5.25

trillion plastic particles on the surface of the ocean, 92% of which are smaller than 5mm in size and categorised as microplastics (Andrady, 2011; Eriksen et al*.,* 2014; Auta et al*.,* 2017). Microplastics are also intentionally manufactured for products including resin pellets, exfoliators, and beads (Cole et al*.,* 2011; da Costa et al*.,* 2016; Auta et al*.,* 2017), or produced through general use of items containing plastics including synthetic clothing and by weathering of larger plastic debris which reduces structural integrity and leads to fragmentation (Browne et al*.,* 2011; Cole et al*.,* 2011). Plastic degradation can occur through physical, chemical, and biological processes (Andrady, 2011; Gewert et al., 2015; da Costa et al., 2016; Alimi et al., 2018; Coyle et al., 2020; Kabir et al., 2022). Plastics are manufactured with synthetic chemicals that give them their durable properties. These chemical additives such as plasticisers, heat stabilisers, slipping agents and antioxidants leach into the marine environment from various plastic polymers (Brydson, 1999; Heudorf et al*.,* 2007; Teuten et al*.,* 2009; Hermabessiere et al*.,* 2017; Hahladakis et al*.,* 2018; Greenshields et al*.,* 2021).

1.7.1 Plastic leaching and plastic odour

Harmful chemicals associated with plastics fall under three main categories: components of the plastic material, by-products of manufacturing, and chemicals absorbed from the environment (Barnes et al*.,* 2009; Rani et al*.,* 2015; Verma et al*.,* 2016; Groh et al*.,* 2019). The possible toxicological effects of plastic can therefore be caused by a combination of these chemicals. Many plastic-derived compounds must be monitored and controlled by government organisations due to their toxicity to organisms and persistence in food webs (Rochman, 2015). Chemicals termed priority pollutants are recognised to impair essential physiological processes in organisms, causing diseases and reproductive complications. In addition to heavy metals and pesticides, these chemicals include polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (El-Shahawi et al*.,* 2010; Hirai et al*.,* 2011; Lithner et al*.,* 2011). The Environmental Protection Agency (US EPA) lists that at least 78% of priority pollutants are associated with plastic waste, while the EU lists 61% to be linked with plastic waste either originating from manufacturing processes or from the environment (Browne, 2015; Rochman, 2015).

Plastics of all types and sizes have the potential to leach and adsorb hazardous substances (Table 1). A particles size and surface area influence leaching and adsorption properties, smaller particles have a higher surface area-to-volume ratio, and thus larger surface area to allow the release or binding of compounds compared to larger ones (Mei et al*.,* 2020). The leaching of additives from polymers can influence their fragmentation behaviour in the marine environment over a longer period of time, which could potentially enhance the leaching or adsorption of harmful substances from the surrounding environment (Jahnke et al*.,* 2017; Luo et al*.,* 2022). Some of the toxic compounds, such as polycyclic aromatic hydrocarbons (PAHs), can form during the production of polystyrene or can be adsorbed to the plastic from the environment, making it a challenge to establish whether a compound has been present in the plastic from manufacturing (Hirai et al*.,* 2011; Rochman et al*.,* 2013).

When assessing the impacts of chemicals on marine life, it is necessary to consider interacting chemicals and synergic effects of the chemicals present in the environment, such as in sediments, the water column, plastics, and those released by organisms (Oehlmann et al*.,* 2009). Most studies to date have evaluated the impacts of plastic debris focusing on their leachates (Gunaalan et al*.,* 2020; Delaeter et al*.,* 2022) or adsorbed contaminants (Rios et al*.,* 2010; Joo et al*.,* 2021). By only examining the influences of individual substances, studies fail to consider the concoction of chemicals and concentrations found in the material (Onwudili et al*.,* 2009; Andrade et al*.,* 2021).

Table 1: The 7 most common plastic types, showing toxicity, and most commonly leached toxins.

Polymer name	**Polyethylene terephthalate**	**High-density polyethylene**	**Polyvinyl chloride**	**Low- density polyethylene**	**polypropylene**	**polystyrene**	**All other plastics: acrylic, fiberglass, nylon, polycarbonate, polyactic acid (a bioplastic)**
Abbreviation	PET or PETE	HDPE	PVC	LDPE	PP	PS	OTHER
How long to decompose under perfect conditions	5 – 10 years	100 years	never	500- 1000 years	20 – 30 years	50 years	Mostly never polyactic acid 6 months
Toxicity level	High	low	high	low	low	high	high
Most commonly leached toxins	Antimony Oxide, Bromine, Diaszomethane, Lead Oxide, and Benzene	Chromium Oxide, Benzoyl Peroxide, Hexane, and Cyclohexane	Benzene, Carbon Tetrachloride, 1,2-Dichloroethane, Phthalates, Ethylene Oxide, Lead Chromate, Methyl Acrylate, Methanol, Phthalic Anhydride, Tetrahydrofuran, and Tribasic Lead Sulfate, Mercury, Cadmium, Bisphenol A (BPA)	Benzene, Chromium Oxide, Cumene Hydroperoxide, and Tert-butyl Hydroperoxide	Methanol, 2,6-di-tert-Butyl-4-Methyl Phenol, and Nickel Dibutyl Dithiocarbamate	Styrene, Ethylbenzene, Benzene, Ethylene, Carbon Tetrachloride, Polyvinyl Alcohol, Antimony Oxide, and Tert-butyl Hydroperoxide, Bensoquinone	BPA, BPS as well as all other toxins mentioned

1.7.1.1 Leaching of harmful substances

The large molecular size of polymers means they are generally regarded to be biochemically inert and non-harmful to the environment (Kyrikou & Briassoulis, 2007; Lithner et al*.,* 2011). However, since polymerization reactions are seldom complete, unreacted residual monomers and small oligomers can still be released into the environment from the plastic (Crompton, 2007; Björnsdotter, 2015). Some plastic monomers, such as ethylene and propylene, are considered safe (Lithner et al*.,* 2011). However, in contrast bisphenol A (BPA) is known to disrupt endocrine functions, whilst vinyl chloride monomers and styrene have both displayed carcinogenic and mutagenic effects (Bartsch & Montesano, 1975; Huff & Infante, 2011; Brandt-Rauf et al*.,* 2012).

Research examining the toxicity of 55 plastic polymers relating to their chemical composition found 29% comprised partially or entirely of monomers classified as carcinogenic, mutagenic, or toxic for reproduction (Lithner et al*.,* 2011; DeMattea et al*.,* 2013). According to their monomer composition, polyurethanes (PUR), polyacrylonitriles (PAN) and polyvinyl chlorides (PVC) were evaluated as the most hazardous polymer types, whereas polypropylene (PP), ethylene-vinyl acetate (EVA), polyvinyl acetate (PVAc) and polyethylenes (PE) were assessed to be the least hazardous (Lithner et al*.,* 2011). While this ranking provides a useful overview, it focuses only on monomers, and fails to take into account the numerous additives used during plastic manufacturing.

The most harmful components of plastics are often compounds, such as solvents, catalysts, softeners, initiators, and other polymerization additives (Lithner et al*.,* 2011; Dube & Salehpour, 2014). Toxic and flammable solvents are difficult to remove from the polymer during the manufacturing process. Solvents used such as methanol, cyclohexane and heptane are toxic to aquatic life, as are catalysts consisting of metals such as zinc oxide, copper chloride, and tributyltin. Initiator's potassium persulfate and benzoyl peroxide cause respiratory and skin irritations (Dowaidar et al*.,* 2007; Lithner et al*.,* 2011; Rochman, 2015).

The plastic production process involves several thousand different additives (Brydson, 1999). Among the most hazardous additive types are brominated flame-retardants, phthalates, and lead compounds used as heat stabilizers (Darnerud, 2003). Brominated flame-retardants such as PBDEs have been found to share structural similarities with polychlorinated bisphenyls (PCBs), well-recognized environmental contaminants found to accumulate in aquatic animals' fat tissues and cause neurotoxic effects and damage thyroid hormone functionality (Darnerud, 2003). The estrogenic compounds present in phthalates can disrupt endocrine functions and reproductive systems of a variety of organisms (van Wezel et al*.,* 2000; Giulivo et al*.,* 2016). Phthalate plasticizers, especially those of low molecular weight, are severely toxic to aquatic life including micro-organisms, algae, invertebrates, and fish (Heudorf et al*.,* 2007).

Although the dangers of plastic leachate are seemingly evident, much ambiguity and debate regarding the extent and rate of uptake by organisms still exists (Burns & Boxall, 2018; Koelmans et al., 2016). The rate and volume of release into the environment has been examined in marine (Staniszewska et al., 2016; Suhrhoff & Scholz-Bottcher, 2016; Rani et al., 2017; Paluselli et al., 2018), freshwater (Suhrhoff & Scholz-Bottcher, 2016; Sun et al., 2019; Liu et al., 2020), and terrestrial contexts (Li et al., 2019). Bioaccumulation has been discovered through field observations (Ryan et al., 1988; Yamashita et al., 2011; Tanaka et al., 2013) and in laboratory studies (Gaylor et al., 2013; Li et al., 2019; Aminot et al., 2020;

Tanaka et al., 2020). Contrary to these findings, earlier research reported microplastics as an insufficient pathway for the uptake of additives in marine species (Koelmans et al., 2014). However, upon further research featuring more accurate concentrations of additives, plastic was concluded to be a successful mechanism for the uptake of additives (Diepens & Koelmans, 2018). This debate highlights the importance of understanding how and to what extent plastic pollution facilitates the uptake of additives and various leachable substances.

1.7.1.2 The process of leaching

During every phase of the plastics life cycle, hazardous substances can be released, including plastic monomers, solvents, additives, and by-products of degradation (Lithner et al*.,* 2011; Katzenberger, 2015; Groh et al*.,* 2019). Chemically unbound additives, particularly those with low molecular weight, leach out of polymer matrixes because they are not covalently bonded (Marcilla et al*.,* 2004). The amount and concentration of chemical substances leaching from plastic varies due to different factors such as particle size, temperature, solvent properties, and polymer structure and can be shaped substantially by the additives in the plastic (Marcilla et al*.,* 2004).

Firstly, how much and what can be leached from plastic depends on the content of the chemicals present. The properties of the polymer, such as structural permeability and the physical state of the polymer- either glassy, rubbery, or crystalline, influence the size of the gaps (Pixton et al*.,* 1994; Hiltner et al*.,* 2005; Choudalakis & Gotsis, 2009). Polymers with rubbery textures have larger gaps and thus higher leachate dispersion rates compared to other polymer types. Chemical properties, such as the size of the chemical in relation to the polymer gaps, affect leaching (Lithner et al*.,* 2012). Additives with small molecular weight have higher volatility and diffusion rates. Linear additives easily migrate into liquid compared to branched ones which volatilise easier to air (Brydson, 1999; Braun et al*.,* 2005).

In addition to the polymer properties, the characteristics of surrounding media can influence leaching rate. The leaching of additives into surrounding water, such as in the marine environment, occurs as seawater infiltrates the polymer structure, where it dissolves the additives inside the polymer matrix and diffuse out to the surrounding environment (Crompton, 2007). Until recently, there have been limited publications on the leaching of additives from plastic debris in the marine environment, with studies featuring various experimental approaches (Bandow et al., 2017; Rani et al., 2017; Sun et al., 2019). However, a much wider understanding of plastic leaching has been established within the pharmaceutical, food safety and waste management industries.

A study testing pellets of virgin plastics: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and high-density polyethylene (HDPE) found leaching of additives and residual monomers from the pellets after only 24 hours in artificial seawater (Björnsdotter, 2015). Leachates were recognized in all plastics, with the exception of polypropylene, including monomers, such as styrene, and oligomers. Similar results were found from research investigating the leaching of additives from household plastic items made of low-density polyethylene (LDPE), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC). Leaching rates were noticeably higher at the start of the experiment and decreased over the duration of testing, however cumulating effects of additives meant the highest concentration was recorded at the end of the experiments.

Leached additives and oligomers were detected from all plastic types including BPA, phthalates, citrates, and styrene oligomers (Zimmermann et al*.,* 2019).

Research has investigated additive leaching from plastic debris to determine the extent and rate of release, bioavailability, and ecotoxicity. Plastics PE, PVC, and PS are the most studied, corresponding with their production volumes, prevalence in the environment, and assumed risk (Lithner et al., 2011; Andrady, 2017). Static leaching experiments are most common and aim to simulate typical exposure conditions (Hermabessiere et al., 2017). In this approach plastic samples are immersed in an aqueous solution over a specified time frame without the media being replaced. Dynamic experiments feature the solution being refreshed periodically or continuously flowing (Suhrhoff & Scholz-Bottcher, 2016; Bandow et al., 2017; Sorensen et al., 2021). While dynamic approaches may be more complex to perform, they can effectively simulate the vast dilution capacity of open ocean environments and ensure that additive release is not limited by solubility or the attainment of equilibrium conditions.

Environmental factors, such as temperature, water turbulence, and micro-organisms, can further influence the leaching process. A study examining variations in the leaching potential of different plastics found water turbulence increased leaching of additives in all tested plastics (Suhrhoff & Scholz-Böttcher, 2016). PBA leaching from polyvinylchloride (PVC) was 11 % higher under turbulent conditions and increased leaching of phthalates was also observed. The highest increase in leaching under turbulent conditions was observed for plastics PVC and PE, which may be due to their flexible composition (Suhrhoff & Scholz-Böttcher, 2016; Fauvelle et al*.,* 2021). Other physical parameters including salinity and UV radiation were studied however these were found to have little effect on leaching behaviour. It was hypothesised that due to continuous exposure to UV radiation throughout the experiment immediate photodegradation of released additives may have occurred, since many of them are UV sensitive (Suhrhoff & Scholz-Böttcher, 2016; Romera-Castillo et al*.,* 2018). Additionally, it has been found that exposure to UV radiation prior to an experiment increased the leakage of hazardous substances from plastics suggesting that weathering can influence leaching properties (Staniszewska et al*.,* 2016; Rai et al*.,* 2021). Polycarbonate plastic litter was treated with UV radiation and immersed in Baltic Sea water for 60 days prior to experiments to determine BPA leaching from weathered versus virgin plastic litter (Staniszewska et al*.,* 2016). During the first days of the experiment, BPA leached from virgin plastic more readily than weathered plastic, but by the end the leaching from weathered plastic was greater. Results concluded that the concentration of BPA in seawater with weathered plastic exceeded 600 ng/L, whereas virgin plastic resulted in a concentration of less than 20 ng/L (Staniszewska et al*.,* 2016).

1.7.2 Info-chemicals

In ocean environments, plastic debris provides a durable substrate for microorganisms, many of which produce info chemicals (Barnes, 2002; Botterell et al*.,* 2020; Lobelle & Cunliffe, 2011). Establishment of biofouling biota, and the formation of biofilms, causes plastic fragments to acquire a chemical signal, which can attract organisms using chemosensory mechanisms to locate, identify, and ingest food (Yoch, 2002; Pohnert et al*.,* 2007; Savoca et al*.,* 2013; Zettler et al*.,* 2013; Botterell et al*.,* 2020).

Studies have observed that the odour emitted from plastic is the driving force behind the attraction as opposed to visual misidentification of plastic as a food source (Cadée, 2002; Schuyler et al*.,* 2014; Savoca et al*.,* 2017). The common slipping agent oleamide shares similarities with a chemical released during the decomposition of arthropods, suggesting crustacean species may mistake oleamide as a food source, creating an olfactory trap (Schirrmacher et al., 2021). Additionally, plastic marine debris host chemicals adsorbed on plastic debris surfaces from the surrounding seawater. A known example of this is biofouling of plastic debris caused by algae. One example of these chemicals is dimethyl sulfide (DMS) produced by phytoplankton (Yoch, 2002), causing fauna to misidentify plastic as a food source. This effect has been studied and observed in zooplankton, fish, hermit crabs, sea turtles and some seabirds (Savoca et al., 2016, 2017; Botterell et al., 2020; Pfaller et al., 2020; Greenshields et al., 2021). Plastic that has been ingested has varying levels of harm and can be a pathway for bioaccumulation of virulent compounds throughout the food web (Gregory, 2009; Cole et al., 2011; Zarfl et al., 2011; Savoca et al., 2017; Trotter et al., 2019; Markic et al., 2020; Miller et al., 2020; Chen et al., 2021).

1.7.3 Combined effects of stressors, specifically, Ocean Acidification and Plastic Odour

There is practical evidence suggesting that pH influences leaching from plastics (Bošnir et al., 2003; Annamalai & Namasivayam, 2017). Bošnir et al. (2003) performed a study assessing the migration of phthalates from plastic bottles to different beverages with the aim of establishing a link between the amount and type of phthalate migration, type of preservative used, and the pH of the sample. The study found bottled carbonated beverages with lower, more acidic pH around 2.7 had higher levels of phthalates observed compared to bottled drinking water around pH 6.5 which has lower observed phthalate concentrations. This suggests very acidic pH and preservatives such as orthophosphoric acid, sodium benzoate and potassium sorbate have a significant role in leaching of phthalates.

The study concluded that phthalate migration seems to be influenced by the drink's pH, the lower the pH value, the greater the phthalate migration (Bošnir et al., 2003).

Shams et al. (2020) investigated the aggregation kinetics and stability of polyethylene and polystyrene nanoscale plastics investigating ranges of pH, salt types and ionic strength applicable to natural marine environments. Findings revealed that ionic strength and salt types had significant effects on the stability of both, polyethylene and polystyrene nanoscale plastics, however, pH did not. The pH ranges investigated were over a range from 2 to 10. Polyethylene nanoscale plastics remained highly negatively charged across the studied pH range indicating that either polyethylene nanoscale plastics do not have an isoelectric point (Ohsawa et al., 1986; Lefèvre et al., 2009; Barany et al., 2012), or they have an isoelectric point below pH 2. In the marine environment pH fluctuates but generally remains between pH 5 to 9 (Wootton et al., 2008; Hofmann et al., 2011; Lauvset et al., 2020). The study concluded that pH is not likely to affect the behaviour of those nanoscale plastics in the aquatic environment (Shams et al., 2020).

In *C. maenas*, pollutants such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals cause substantial physiological costs to the individual (Lundebye & Depledge, 1998a,b; Dissanayake et al., 2010) including reduced metabolic scope, limited energy available and thus impaired behaviour and performance (Dissanayake et al., 2010). Additionally, there is ample data supporting significant developmental and physiological costs in invertebrates associated with low pH environments (Pörtner et al., 2004; Orr et al., 2005; Briffa et al., 2012). High CO_2 conditions elevate the cost of maintaining an internal acid–base balance and leads to an increased metabolic load for marine species to sustain (Pörtner et al., 2004; Spicer et al., 2007). As seen with exposure to chemical pollutants, changes in underlying physiological condition as a result of low pH environments is likely to reduce behavioural competence (Dissanayake & Ishimatsu, 2011).

1.8 Study organism: the green shore crab *Carcinus maenas*

The green shore crab *C. maenas* is a highly resilient epibenthic crustacean species with a wide range of thermal and salinity tolerances and extensive phenotypic plasticity (Tepolt & Somero, 2014; Young & Elliott, 2019) (Figure 6). These features enable extensive distribution and a variety of potential habitats across sheltered soft and hard environments within and outside of their native range (Baeta et al*.,* 2005; Young & Elliott, 2019), making them one of the top 100 most successful, and harmful, globally invasive marine species (Lowe et al*.,* 2000; GISD, 2021). The shore crab is well studied and extensively used in ecotoxicology experiments. As a common intertidal species, *C. maenas* are abundant, easily identified, measured, and marked making them highly convenient for experimental studies (Crothers, 1968; Rodrigues & Pardal, 2014; Young & Elliott, 2019).

Figure 6: Photo of *Carcinus maenas* from Lamb and Hanby (2005).

The native range of *C. maenas* extends the northeast Atlantic coastline, coastal areas of Europe and Africa, from Iceland, Norway and the British Isles, to Mauritania in northern Africa (Carlton & Cohen, 2003; Darling et al*.,* 2008; Young & Elliott, 2019). *C. maenas* is considered to be one of the most prolific globally invasive species, with at least two well-studied invasions in the north-western Atlantic and north-eastern Pacific as well as populations established in Argentina, Australia, and South Africa (Grosholz & Ruiz 1996; Grosholz, 2002; Carlton & Cohen, 2003; Hidalgo et al., 2005; Compton et al*.,* 2010).

C. maenas uses chemical signalling and chemoreception to navigate its environment and control olfactory-mediated behaviours such as foraging, mating and prey detection. Extended antennules display a characteristic flicking motion which serves to enhance chemosensory function when foraging for food (Schmitt & Ache, 1979). Funnel canals located on the walking legs respond to the stimulus of prey beneath them and the prey is then grasped within the crabs' chelae (Crothers, 1968). Mating behaviours displayed by *C.*

maenas is distinct from both feeding and typical locomotory movements. When sexually stimulated through olfaction, receptive *C. maenas* males elevate themselves on extended Pereopods 2 to 4 with the long axis of the propodus of the chelipeds pointing forward at an approximate 90˚ angle to the front of the carapace and may commence a searching motion (Bamber & Naylor, 1995). Often Pereopods 5 are held above the level of the carapace behind the crab (Bamber & Naylor, 1995). These processes can be disrupted by ecological changes such as ocean acidification and chemicals released by plastics through leaching (Olsén, 2010; Briffa et al*.,* 2012; Porteus et al*.,* 2021).

1.9 Potential for adaptation or acclimatisation

Adaptation and acclimation are two means for species developing tolerance to a new or changed environment (Cominassi, 2019). Adaptation requires genetic variation within the population, with the selection of features or adaptive traits, which enable individuals to survive or reproduce more successfully in certain environmental conditions (Garcia de Leaniz et al*.,* 2007; Edelaar et al*.,* 2008). Acclimation is a form of phenotypic plasticity where a genotype is expressed on phenotype fitting to the environment enabling the individual to maintain or improve its performance and often survival under new conditions (Whitman & Agrawal, 2009).

The effects of ocean acidification are typically studied over a short-term time period however, realistically, shifts in seawater chemistry will take place over several generations. Adaptation of populations of species with shorter lifespans have been considered as a potential outcome (Melzner et al*.,* 2009; Ofori et al*.,* 2017), however, a more common finding is that short-term exposure to future ocean conditions may not be a sufficient evolutionary force resulting in potential for rapid adaptation, and that adaptation is a more plausible possibility over many generations (Uthicke et al*.,* 2019).

Marine species can experience climate change associated stress conditions throughout their lifespan and over generations. It is important to consider adaptive potential occurring after acclimatising over a longer time frame (Sunday et al*.,* 2014; Foo & Byrne 2016; Senner et al*.,* 2018). Environmental properties of the ocean such as pH, salinity, temperature, and oxygen vary substantially during seasonal, tidal, and diel cycles, as well as across geographical location and solar and lunar interactions (Morris & Taylor 1983; Aagaard, 1996). These routine physicochemical variations require constant biochemical, physiological, and behavioural adjustments. Organisms such as *C. maenas* inhabiting these environments are often highly eurythermal and demonstrate considerable acclimatisation potential (Fangue et al*.,* 2006; Madeira et al*.,* 2012). Organisms inhabiting highly stable environments are likely to have narrower thermal tolerances and reduced acclimation abilities (Peck et al*.,* 2014; Tepolt & Somero, 2014).

Tolerance and acclimatization capacities have been seen to differ between closely related species that have evolved in diverse environments (Stillman & Somero, 2000; Stenseng et al*.,* 2005; Tepolt & Somero, 2014). For instance, porcelain crabs inhabiting environments close to their upper thermal constraints struggled to acclimate outside of those limits. Populations inhabiting cooler environmental conditions were able to modify their optimal thermal tolerances substantially beyond their usual limit after acclimation to warmer temperatures (Stillman, 2003; Calosi et al*.,* 2008; Bozinovic et al*.,* 2011).

A key ecological study system for examining intraspecific variations in environmental sensitivities is invasion biology as described in Tepolt and Somero (2014). Widespread invasive species are often successful in establishing populations across a range of habitats with wider abiotic variations and present key opportunities for analysing differences in genetic variation and acclimatisation potential (Chown et al*.,* 2007; Sorte et al*.,* 2010; Lockwood & Somero, 2011; Tepolt & Somero, 2014).

Invasion success is largely determined by the capability of a species to acclimatise to new conditions (Yeh & Price, 2004; Smith et al., 2009; Nyamukondiwa et al*.,* 2010; Tepolt & Somero, 2014). Invasive species such as fruit flies, *Drosophila subobscura,* have established widespread populations across diverse environments in its non-native range (Miura, 2007; Moran & Alexander, 2014). Species with high levels of genetic variation and short generation time, are often able to readily adapt to new environments.

C. maenas is also considered a classic model of a successful invasive species with non-native populations established in Japan, Australia, Argentina South Africa, and both North American coastlines (Carlton & Cohen, 2003; Hidalgo et al*.,* 2005). Tepolt and Somero (2014) observed high thermal tolerance and acclimatory plasticity in the shore crab. They found individuals to be extremely tolerant of both heat and cold, with a stronger ability to survive higher temperatures than native marine crustaceans inhabiting the same environment. It is becoming increasingly evident that invasive species are characterised by a higher thermal tolerance than related non-invasive species (Schneider, 2008; Sorte et al*.,* 2010; Lenz et al*.,* 2011; Lockwood & Somero, 2011; Zerebecki & Sorte, 2011).

Impaired chemical signalling due to ocean acidification lends to the prospect of organisms facing increased risk of extinction. However, adaptation to detect chemical cues under acidified conditions may be possible. For long-term adaption by evolution to successfully occur, there must be a level of individual variability allowing an advantageous trait, allowing for better tolerance to acidified conditions, to be inherited.

In the case of short-term adaption, evolution is not essential where there is adequate plasticity to accommodate for the change in environment condition (Susoy et al*.,* 2015). Research focusing on microorganisms and metazoan species representing Crustacea, Bryozoa and Polychaeta groups has revealed successful short-term adaptation to high pCO_2 environments (Melzner et al., 2009; Calosi et al, 2013). Individuals were relocated from stable pH environments to CO_2 vents with significantly lower pH (ranging from 6.8 – 7.7 pH). While the species were able to exist in these vent systems, trade-offs are unsurprisingly incurred, with no clear observations of breeding success (Rodolfo-Metalpa et al*.,* 2010; Burrell et al*.,* 2015; Turner et al*.,* 2015).

As *C. maenas* occupy various shoreline types, many get isolated in shallower waters so it would be likely that many individuals used in experimental studies are already likely to be adapted to fluctuations in temperature, salinity and light intensity due to their natural environmental conditions. High individual variation and phenotypic diversity could promote adaptation to future ocean acidification and changes in the marine environment. By comparing behavioural results to individuality data, we can identify if the variability in certain characteristics in *C. maenas* enable those individuals to be more tolerant, better adapted or more susceptible to the impacts of microplastics and pH induced stress.

1.10 Individuality

Individual variation such as morphological differences and heterogeneity in behaviour has become a key focus of studies relating to social behaviour due to its potential to influence animal communities (Sih et al*.,* 2004; Dall et al*.,* 2012; Wolf & Krause, 2014; Farine et al*.,* 2015). Numerous studies have found that the behavioural response of an animal is heavily impacted by individual characteristics such as sex, size and weight and social hierarchy which need to be considered when studying the effect of an external factor on behaviour (Blueweiss et al*.,* 1978; Dall et al*.,* 2004; Hayden et al*.,* 2007; McNamara & Houston*.,* 2009; Anthes et al., 2010; Schuett et al*.,* 2010; Wolf et al*.,* 2011; Kelley et al*.,* 2015; Eldøy et al., 2021; Thoré et al*.,* 2021).

1.10.1 Sex

Male and female individuals have sex specific responses to pheromone cues due to varying interests in the stimuli. Males react strongly to female sex pheromones uridine diphosphate (UDP) and uridine triphosphate (UTP) in comparison to females (Hayden et al*.,* 2007; Hardege et al*.,* 2011; Clark, 2017). It is known that female shore crabs display stronger responses to food odours than males throughout the year with males showing significantly reduced responses to feeding stimulants during their reproductive season in the summer months (Hayden et al*.,* 2007).

Sex specific changes to behavioural responses to odour cues under low pH conditions was found by Richardson et al. (2021), who observed detection and response behaviour of *C. maenas* in response to prey and predator cues. The study showed an altered response at pH 7.6 with males detecting prey cues faster than females, suggesting that there may be physiological or morphological differences between sexes. However, results also showed that in response to odour of the common cuttlefish (*Sepia officinalis*), a predator species, detection behaviour was not altered in males or females (Richardson et al., 2021).

Thoré et al. (2021) found sex specific behavioural variation in the freshwater fairy shrimp (*Branchipodopsis wolfi), a*lthough larger than males, females were less active. Additionally, activity was negatively associated with size amongst females, whereas an opposite relationship was found for males. Trade-offs like these often aid in antipredator tactics to avoid detection by visual predators.

1.10.2 Size and weight (carapace width)

Body size is recognized as a key component of evolutionary fitness as it can impact physiological performance, behaviour, anatomy, reproduction, and longevity (Hines, 1982, 1988; Naganuma & Roughgarden, 1990; Hart & Bychek, 2011; Kelley, 2013; Kelley et al*.,* 2015). In stone crabs (*Hapalogaster dentata*) it was observed that larger males had a significantly higher fertilization success than smaller male conspecifics when mating with females (Sato & Goshima, 2007). A larger body size is assumed to be advantageous in competition and utilisation of resources (Brown & Maurer 1986; Bonner, 1988), foraging and predator escape (Reimchen, 1991; Scharf et al., 2000; Cook et al., 2013), as well as greater ability to endure extreme environmental conditions (Peters, 1983; Peters & Wassenberg, 1983; Smith et al., 1991; Sogard, 1997; Portner, 2002; Brandl et al., 2020). It is known that in polychaete individuals kept at low pH are on average smaller than individuals reared in current oceanic pH conditions (Sokołowski et al*.,* 2020). Larvae cultured in low pH had reduced sizes at metamorphosis, abnormal growth, and resulted in juveniles of a smaller size (Kroeker et al*.,* 2013; Lane et al., 2013; Espinel-Velasco et al., 2018, 2021; Díaz-Castañeda et al., 2019).

Studying the effect of body size is complex due to larvae and juveniles typically being smaller than mature adults, making it a challenge to disentangle body size and development as different factors. In many species maturity is determined in relation to size (Crothers, 1967), in *C. maenas* males often reach maturity by 21 mm, and in females around 28 mm, with individuals below these measurements classed as juveniles (Audet et al*.,* 2008). Sexual maturity can vary with size, male shore crabs reach sexual maturity at around 30–35 mm carapace width; however, as the species is highly competitive, individuals measuring below 60 mm are unlikely to mate (Styrishave et al*.,* 2004). *C. maenas* are generally larger in both the north-western Atlantic and north-eastern Pacific non-indigenous populations compared to those in the native European population (Thresher et al*.,* 2003; Young & Elliott, 2019).

Laboratory trials studying shore crab larvae from invasive populations on the Atlantic and Pacific coasts of North America suggest that juvenile crab larvae have a narrower thermal tolerance than adults, with successful development occurring only between 10 and 22.5°C (deRivera et al*.,* 2007). However, in contrast, juvenile *Carcinus* in the Mondego estuary showed a higher tolerance to lower salinities than adults and larvae in the population. Juvenile *Carcinus* were located in upriver areas and observations confirmed that as individuals increased in size, they began a migration downstream towards the mouth, into more saline waters (Baeta et al*.,* 2005; Monteiro et al*.,* 2021).

1.10.2.1 Size relating to dominance and hierarchical factors

In crustaceans, dominance hierarchies are determined and maintained largely by size (Jachowski 1974; Fielder & Lee, 1983; Barki et al., 1991; Huntingford et al*.,* 1995; Goessmann et al., 2000; Gilpin & Chadwick, 2020), and fights between individuals are usually resolved by the smaller opponent retreating (Glass & Huntingford, 1988; Sneddon et al*.,* 1997; Huber et al., 2002; Edwards et al., 2005). Encounters between male shore crabs (*C. maenas*) are aggressive and observations suggest fights are significantly more intense in the presence of females (Sneddon et al*.,* 2003) than in the presence of food. Size is the single most important factor in determining the outcome of these conflicts, large males with carapace width over 60 mm show higher dominance than smaller males and thus higher success rate in winning fights and approaching receptive females (Berrill & Arsenault, 1982; Styrishave et al*.,* 2004).

During fights dominant males (winners) initially climb on top of their opponents, followed by the weaker crab (losers) retreating from the fight (Sneddon et al*.,* 1997). Winners of fights are more likely to win again, and losers are more likely to lose again; this is described as the winner-loser effect. Losing a fight was also seen to impact the responses of males towards pheromone cues (Fletcher & Hardege, 2009), which can greatly influence community and population structure (Chase et al*.,* 1994; Daw et al*.,* 2002).

Fletcher and Hardege (2009) observed the responses of male *C. maenas* after an antagonistic interaction and established a link between the outcome of social interactions and the animal's response to reproductive chemical cues. 40% of losers failed to respond to the female sex pheromone, with both winners and losers showing a delayed response after a conflict. Individuals exposed to pheromone odour cues after a loss in a fight performed fewer mating behaviours. Status-dependent responses seen here in males indicate the presence of social structures and social hierarchy-driven mating tactics to potentially reduce the number and intensity of costly fights during the short reproductive window (Fletcher & Hardege, 2009). A similar study by Johnson et al. (2008) staged fights between Norway lobsters (*Nephrops norvegicus)* and observed that only the losers of the fights reduced their aggression from the first to second encounter.

1.10.3 Injury

C. maenas is a known aggressive species so intraspecific and interspecific competition is to be expected (see above section 1.10.2.1). Fighting behaviour can also lead to injuries so body condition of individuals can vary depending on severity of the damage often visible by missing limbs.

In crustaceans there is generally a high size to body weight ratio regarding cheliped dimensions (Stein, 1976; Lee & Seed, 1992). Chelipeds have been widely studied for their use in foraging activities however it has been noted that cheliped size and structure is not driven by foraging alone and plays a prominent role in success and efficiency of feeding, reproductive behaviour, and antagonistic interactions (Lee & Seed, 1992). Cheliped movement is considered the main display and expression of aggressiveness (Wright, 1968). *Carcinus* with larger chelae have selective advantages when competing for mates and in antagonistic interactions within the group, owing to higher success and significant importance in mating behaviour (Lee & Seed, 1992; Sneddon et al*.,* 1997). The size and spread of chelipeds is considered more influential in determining the response pattern of hermit crab individuals than general body size (Yoshino et al*.,* 2011). It was observed that mating pairs are established after a fight has occurred between males (Lee & Seed, 1992). Aggression and fighting behaviour naturally increase during the mating season, essentially promoting intrasexual selection among males, as only those successful in winning fights and defending the moulting female will reproduce.

it is known that damage or loss of limbs decreases male pairing success and male *C. maenas* without functioning chela had reduced ability to compete for and protect a moulting female (Sekkelsten, 1988; Abello et al*.,* 1994). Whilst injured crabs will require energy to rebuild lost limbs and to cope with the physiological stress of an injury, crabs in “optimal condition" with no injuries have greater metabolic needs and so require higher cardiac activity (Aagaard, 1996; Rovero et al., 2000). Additionally, larger cheliped size has been related to cause disproportionately higher metabolic rates during high activity tasks such as swimming, suggesting that there is a trade-off.

In laboratory studies often opponents are sized matched in order to remove bias from the outcome of a fight when examining external factors (Huber et al*.,* 2001; 2002; Johnson et al*.,* 2008), however this does not present an accurate representation of fights occurring in the natural environment, where the larger individual would most likely be the winner of the antagonistic interaction.

Furthermore, it is common for individuals with injuries to be excluded from fighting experiments, although this is a straightforward way to reduce the influence of body condition on results, it does not reflect real life situations in the field. Including individuals in suboptimal conditions provides more accurate and realistic results for ecological studies as individuals in the natural environment often experience damage or injury through fights and predation.

1.10.4 Colour variation (carapace colour)

Throughout their ontogenetic development, *C. maenas* presents a variation in colouration, exhibiting a range of carapace colours whilst undergoing moults allowing for growth (Styrishave & Andersen, 2000). The difference in morphotype indicates the stage of intermoult: newly moulted crabs being green in colour and individuals with a longer intermoult period presenting as red (Reid et al*.,* 1997; Monteiro, 2019).

The varying colour morphs of *C. maenas* (Todd et al*.,* 2006; 2012; Stevens et al*.,* 2014) allow distinguishing the different stages of moult phases (McGaw et al*.,* 1992; Styrishave et al*.,* 2004). Colouration can vary from a red to green carapace with darker marbled appearance and a lighter coloured abdomen (Crothers, 1968; Reid & Aldrich*,* 1989; McGaw et al*.,* 1992). Moulting is age and temperature dependent (Young & Elliot, 2019) and as in many decapod crustaceans coincides with reproduction (Steel, 1980; Adiyodi, 1985; Nelson, 1991; Cuzin-Roudy, & Buchholz, 1999). Before and during the moult female shore crabs release sex-pheromones predominantly in the urine, which attract males and induce mating behaviours such as guarding (Bamber & Naylor, 1996; Hardege et al*.,* 2002, 2011). In female *C. maenas* the first sex pheromone was identified as the nucleotide uridine diphosphate (UDP) (Hardege et al*.,* 2011). Further examination of female urine samples over the moult cycle revealed a pheromone bouquet with UDP as well as uridine triphosphate (UTP) being detected. A combination of the two nucleotides at a ratio of 4:1 UDP:UTP induces the male mate guarding behaviour (cradling) and elicited an even stronger mating response than either UDP or UTP in isolation (Fletcher et al*.,* 2021). This compound changes over the moult period pre and post ecdysis relating to the physiological state of the female releasing it (Fletcher et al*.,* 2021).

Colour-dependent variation relating to distribution of *C. maenas* individuals within populations in combination with size and sex has been investigated (Hunter & Naylor, 1993; Warman et al*.,* 1993; Aagaard et al*.,* 1995; Aagaard, 1996). Research has analysed various aspects of *C. maenas* physiology and morphology with regard to differences in carapace colour (Reid & Aldrich, 1989; Kaiser et al*.,* 1990). For example, in males, red individuals have thicker, more robust carapaces compared to green individuals (McGaw et al*.,* 1992).

Green crabs show higher levels of activity compared to red crabs (Hunter & Naylor, 1993; Warman et al*.,* 1993; Aagaard et al*.,* 1995) which may be due to red coloured individuals needing to fulfil increased metabolic demands of growing tissues in prolonged intermoult (Aagaard, 1996). Reid and Aldrich (1989) found red shore crabs had significantly higher “excited” oxygen uptake rates and a lower ability to withstand environmental stress such as low salinity and hypoxia than the green crabs.

2. Chapter 2: The impact of reduced pH and plastic odour upon the green shore crab *Carcinus maenas*

2.1 Introduction to study

Marine ecosystems face numerous human induced stressors including overexploitation of natural resources, habitat destruction, invasive species, pollution, and climate change, all of which are known to negatively impact species functionality (Claudet & Fraschetti, 2010; Gallardo et al*.,* 2017; Cordier et al*.,* 2019; Montero-Serra et al*.,* 2019; Pörtner, et al*.,* 2019; von Schuckmann et al*.,* 2020; Lyu et al*.,* 2021). These environmental stressors rarely act in isolation, and instead often act together (Manríquez et al*.,* 2021). There has been an exponential interest in research aiming to unravel and predict the consequences of ocean acidification on marine species, populations, and ecosystems (Davidson, 2013); as well as the inescapable issue that is plastic pollution. However, species face exposure to these stressors simultaneously.

It is known that individual variations within a species population have varying responses to stressors e.g., sex, size, hierarchy, colour (Bell et al*.,* 2010; Koolhaas et al*.,* 2010; Pankhurst & Munday, 2011). However, the anticipated ecological and fitness consequences for species if plastic and/or pH impacts their olfactory systems are unknown (Hoet et al*.,* 2004; Savoca et al*.,* 2017). The lasting impacts on population structure and ecological systems could be detrimental if functional traits are impacted. Understanding the effects of multiple stressors and unravelling multiple-stressor effects is challenging. Marine stressors can interact and cause synergistic, additive, or antagonistic effects, but experimental research on crustaceans encountering stressors simultaneously are limited (Sokolova, 2013; Curry, 2020; Stein & Harzsch, 2021). Studying the diversity in the individual behavioural response relating to individual characteristics within the species population may be key to species endurance in a changing environment (Tepolt & Somero, 2014).

The present study aims at studying the responses of the shore crab, *C. maenas* a decapod crustacean, when facing combinations of low pH and plastic odour. The effects of plastic derived odour and ocean acidification as combined stressors affecting shore crab olfaction, behaviour and consequently fitness have been examined. Additionally, objectives also consider if individuality factors (size, sex, colour, and injury) are influential amongst results.

This study aimed to address the following questions:

(i) How does *C. maenas* respond to acidification and plastic odour in isolation?

(ii) How does *C. maenas* respond to the combined effects of acidification and plastic odour?

(iii) How do the impacts of acidification and plastic odour interact?

The shore crab *C. maenas* was selected as an appropriate study species for this research as there is sufficient data that document responses to feeding and reproductive cues in the form of increased rates of antennule flicking, making this species a suitable experimental organism for this study (Ropes, 1968; Lyons et al., 2012; Roggatz et al*.,* 2016). There is knowledge of the chemical ecology of *C. maenas* (Sneddon et al*.,* 2003; Hayden et al*.,* 2007; Hardege et al*.,* 2011; Rodrigues & Pardal, 2014) focusing on both feeding and reproductive behaviours (Ekerholm & Hallberg, 2005; Bublitz et al*.,* 2008; Edgell & Hollander, 2011; Fletcher et al*.,* 2021). Behavioural studies on this species were performed with known synthetic cues which enables the use of quantitative evaluation of behavioural changes in individuals (Bublitz et al*.,* 2008; Hardege et al*.,* 2002, 2011; Fletcher et al*.,* 2021). The impacts of temperature and salinity on *C. maenas* have been described (Therriault et al*.,* 2008; Appelhans et al*.,* 2012; Monteiro et al*.,* 2021), making animal husbandry and culture procedures simple to maintain.

2.2 Aims and objectives

Current research suggests that ocean acidification will modify the structure of a variety of olfactory chemical cues and will affect crustaceans' ability to detect and respond to chemical cues in the environment (Leduc et al*.,* 2013; Roggatz et al*.,* 2016; Ashur et al*.,* 2017; Schirrmacher et al*.,* 2021). With only a very small number of studies utilising known synthetic compounds, evidence for this phenomenon is limited (Porteus et al*.,* 2021). With the increase of plastic odour and leachates polluting the marine environment, this project aims to observe detection ability and response time of *C. maenas* when exposed to synthetic and natural cues under combined plastics exposure and acidified ocean conditions.

Experiments will be conducted at three different pH levels simulating current, projected, and extreme localised cases of ocean acidification. This study will observe behavioural responses in relation to feeding and reproductive cues at varying levels of pH as well as combined effects when exposed to plastic odour. Individual variability will be analysed to investigate if certain factors: size, sex, colour and injury, influence susceptibility or resilience of marine crustaceans to ocean acidification.

2.3 Hypotheses

To explore the impacts of low pH and plastic odour, the following hypotheses were tested throughout this study:

1. At low pH (7.6 and 7.2) *C. maenas* will show a reduced response to a) feeding cue (glutathione) and b) synthetic reproductive olfactory cues (UDP/UTP) than individuals tested at normal pH 8.2.

Null hypothesis: At low pH C. maenas will show no difference in response to the synthetic olfactory cues UDP/UTP and glutathione compared to individuals at a normal pH.

2. Individuals tested with the presence of plastic odour will show a reduced response to olfactory cues (UDP/UTP and glutathione) than individuals tested without the presence of plastic odour.

Null hypothesis: With the presence of plastic odour C. maenas will show no difference in response to the synthetic olfactory cues UDP/UTP and glutathione compared to individuals tested without the presence of plastic odour.

3. At low pH individuals tested with the presence of plastic odour will show a more reduced response to olfactory cues (UDP/UTP and glutathione) than individuals tested with the presence of plastic odour at normal pH.

Null hypothesis: At low pH C. maenas individuals tested with the presence of plastic odour will show no difference in response to the synthetic olfactory cues UDP/UTP and glutathione compared to individuals tested with the presence of plastic odour at normal pH.

4. Individuals will have variability in cue detection and response relating to size, sex, colour, and injury.

Null hypothesis: There will be no individual variability in cue detection and response relating to size, sex, colour, and injury.

3. Methodology

3.1 Animal collection

C. maenas were collected on the 4th of June 2021 from the British coast at the rocky shore in Whitby, North Yorkshire (Figure 7). All collections were carried out in non-protected areas with no conservation designation, that are frequently used by the University of Hull and fellow researchers. Whitby was chosen for crab collecting as *C. maenas* populations are plentiful and easily located.

Figure 7: Location of the collection site, the rocky shore in Whitby, North Yorkshire where *Carcinus maenas* specimens were collected (pictures from Google images 2022, Google Maps, 2022; Wikipedia contributors, 2022) [Accessed 19/04/22]

The most effective way to collect animals was by using crab drop nets with bait to attract individuals that could then be pulled out of the water and collected in buckets. Intermoult adults, both males and non-ovigerous females were collected, identified, and sexed based on the observation of abdomen morphology. Males feature a triangular abdomen, while females display a rounded abdomen of equal breadth for most of its length (Figure 8, bottom A, B- males and C, D- females). 100 crabs were collected in total, 60 males (3.5 – 8.5 cm) and 40 females (3.5 – 6.5 cm) and placed into separate labelled cool boxes lined with cooling blocks to lower internal temperature and reduce metabolic rate. Collected animals were transferred to tanks in the culture room at the University of Hull within 6 hours of collection. These methods of collection, transportation and removal have been used and proved to be effective in other previous projects conducted at the University of Hull.

3.2 Culture maintenance

All experimental animals were kept in holding tanks supplied with circulating natural sea water (pH 8.2, temperature 15-20˚C, salinity 32 to 34%) where they were monitored daily under controlled conditions. Daily pH readings were taken to monitor the carbonate chemistry (measured using an Orion 8103SC pH electrode calibrated on the National Bureau of Standards (NBS) scale. Males and females were housed separately in groups ranging between 3 - 6 animals per holding tank (50 cm x 40 cm x 25 cm). *C. maenas* remained in the aquaria facilities until their natural death, typically up to 1 year. During this time in captivity, cannibalism on soft moulting animals was expected to be a common cause of death for crabs kept together in tanks (Breteler, 1975). To reduce predation during moulting, crabs were grouped by size to avoid predation of larger older crabs on the smaller juvenile crabs.

When moulting, death or injury occurred, it was marked on the crab database spreadsheet and the individual was re- marked as described below in section 3.4.1 Marking Individuals.

3.2.1 Feeding

C. maenas were fed on mussel (*Mytilus edulis*) flesh twice weekly as a standard, prior to biological assays and for the duration of the experimental period. Crabs were only used for experimental assays at least two days after feeding to standardise hunger levels at the time of the assays, especially as some cues were associated with feeding. Crabs were fed at the end of the days with bioassays and not tested in assays for at least two days. The animals were fed blue mussel *Mytilus edulis* flesh as recommended by Ameyaw-Akumfi & Hughes (1987). This was prepared by placing approximately 200g of frozen cooked mussels into a container containing 100ml of water and allowed to defrost. Once the mussels had defrosted the flesh was cut into pieces of around $1cm^3$ in size. Food was distributed evenly allowing for one piece per individual, care was taken to spread out the pieces to minimise fighting over food placed in one area.

3.3 General experimental procedures

3.3.1 Ethics statement

All experimental work using *C. maenas* was undertaken following appropriate ethical approval through the University of Hull ethics committee (reference code: UO20). The collection and housing of the organisms used in this project and the behavioural bioassays used were covered ethically by the chemical ecology research group’s general ethics form.

After the experiments, organisms were kept in aquaria at the University of Hull for future use.

3.4 Assessment of Animals: Measuring and observing individuals

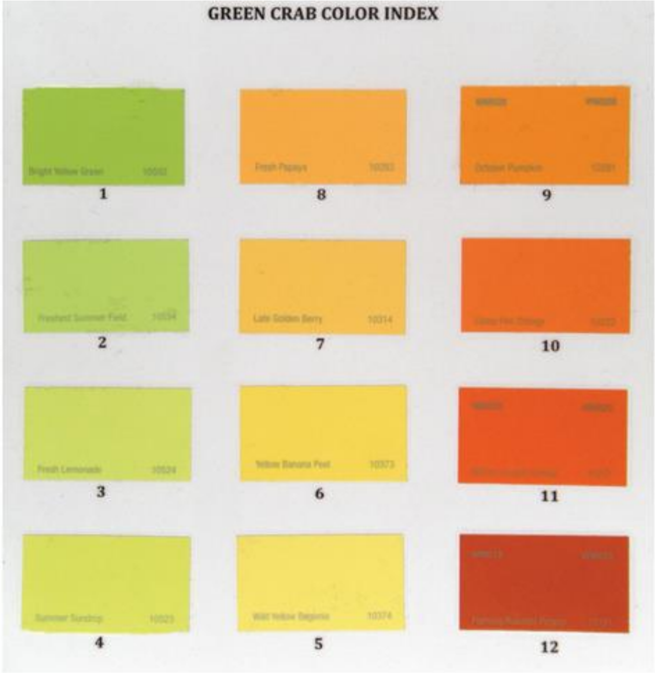
Prior to and during data collection, a series of measurements and observations were recorded for each individual crab. Size and weight of each individual was recorded to determine data trends relating to body size (Appendix Table 1).

C. maenas individuals were placed into a container and paper towels were used to absorb and remove excess surface water to ensure only the crabs body weight was being measured. The individual was moved to a separate container placed on 3-point scales which was re-set to remove the weight of the container from the measurement. As gill chamber water could not be drained, the wet weight of the crab was recorded in grams (g). This method was repeated for all individuals.

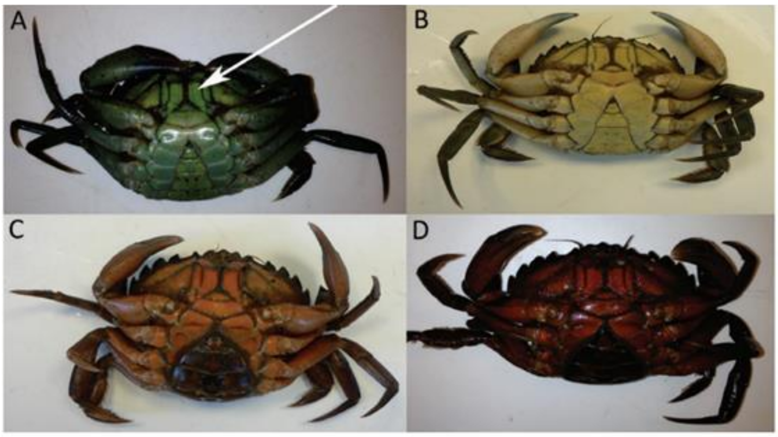
Size was measured as carapace width. Measuring the widest point of the carapace, the measurement was recorded to the nearest 0.1 cm. For this study individuals were categorised as ‘large’ or ‘small’ based on mean carapace width, individuals measuring 6cm and under were classified as ‘small’ and those over 6cm were classified as ‘large’.

At the same time as the above procedure the animals were examined for their body condition. Body condition relates to the physical condition of the animal accounting for any damage to the individuals form including loss of appendages, cheliped, or antenna. If the individual was in less-than-optimal condition it was deemed as ‘compromised condition’, if the individual was in optimal condition, it was deemed as ‘good condition’.

Sex was distinguished as described above in section 3.1 Animal collection, and colour was determined using a colour index chart as defined by Lee et al. (2005) ranging from green (1) to shades of yellow and orange (2–9) to red (10) (Figure 8). For the analyses, crabs were categorized as either green (colour codes 1–5) or red (colour codes 6–10)



Color index used to quantify ventral coloration in *Carcinus maenas*. Color chips are sections of paint swatches. Color of third maxillipeds was matched to a color chip to determine the appropriate color index (CI) value.



Examples of *Carcinus maenas* ventral coloration. Arrow points to third maxillipeds that were used to determine color index (CI) values. A, green male (CI = 1); B, yellow male (CI = 6); C, orange female (CI = 9); D, red female (CI = 12).

Figure 8: Charts created by Lee et al. (2005) from Young et al. (2017). Colour index chart (top image) and examples of *C. maenas* colouration and differentiation of sex (Bottom).

3.4.1 Marking individuals

For the data recorded to be successfully linked to individual variability, a database of all individuals used in the study was created, containing the sex, weight, carapace width, colour, and condition (Appendix Table 1). This spreadsheet was updated regularly and prior to bioassays. To link this data to individuals nail varnish was used to mark the carapace with a unique number and colour, depending on sex. Earlier studies established no effect of nail varnish on the behaviour or mortality of *C. maenas* (Lee et al*.,* 2005; Sturm et al*.,* 2006). Markings were reapplied in the case of an individual moulting (Figure 9).

Figure 9: Markings made with nail varnish on *Carcinus maenas* individuals, here pictured Females 7 – 12.

3.5 Development of methods and preliminary testing

To try and develop a bioassay that tested a single crab and could be repeated for different crabs, pH treatments and odour cues, various small-scale preliminary bioassays were carried out. Preliminary bioassays were selected and used to determine the duration and effectiveness of the cues and responses to differing cue concentrations. Experimental methods were altered if not successful.

In order to investigate how a change in pH affects chemical cues, a concentration threshold had to be determined to ensure pH would have an effect when experiments were repeated at different pH treatments. The chemical odour cue glutathione (GSH, 70-18-8, Sigma-Aldrich) was selected as the feeding cue as it is a known chemical foraging cue (Loomis, 1955; Reimer, 1972), particularly well-studied in Portuguese man o' war jellyfish (*Physalia physalis*) and hydra (Lindstedt, 1971; Bellis et al., 1992; Kulkarni & Galande, 2014; Lenhoff, 2018). Concentration threshold for glutathione was tested on crabs with concentration gradient of between 10^{-6} M to 10^{-2} M. UTP/UDP concentrations and glutathione concentrations were both 10^{-3} M. These concentrations were initially tested as previous similar studies at the University of Hull found reactions of organisms to experimental concentrations within this concentration gradient.

Crabs were tested for behavioural responses to the cues prior to experiments. *C. maenas* males perform distinct posing and cradling behaviours in response to female pheromone cues (Berrill & Arsenault, 1982). Posing is typically displayed by the individual rising onto the tips of its pereopods with the fifth pereopods raised to carapace height and chelipeds extending 90 degrees from the body. Cradling is described as the crab grabbing or mounting the 'female', in this case; the tea strainer containing the cue. Observations of these behaviours were considered a positive mating response. As testing started in the summer (July – September) crabs were deemed sexually active as the males responded strongly to the female pheromone cue by performing typical mating behaviour. For this reason, experiments testing response to pheromone cues were completed first, followed by the conditions testing food and plastic odour. To assess when the crabs had reached the end of the reproductive season later in the year, the same preliminary tests for response to pheromone odour were repeated. When male crabs no longer responded to the pheromone cue with mating behaviour, they were considered to be winter crabs, this was observed through testing during the months of October- February.

When carrying out trial experiments for the odour cues the following set up was used (Figure 10). 0.5ml of the cue was pipetted onto a sponge and placed into one end of the tank. The individual was isolated in the centre of the tank by a plastic tube, with the odour cue on one side and a blank cue on the other side. The positioning of the odour cues was chosen at random. These trial experiments were repeated for all the odour cues used in the

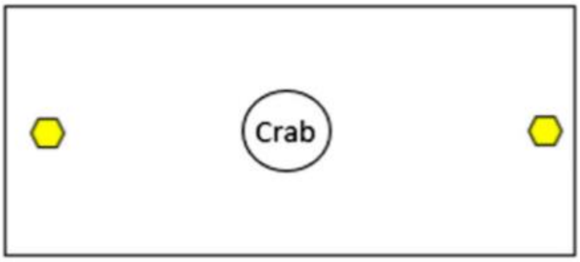


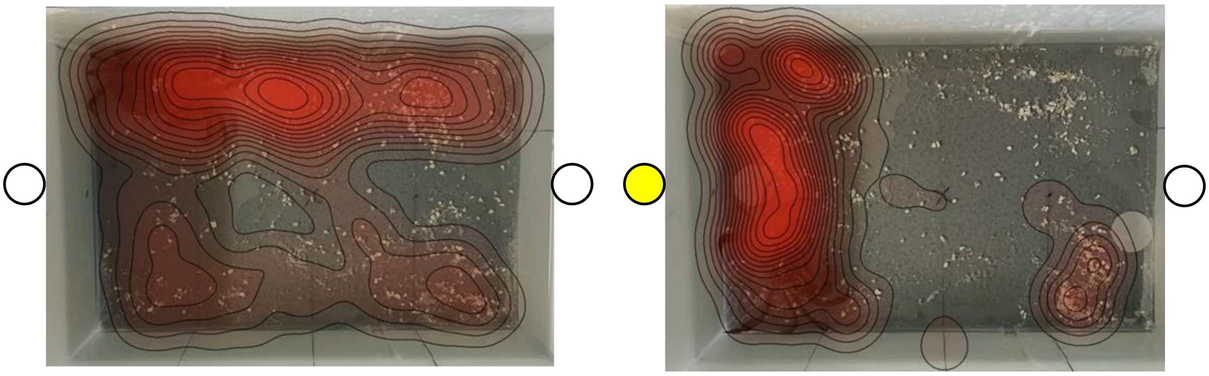
Figure 10: The experimental set up used during preliminary testing and for Lolitrack^{TM} video analysis. *Carcinus maenas* isolated in the centre of the tank (58 cm x 31 cm x 31 cm) by a tube (10 cm diameter), and the two odour cues positioned at each side; one of which was selected at random as the required odour cue. Diagram from Richardson et al. (2021).

experiment: Glutathione, UDP/UTP and plastic odour. These results decided the final experimental concentration of each cue.

To assess the crab's response to plastic odour, the response to three different plastics was tested, using the same methods for creating plastic odour water as described below in section: 3.8 Preparation and storage of experimental cues (see page 71).

The plastics tested were Polypropylene (PP), Polyethylene (PE), and Polyvinylchloride (PVC). Response was measured by observing the time taken for antennule flicking and observations of behaviour. If the crab moved towards the odour cue and stayed in proximity for the duration, we regarded this as a positive response. Lolitrack^{TM} video analysis software was used to create heatmaps of individuals and their movement within a small rectangular tank (size- 58 x 31 x 31 cm), with approximate 11cm depth of artificial sea water (salinity 35%, 19˚C). A tripod was positioned 83 cm above the tank, where a camera (iPhone) was held to record responses. The two 4.25cm diameter filter paper circles were soaked with solution, one with 2ml of plastic odour solution and the other with 2ml of seawater as a control. The filter paper was then secured in place at opposite ends of the tank with equal sized stones and were frequently repositioned to reduce bias. The crab was placed into the tank in a holding tube and acclimatised for 20 seconds. The crab was then released and recorded for 2 minutes. This was repeated in pH conditions 8.2 and 7.6. From these trial experiments PE was found to be the most effective and this was used to create the plastic odour for the plastic odour cues used in the study (Appendix Table 2).

Video footage was inputted into Lolitrack™ software to produce individual heatmaps and data outputs. This allowed prediction of whether plastic would be an attractant or deterrent and if response changed under low pH (Figure 11).


Control

With plastic odour cue

Figure 11: Heatmaps created with Lolitrack™ showing an example of movement and response to control cues (A) and plastic odour cue (B). (A) shows movement of a Male in a static tank with two Control odours (artificial seawater) at each end pH 8.2. (B) shows movement of the same Male when a plastic odour (yellow) was added to one end of the tank pH 8.2.

Once all the concentrations were found and the cues were created, a preliminary test was conducted to assess the cue diffusion rate and the amount of time an odour was detectable. This test was carried out in a Y shaped olfactometer flume (180cm x 90cm x 45 cm) (Figure 12). Food colouring was added to several test carboxycellulose gels (described below page 71), which were placed into the arms of the Y shaped tank. The duration of time taken for the food colouring to diffuse to the other end was tested and recorded at various flow rates. The results (Appendix Table 3, Table 4) showed that it took approximately 5 minutes for food colouring to diffuse to the other end of the Y-shaped tank. The same process was tested with the odour cues through preliminary bioassays, and it was concluded that the cues were detectable for approximately 2 hours before needing to be replaced.

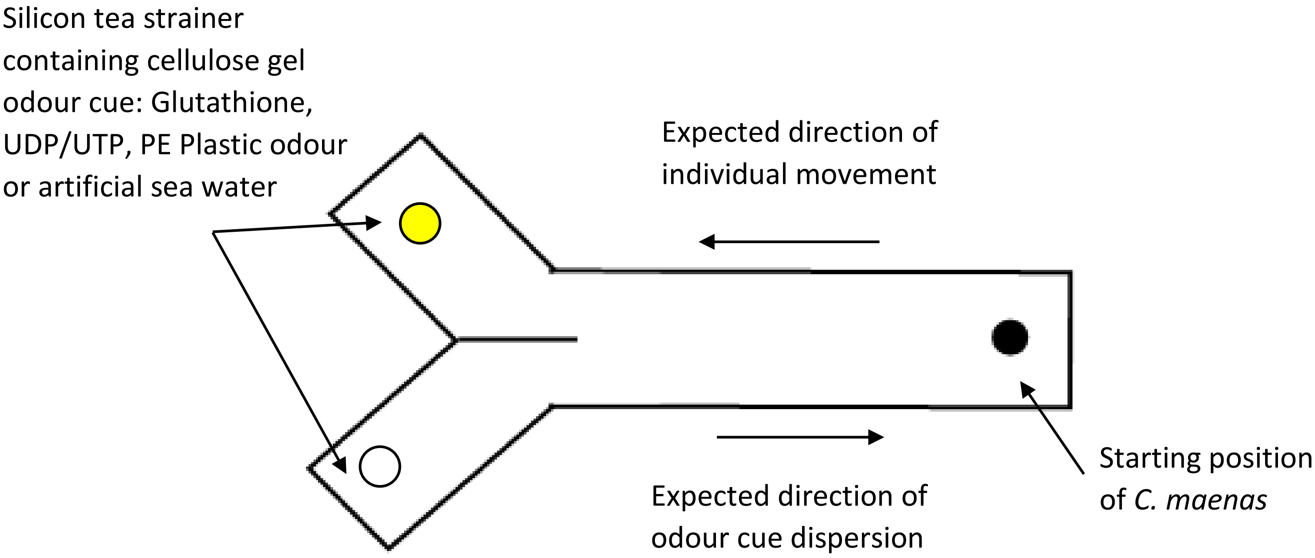


Figure 12. Arial view of the experimental set up of Y- shaped olfactometer used during bioassays (dimensions 180cm x 90cm x 45 cm). The crab *C. maenas* contained at the far end of the tank (black circle) by a PVC basket (15 cm length), and the two cues randomly positioned at the end of each branch of the tank; one containing the desired odour cue.

To standardise observational data for the response of the crab, all behaviours observed throughout preliminary testing were identified and defined (Table 2).

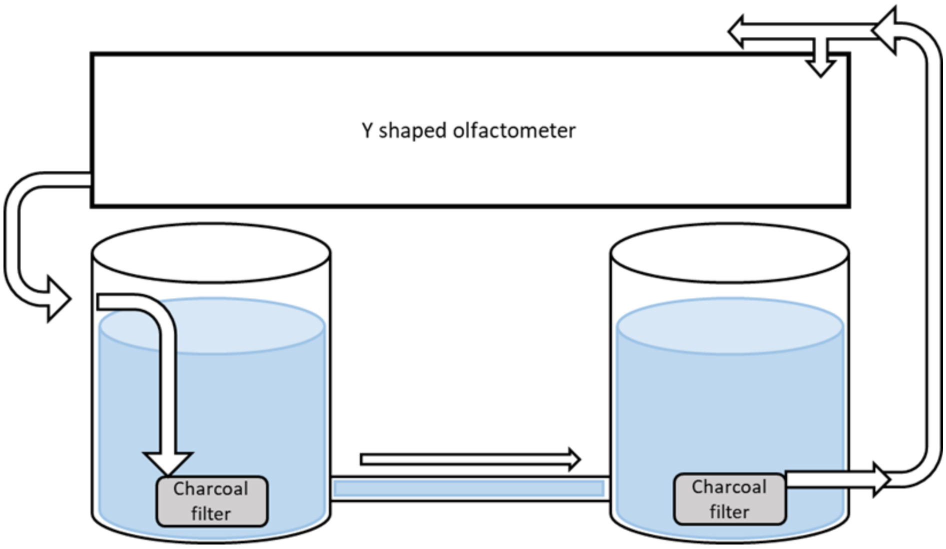


Figure 12 A. Re-circulating system set up for behavioural assay design. Two 210L water butts connected via tubing were used to pump artificial seawater into the Y shaped olfactometer. Charcoal filters were fitted to remove any previously released odours from the water.

Table 2. Description of behavioural acts observed throughout preliminary testing and experiments. Standardised definitions of behaviours were agreed on for observations recorded during bioassays.

Behaviour	**Description**
Antennular flicking	Rapid flicking or twitching of antennules
Wafting	Mandibles moving or rubbing together
Grabbing	Using chelipeds to grab or move the cue probing / grasping
Cradle	Holding the cue underneath in cradling action
Burial	Buried into the sediment either at the start of the experiment or next to the cue
Continuous activity	Moving around the tank or cue but not interacting with the odour cue
No visible reaction	No visible reaction or no behaviours shown
Cue being chosen	Individual reaching the location of a cue and displaying any behaviour or interaction towards the cue

3.6 Behavioural assays

All bioassays were conducted in the same room and the tanks were positioned prior to experiments and remained in the same position throughout the duration of data collection. Artificial sea water was made the day of experiments and temperature, salinity, pH and light intensity was recorded prior to experiments to ensure the parameters for testing were uniform across all bioassays.

Two identical Y-shaped olfactometer flumes were positioned side by side with split flow tubes allowing artificial seawater to enter each branch of the tank at the same flow rate of 500ml per minute, and 1 litre per minute exiting at the other end. Identical flow rates ensured that odours from the cues diffused at an equal rate and removed any odours from previous testing. Each Y-shaped flume was filled up to a 12 cm mark with artificial sea water, and pH was adjusted so one measured at pH 8.2 and the other pH 7.6: this modelled current and predicted ocean pH conditions. One tank was then used for pH 7.2 conditions after other experiments had been completed. pH was regularly measured throughout experiments and before the start of the bioassay to ensure pH was maintained at the correct level throughout testing and could be readjusted if necessary using sodium hydroxide or hydrochloric acid. The Y shaped olfactometers were positioned side by side, to ensure temperature and light intensity remained consistent for all pH conditions. The flumes were made from opaque non-reflective material to reduce reflections that may cause distress and affect results, as behaviour can be impacted by factors such as background noise, light intensity, and vibrations (Lewejohann et al., 2006; Simčič & Brancelj, 2007; Chiesa et al., 2010; Erbe, 2012; Wale et al., 2013; Filiciotto et al., 2016; O'Connor et al., 2019; Scott et al., 2020; Wilson et al., 2021). On the bottom of the flume 2 cm of sediment was added to mimic natural environment conditions. Silicon tea strainers were used to hold the cellulose chemical cues in place and buried into the sediment to prevent them from floating to the surface of the water. These were positioned at the end of each branch and provided a source of the odour cues, and as a physical object the crabs could grab.

A continuous flow set up was operating with two sea water butts holding the artificial seawater made up the day of experimental trials, and a charcoal filter in the pump to prevent odours being transferred in water (Figure 12 A). The two sea water butts were connected using a tube to make a continuous cycle, these were recirculating systems with charcoal filters to remove previously released odours. Artificial sea water entered through split flow tubes at the end of each branch at a flow rate of 500ml per minute. Artificial seawater exited at the other end of the flume at 1 litre per minute, this maintained the water level within the flumes and identical flow rates ensured that odours from the cues diffused at an equal rate.

Specimens of *C. maenas* were individually marked a few days prior to experiments for individual identification (Figure 9). However, in order to account for any change to individuals through injury, loss of limbs etc. crabs were weighed, and carapace width measured individually again and checked against the previous measurements before the experiment began. For each of the bioassay trials 40 randomly allocated *C. maenas* individuals were selected, 30 Males and 10 Females (Appendix Table 5). Males and females were tested separately with the 30 males tested first and 10 females tested second (Appendix Table 6). Silicon tea strainers were used to hold the cellulose odour cues in place and buried into the sediment to prevent them from floating to the surface of the water.

These were positioned at the end of each branch of the Y shaped olfactometer and provided a source of the odour cues, and as a physical object the crab could grab (Figure 12). Positioning of the cues was undertaken at random. The individual crab was placed into an overturned PVC basket inside the Y shaped olfactometer experimental set up for an acclimatisation period.

The acclimatisation period was for a duration of 2 minutes, by this time individuals had settled and showed no signs of stress or discomfort. After the acclimation period, the basket holding the crab was removed, and a timer was started. The crab was observed for a duration of 5 minutes and any behaviours were recorded upon observation: initial reaction (antennule flicking), time taken for individual to reach a cue at the end of either branch of the flume, which cue the crab ‘chose’ and any behaviours performed at the cue (Appendix Table 6). The behavioural reactions observed included initial detection: increased antennule flicking, maxilla fanning and reaction at cue: wafting, grabbing, burial, and continuous activity (Table 2).

If an individual did not reach a cue or perform any categorised behaviours this was also recorded. Once the 5-minute experiment duration was completed, the individual was removed from the Y shaped olfactometer and replaced into the holding tank.

Due to the required number of crabs for each pH treatment and cue, crabs were placed back into culture tanks after experiments were carried out. This ensured they could be re-used with a different cue on a different day. Different cues were tested on different days meaning there would always have been at least two days between the different cues to minimise the chance of cue recognition. There was no chance of the same crab being used twice in the same day as they were marked and after experiments crabs were placed in a separate holding tank until all experiments had been carried out before been returned to the culture tanks (Appendix Table 5).

3.7 pH treatments

C. maenas were cultured at pH 8.2 from the date of collection throughout the entirety of the study. Crabs were tested at the culture pH 8.2 as well as at pH 7.6 and 7.2 to test responses under low pH conditions. Where the test pH was different to the culture pH, crabs were not given any prior acclimation period other than the two-minute acclimation period as part of the experimental design. These three test pH treatments were selected for this experiment as pH 8.2 is used as a control level as it replicates the existing ocean pH level, pH 7.6 to create predicted future pH, and extremely low pH 7.2 to simulate extreme localised cases such as upwellings or CO_2 vents (de la Haye et al*.,* 2012; Chan et al*.,* 2017). Within these three pH conditions there were six treatments (cue combinations):

i. Plastic odour polyethylene (PE) v food odour glutathione (GSH)
ii. Plastic odour polyethylene (PE) v pheromone odour uridine diphosphate (UDP) and uridine triphosphate (UTP)
iii. Plastic odour polyethylene (PE) v control
iv. food odour glutathione (GSH) v control
v. pheromone odour uridine diphosphate (UDP) and uridine triphosphate (UTP) v Control
vi. food odour glutathione (GSH) v pheromone odour uridine diphosphate (UDP) and uridine triphosphate (UTP)

Food odour cues were made with the recognised feeding odour glutathione (GSH) (Loomis, 1955; Reimer, 1972), sex pheromone cues were made with a combination of uridine triphosphate (UTP) and uridine diphosphate (UDP), major components identified in female *C. maenas* urine (Hayden et al*.,* 2007; Hardege et al*.,* 2011). Artificial seawater was used for experiments, in the holding tanks between experiments, and for control cues in replacement of odour cues. Artificial seawater was selected as opposed to natural seawater for use in experiments as it could be presumed that there would be no pre-existing olfactory cues within samples. To prepare artificial seawater approximately 35 grams of salt was dissolved for every 1 litre of water, to align with typical ocean salinity. Artificial seawater measured 35 PSU in salinity and a temperature of 18 ± 0.5°C. Salinity was measured using HI96822 Refractometer (Hanna Instruments). pH levels of samples were achieved by bubbling CO_2 through samples using a pressurised canister, and then using 0.1 M sodium hydroxide (NaOH) or 0.1 M hydrochloric acid (HCl) for more precise adjustments. This was carried out in the same laboratory facilities where bioassays were completed at the University of Hull. The seawater was monitored throughout the course of each series of experiments and readjusted when the pH began to change, again using NaOH or HCl. The pH of the cues was increased/decreased using 0.01 M sodium hydroxide or 0.01 M hydrochloric acid in the same manner as the seawater.

3.8 Preparation and Storage of experimental cues

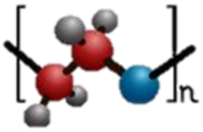
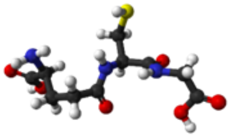
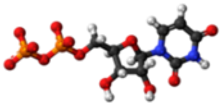
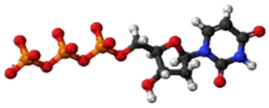
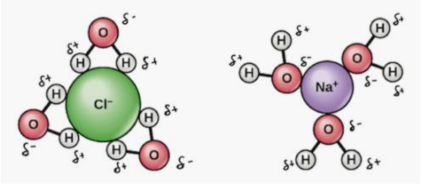
The experimental cues used in the bioassays were categorised into synthetic cues, plastic odour cues and controls. The synthetic cues used were glutathione (GSH) a food cue at 10^{-3} M concentration from Sigma-Aldrich. Pheromone odour cues were 4:1 uridine diphosphate

(UDP) to uridine triphosphate (UTP) at a concentration of 10^{-3} M, as the female pheromone cue (Hayden et al*.,* 2007; Hardege et al*.,* 2011). The control cues used were made with artificial seawater to provide a comparison to the odour cues.

Using the same methods as Savoca et al*.* (2016, 2017), polyethylene plastic (PE) beads with a size of 3 mm in diameter were used. The 'Plastic odour solution' was created by shredding 15g of the raw PE beads using a small electrical blender. The PE beads were blended for approximately 2 minutes. Then, 500ml of artificial seawater (pH 8.2) was added, this was the same seawater used in the experimental treatments. The plastic water solution was then blended for a further 2 minutes and was then transferred into a sealable glass beaker. The plastic was left to soak in the water stirring occasionally and left in a fridge for 6 hours. After 6 hours the mixture in the beaker was strained into a glass beaker through Whatman filter paper to remove particles greater than 25µm. After filtration the solution was stirred, and the solution was used to make carboxycellulose gels.

The cellulose odour cues were made by mixing carboxycellulose powder with artificial sea water and the required concentration of the odour (Table 3), then mixed well before pouring into ice cube trays and freezing overnight at -20˚C. All cues were freeze-dried using liquid nitrogen and stored in a -20˚ freezer until required for use in bioassays.

Table 3. Experimental cues used in bioassays, and their structural composition.

Plastic odour cue	Polyethylene (PE)	 Liliya (2021) Polyethylene molecule [computer generated model] Available online: https://www.alamy.com/polyethylene-polythene-pe-polyethene-polymethylene-molecule-it-is-polymer-of-ethylene-the-most-common-plastic-molecule-model-vector-illustra-image457382054.html [Accessed 17/11/21].
Food cue	Glutathione (GSH)	 Mills, B. (2008) Ball-and-stick model of the glutathione molecule [computer generated model] Available online: https://commons.wikimedia.org/wiki/File:Glutathione-from-xtal-3D-balls.png [Accessed 17/11/21].
Pheromone cue	Uridine diphosphate (UDP) Uridine triphosphate (UTP)	 Jynto (2011) Ball-and-stick model of the uridine diphosphate molecule [computer generated model] Available online: https://en.wikipedia.org/wiki/File:Uridine_diphosphate_anion_3D_ball.png [Accessed 17/11/21].  Jynto (2011) Ball-and-stick model of the uridine triphosphate molecule [computer generated model] Available online: https://en.wikipedia.org/wiki/File:Uridine_triphosphate_anion_3D_ball.png [Accessed 17/11/21].
Control cue	Filtered seawater	 OpenStax (2015) Image of saltwater molecule modified from "Water: Figure 3". by OpenStax College, Biology. Available online: https://cnx.org/contents/GFy_h8cu@9.87:pPjfgsd4@9/Water [Accessed 18/11/21].

3.9 Data analysis methods

Statistical analysis was performed using R Studio v1.3. Raw data (Table 4, Appendix Table 6) was logged on an excel spreadsheet with a simple layout and clear data columns. This spreadsheet was saved as a comma separated values file (.csv) in order to be uploaded to R Studio for data analysis to be completed.

Table 4. Partial example of spreadsheet showing headings and a few data entries. Description of raw data layout when recorded from bioassay observations. Completed test sheet included in appendices (Appendix Table 6).

crab ID #	size (cm)	Initial reaction (min)	Time to cue (min)	Cue chosen	reaction
28 M	6.8	00:04	02:25	GSH	Grab
29 M	6	00:02	00:26	GSH	Grab
30 M	5.4	00:05	04:41	PE	Waft
31 M	5	00:02	00:19	PE	Grab
32 M	5.6	00:02	-	-	Buried

Behavioural assays were limited to 5 minutes, if the individual did not reach either cue within the designated time, a time to cue of 300 seconds was given. Therefore, some individual bioassays were censored. To account for this, the effects of sex, size, colour, injury, and pH on time to cue were analysed using survival analysis or time-to-event analysis as described in Clements et al. (2021). This method can be applied to data that records the amount of time taken until an event of interest occurs, in the case of this study the event was reaching a cue, and takes into account 'censored' data points in which the event of interest did not occur within the defined time period, here individuals that did not reach a cue within the 5 minute bioassay observation period (Schober & Vetter, 2018; Clements et al*.,* 2021). For T-tests the p-value was set at 0.05 meaning for a significant difference to be observed and the null hypothesis rejected a p-value of <0.05 was required. The heatmaps and video footage was analysed on Lolitrack^{TM} software.

4. Results

4.1 Response to feeding and reproductive cues

> *Hypothesis: At low pH (7.6 and 7.2) C. maenas will show a reduced response to a) feeding cue (glutathione) and b) synthetic reproductive olfactory cues (UDP/UTP) than individuals tested at normal pH 8.2.*

There was a significant difference in initial reaction time for feeding and pheromone cues between pH 8.2 and 7.2 (P = 0.0017) (T-test between pH 8.2 and 7.2) (Figure 13 A). Initial reaction time was observed to be significantly slower for both pheromone (UDP/UTP) and feeding (GSH) cues as pH decreased (Figure 13 A), suggesting that reception of the cue is impaired by low pH. (P = < 0.000013) (T-test between pH 8.2 and 7.2 for pheromone cue), P = < 0.00001 (T-test between pH 8.2 and 7.6 for pheromone cue), (P = < 0.00001) (T-test between pH 8.2 and 7.2 for glutathione feeding cue), and (P = < 0.00001) (T-test between pH 8.2 and 7.6 for glutathione feeding cue) (Figure 13 A).

Time taken to reach the cue increases as pH decreases to 7.2. There was a significant difference in time to reach cue for both feeding and pheromone cues between pH 8.2 and 7.2 (P = < 0.00001) (T-test between pH 8.2 and 7.2 for pheromone cue), (P = 0.000355) (T-test between pH 8.2 and 7.2 for glutathione feeding cue) (Figure 13 B). However, there was no significant difference between pH 8.2 and 7.6 for both pheromone and feeding cues (Figure 13 B).

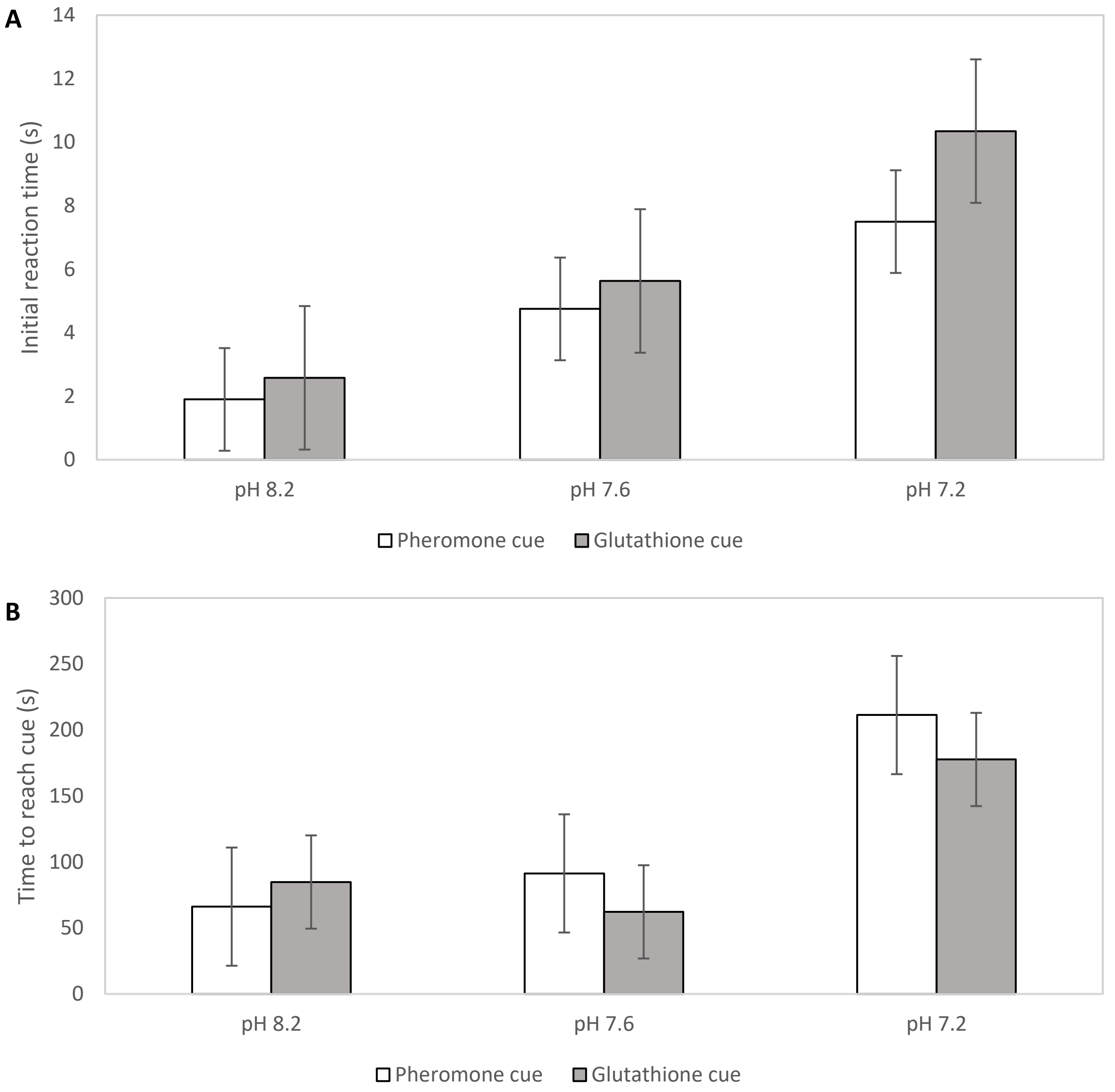


Figure 13: Initial reaction time in seconds A, and time taken to reach a cue in seconds B, for bioassays testing feeding GSH (Glutathione) and reproductive UDP/UTP (Uridinediphosphate and Uridinetriphosphate) cues in the three pH treatments 8.2, 7.6 and 7.2. Results based on mean values with standard error bars. In each pH treatment, number of replicates (n) = 40, additional individuality data presented in Appendix Table 5 and Table 1.

In the condition food cue (GSH) v pheromone cue (UDP/UTP), results showed that *C. maenas* had a strong preference to the pheromone cue in pH 8.2, however when this was repeated in pH 7.6 this preference switched and GSH was chosen over the pheromone cue (Table 5). In pH 7.2 50% of individuals remained at the starting end of the tank for the study duration without making a choice. From the individuals who did make a choice GSH was again favoured over the pheromone cue (Table 5). The result of the condition pheromone v control showed that pheromone was chosen over the control cue in both pH 8.2 and 7.6. However, in pH 7.2 60% of individuals chose the control cue (Table 5). From these results there is a clear pattern that in extreme low pH 7.2 treatments individuals made significantly less choices than in the other two pH treatments (Table 5).

Table 5. Percentage of shore crabs that interacted with food and pheromone odour cues across experimental conditions in current pH (8.2) low pH (7.6) and extreme pH (7.2) treatments. N/A refers to percentage of individuals that did not reach either cue in the 5-minute bioassay time.

pH	**GSH V Control**			**GSH V Pheromone**			**Pheromone V Control**		
	GSH	Control	N/A	GSH	Pheromone	N/A	Pheromone	Control	N/A
8.2	25	57.5	17.5	30	57.5	12.5	55	30	15
7.6	37.5	55	7.5	42.5	37.5	20	45	37.5	17.5
7.2	22.5	30	47.5	37.5	12.5	50	17.5	22.5	60

4.2 Response to plastic odour cues

Hypothesis: Individuals tested with the presence of plastic odour will show a reduced response to olfactory cues (UDP/UTP and glutathione) than individuals tested without the presence of plastic odour.

Hypothesis: At low pH (7.6 and 7.2) individuals tested with the presence of plastic odour will show a more reduced response to olfactory cues (UDP/UTP and glutathione) than individuals tested with the presence of plastic odour at normal pH (8.2).

In order to determine whether plastic odour influenced the response to olfactory cues a series of t-tests were performed to compare conditions with plastic odour and those without. There was a significant difference between initial reaction time in conditions with plastic odour tested at pH 8.2 and conditions without plastic odour at pH 8.2 (T-test: $p = <0.00001$).

Initial reaction time was observed to be slower with plastic odour present for both feeding and reproductive cues across all pH treatments (Figure 14 A and B). The initial reaction time for detecting the pheromone cue (UDP/UTP) increased by an average of 2 seconds with the addition of a plastic odour cue across the pH treatments. The initial reaction time for detecting the feeding cue Glutathione (GSH) increased by an average of 3.9 seconds with the addition of a plastic odour cue across the pH treatments. Time taken to reach the cue was not affected by the presence of plastic odour as there was no significant difference (Figure 14 C and D).

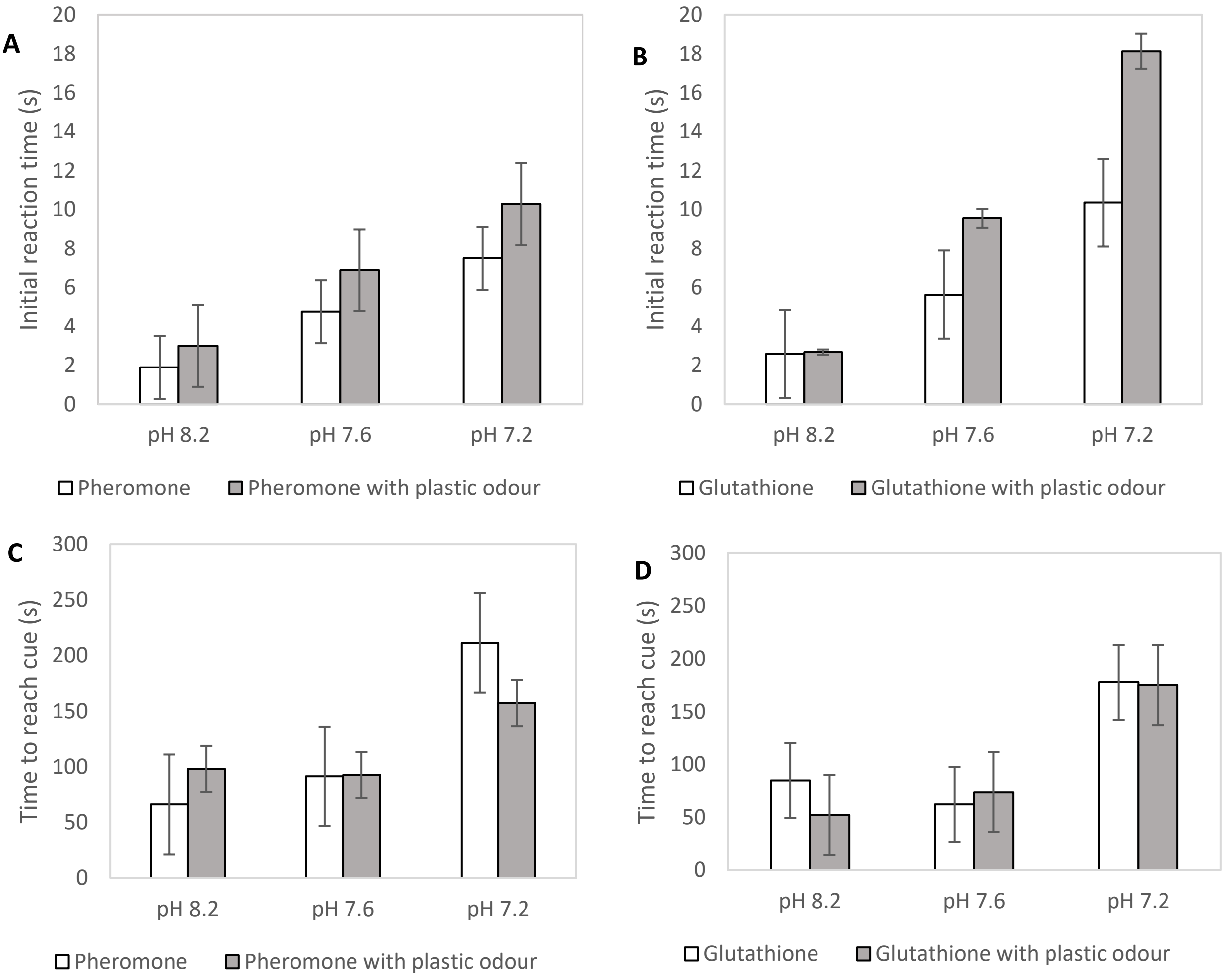


Figure 14: Comparison of initial reaction time and time taken to reach cue with the presence of plastic odour (grey) and without (white) for bioassays testing feeding and reproductive cues (GSH and UDP/UTP). With initial reaction time to pheromone cues (A), initial reaction to Glutathione feeding cues (B), time taken to reach cue in pheromone bioassays (C), time to reach cue in Glutathione feeding cue bioassays (D). Results based on mean values with standard error bars. In each pH treatment n = 40, additional individuality data presented in Appendix Table 5 and Table 1.

When comparing responses across all six conditions at each of the three test pH treatments there was a significant difference (p = 0.014469) between initial reaction time in conditions with a plastic odour and those without (Figure 15 A). Time taken to reach the cue across the six conditions at each pH test treatment showed no clear trends or patterns regarding the presence of plastic odour or not. However, it was clear that in the extreme low pH 7.2 time to reach cue was significantly longer than the other pH treatments (Figure 15 B).

In order to determine whether pH had an influence on the response to plastic odour a series of t-tests were performed to compare against conditions with plastic odour between pH levels. There was a significant difference in initial reaction time in conditions with plastic between pH 8.2 and 7.6 (T-test, p = <0.0001). There was also a significant difference in initial reaction time in conditions with plastic between pH 8.2 and 7.2 (T-test, p = <0.0001). For time taken to reach the cue there was no significance between pH 8.2 and 7.6, however, in extremely low pH 7.2 there was a significant difference in time taken to reach cue (T-test, p = 0.000213).

In the condition Plastic vs Food (PE v GSH) results showed a mix of preference to plastic odour cue and the glutathione feeding cue with no clear preferred choice (Table 6). As pH was reduced the percentage of crabs that did not respond increased. From the individuals who did make a choice GSH was favoured over the plastic odour cue however only slightly and not sufficient to be significant.

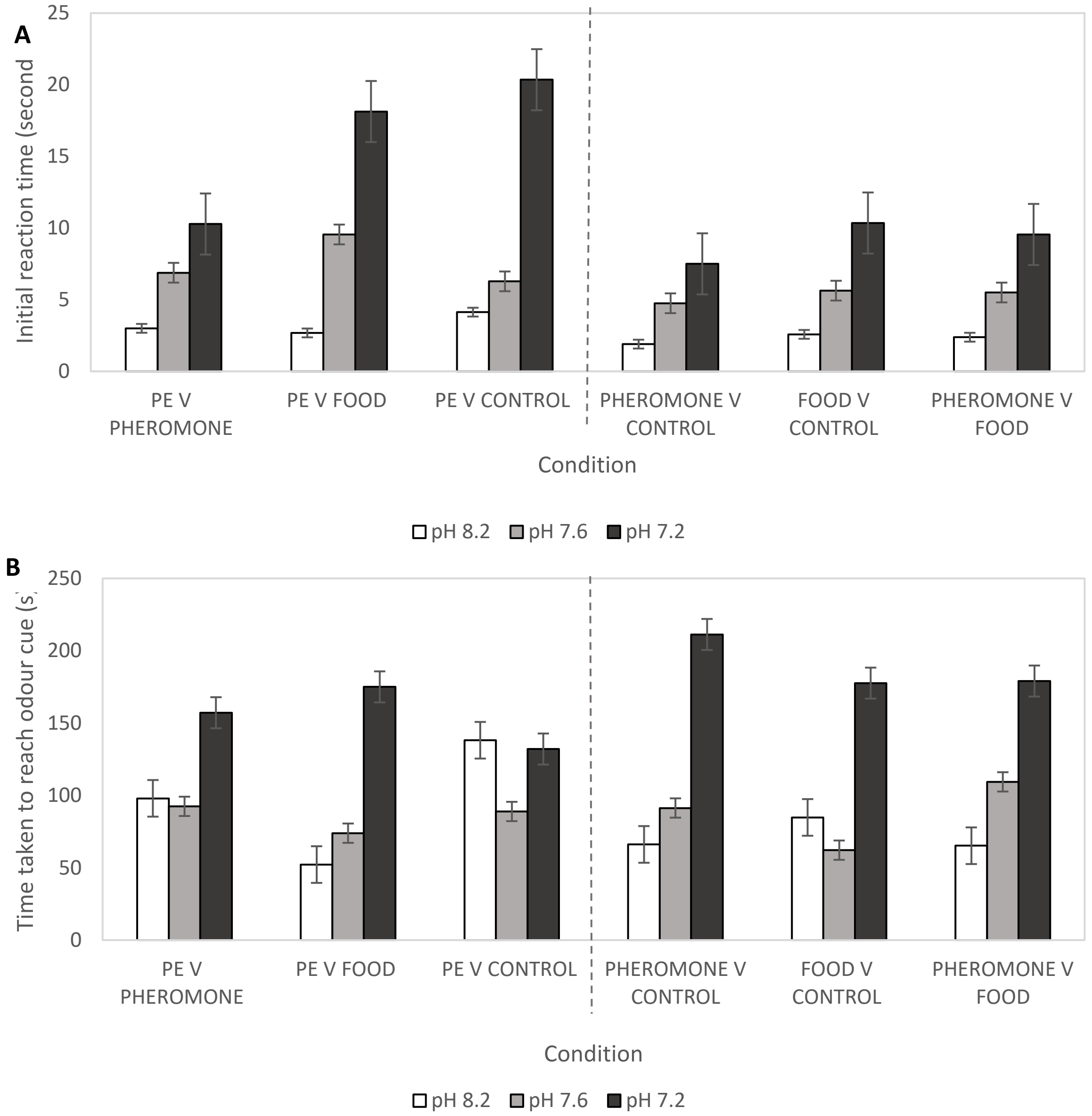


Figure 15: Responses to olfactory cues across all six conditions at each of the three test pH treatments (Conditions testing odour cues against plastic odour displayed on the left of the dotted line and conditions testing odour cues without plastic odour on the right). Shows the mean values for initial reaction (A) and time taken to reach the cue (B). Results based on mean values with standard error bars. Each bar represents n = 40, additional individuality data presented in Appendix Table 5 and Table 1.

The result of the condition PE v Pheromone showed that in the treatment pH 8.2, 50% of crabs chose the pheromone. However, in pH 7.6 this was reversed as 50% of individuals then chose the plastic odour cue. In pH 7.2 responses were mixed and no significant results (Table 6).

Table 6. Percentage of shore crabs that interacted with odour cues across experimental conditions with plastic odour in current pH (8.2) low pH (7.6) and extreme pH (7.2) treatments.

pH	**PE V Control**			**PE V GSH**			**PE V Pheromone**		
	PE	Control	N/A	PE	GSH	N/A	PE	Pheromone	N/A
8.2	22.5	40	37.5	52.5	37.5	10	30	50	20
7.6	37.5	40	22.5	40	42.5	17	50	32.5	17.5
7.2	42.5	32.5	25	30	35	35	35	37.5	27.5

By comparing the choice of cue made by individuals across all conditions it was clear that decisions became altered under extremely low pH conditions (Figure 16). The percentage of crabs which did not respond within the 5-minute bioassay time increased in extreme low pH 7.2 conditions, this trend was most apparent in the treatments not containing plastic odour (Figure 16 D, E, F).

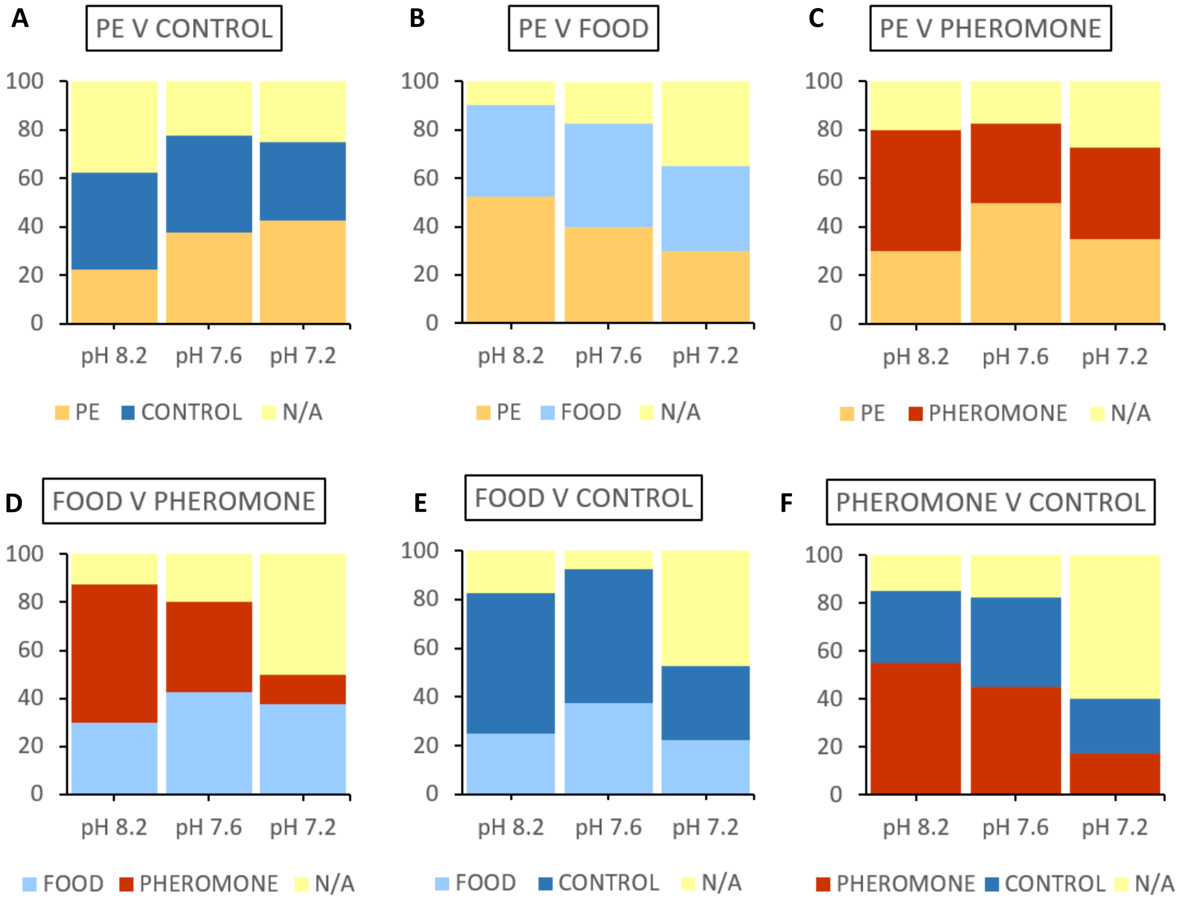


Figure 16: Comparison of choice % of olfactory cues reached across all six bioassay conditions at each of the three test pH treatments. In each pH treatment n = 40, additional individuality data presented in Appendix Table 5 and Table 1.

4.3 Individuality

Hypothesis: Individuals will have variability in cue detection and response relating to sex, size, colour, and injury.

Variances between individuals were observed throughout data collection. To verify statistically significant variance between individuals, data regarding initial reaction for each individual was tested using a Kruskal-Wallis test (Appendix Table 7).

The Kruskal-Wallis test results indicated that the initial reaction times show significant variability, which is dependent on different individuals. Tests were carried out to determine whether this variance between individuals shared a relationship to a simple measurable morphologic trait: sex (male or female), body-size measured by carapace width (cm), colour morph (red or green) and whether the individual had any injuries.

4.3.1 Sex: comparison of response of male and female *C. maenas* to feeding and reproductive cues

General observations made during bioassays noted sex specific differences in response to cues. Overview of choices made by male and female individuals (Appendix Figure 1.) shows that males respond much stronger to female sex pheromone UDP.

Results showed sex dependant responses. There was statistical significance between cue chosen and sex of crab (p=0.00012) (shared data Ohnstad, 2022) (Appendix Figure 1.) and between the sex of the crab and the behaviour shown at the chemical cue (p=0.0015) (Table 7).

Table 7. Statistical output for Kruskal Wallis test, testing for individuality relating to sex

Testing for individuality relating to sex			
	χ 2	df	p-value
Kruskal-Wallis test: sex and cue chosen	14.727	1	0.00012
Kruskal-Wallis test: sex by behaviour	19.566	5	0.0015

Initial reaction times for male and female individuals showed no significant difference (Figure 18).

Time taken to reach cue for male and female individuals showed no significant difference, however in low pH on average females took longer to reach a cue than males (Figure 19).

However, there may be a sex dependent response to plastic odour. On average female individuals had a much wider range of distance than males, and were showing less interest in the plastic odour, particularly in pH 8.2 (Appendix Figure 2.).

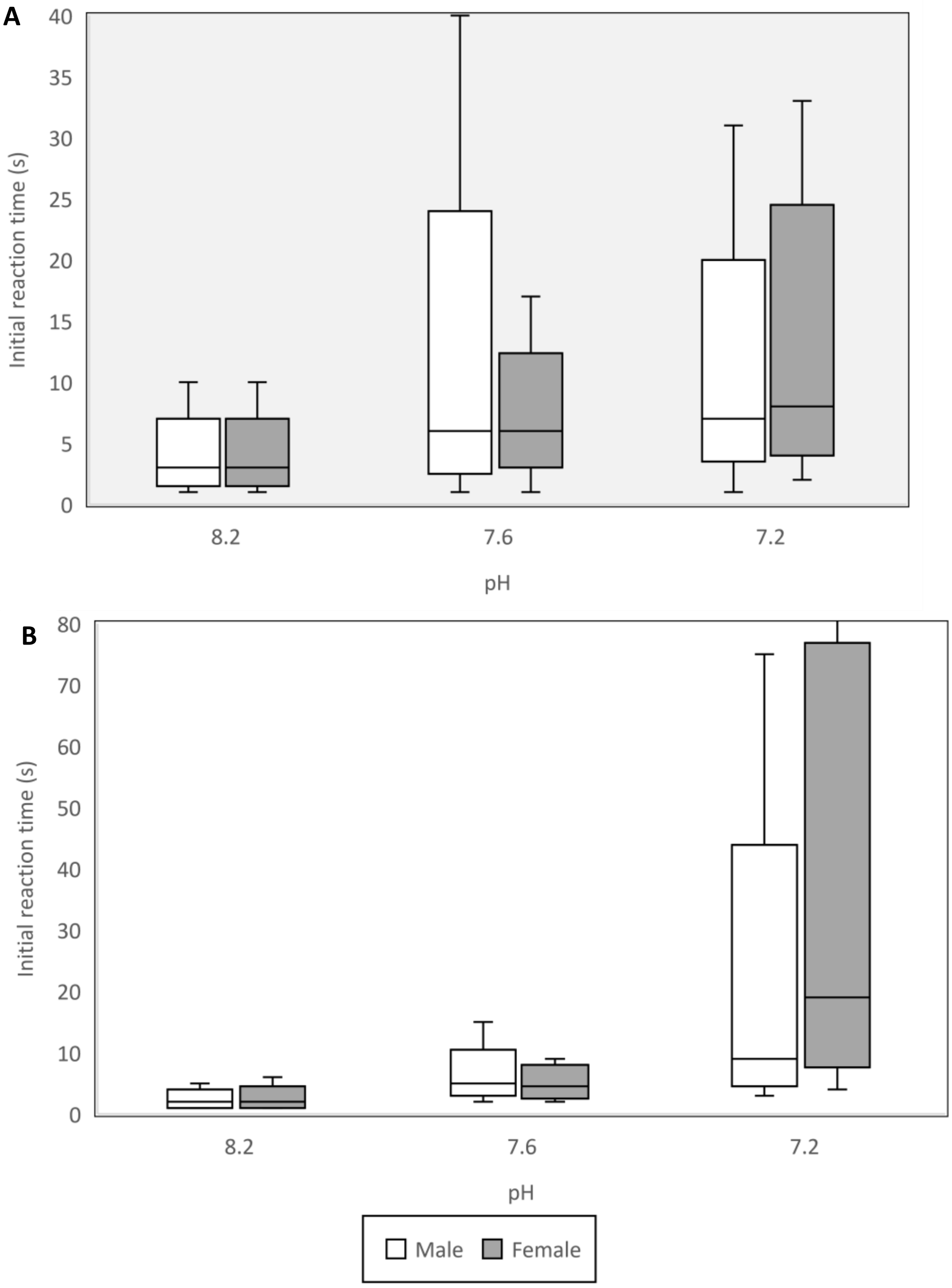


Figure 18: Initial reaction times for male and female *C. maenas* across three pH treatments (8.2, 7.6 and 7.2). A (light grey background) pooled results from bioassays tested containing plastic odour (PE v GSH, PE v UDP/UTP) excluding those tested against control cues, B (white background) pooled results from bioassays tested without plastic odour (GSH v UDP/UTP) excluding those tested against control cues. In each pH treatment n = 80, additional individuality data presented in Appendix Table 5 and Table 1.

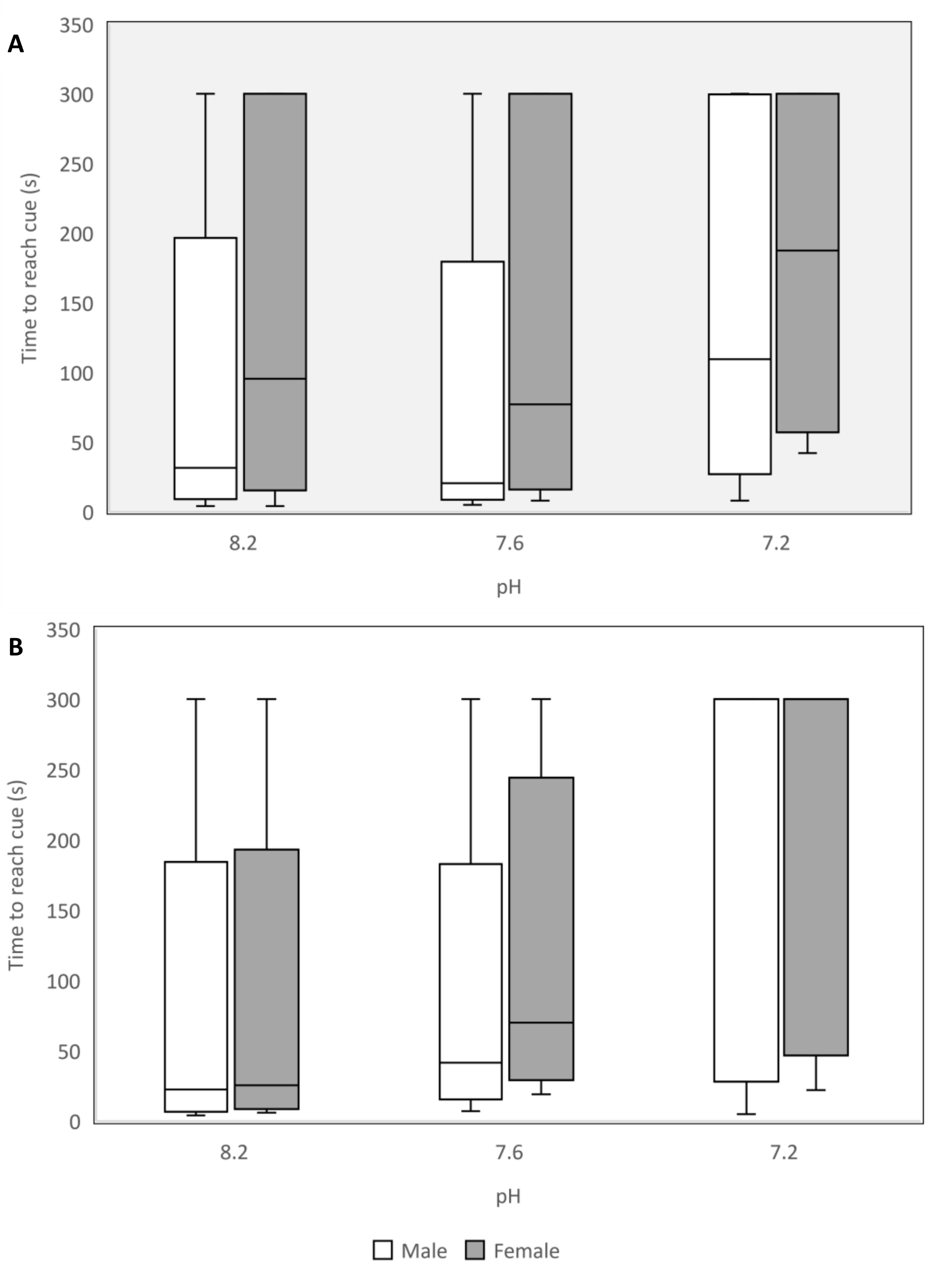


Figure 19: Time taken to reach a cue for male and female C. maenas across three pH treatments (8.2, 7.6 and 7.2). A (light grey background) pooled results from bioassays tested containing plastic odour (PE v GSH, PE v UDP/UTP) excluding those tested against control cues, B (white background) pooled results from bioassays tested without plastic odour (GSH v UDP/UTP) excluding those tested against control cues. In each pH treatment n = 80, additional individuality data presented in Appendix Table 5 and Table 1.

4.3.2 Size: comparison of response of small and large *C. maenas* to feeding and reproductive cues

There was statistical significance between the size of the crab (cm) and the initial reaction time (s), p=0.047 (Figure 20 B) (Table 8). Additionally, there was significant difference between size of the individual and the cue chosen (p=0.0016) (Table 9) (shared data Ohnstad, 2022).

Table 8. Statistical output for Kruskal Wallis test, testing for individuality relating to size and initial reaction

Testing for individuality relating to initial reaction			
	χ 2	df	p-value
Kruskal-Wallis test: Initial reaction by size	36.673	24	0.04716

Table 9. Statistical output for Kruskal Wallis test, testing for individuality relating to size

Testing for individuality relating to size			
	χ 2	df	p-value
Kruskal Wallis: size and cue chosen	49.527	24	0.001626

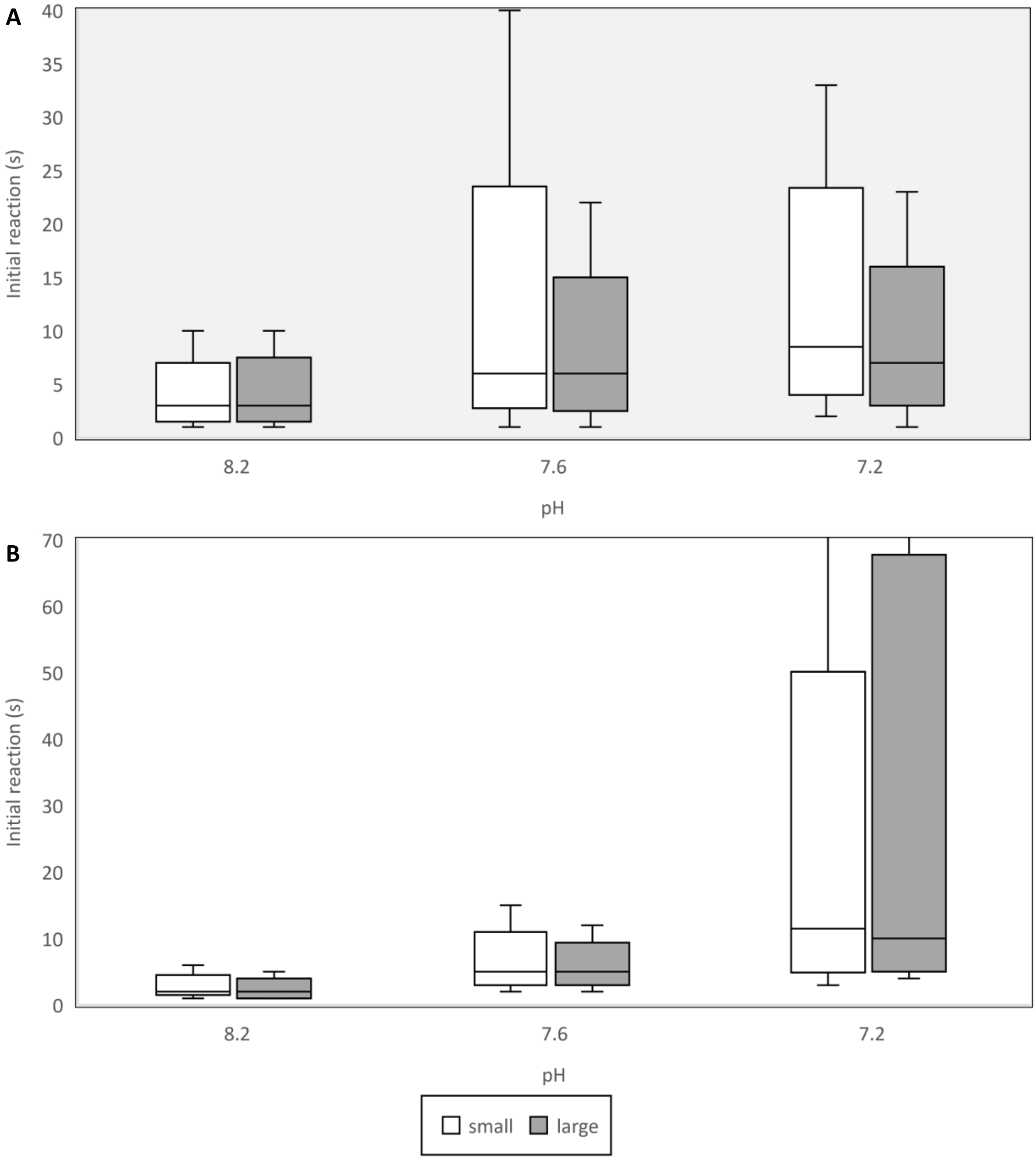


Figure 20: Initial reaction times for small and large C. maenas across three pH treatments (8.2, 7.6 and 7.2). A (light grey background) pooled results from bioassays tested containing plastic odour (PE v GSH, PE v UDP/UTP) excluding those tested against control cues, B (white background) pooled results from bioassays tested without plastic odour (GSH v UDP/UTP) excluding those tested against control cues. In each pH treatment n = 80, additional individuality data presented in Appendix Table 5 and Table 1.

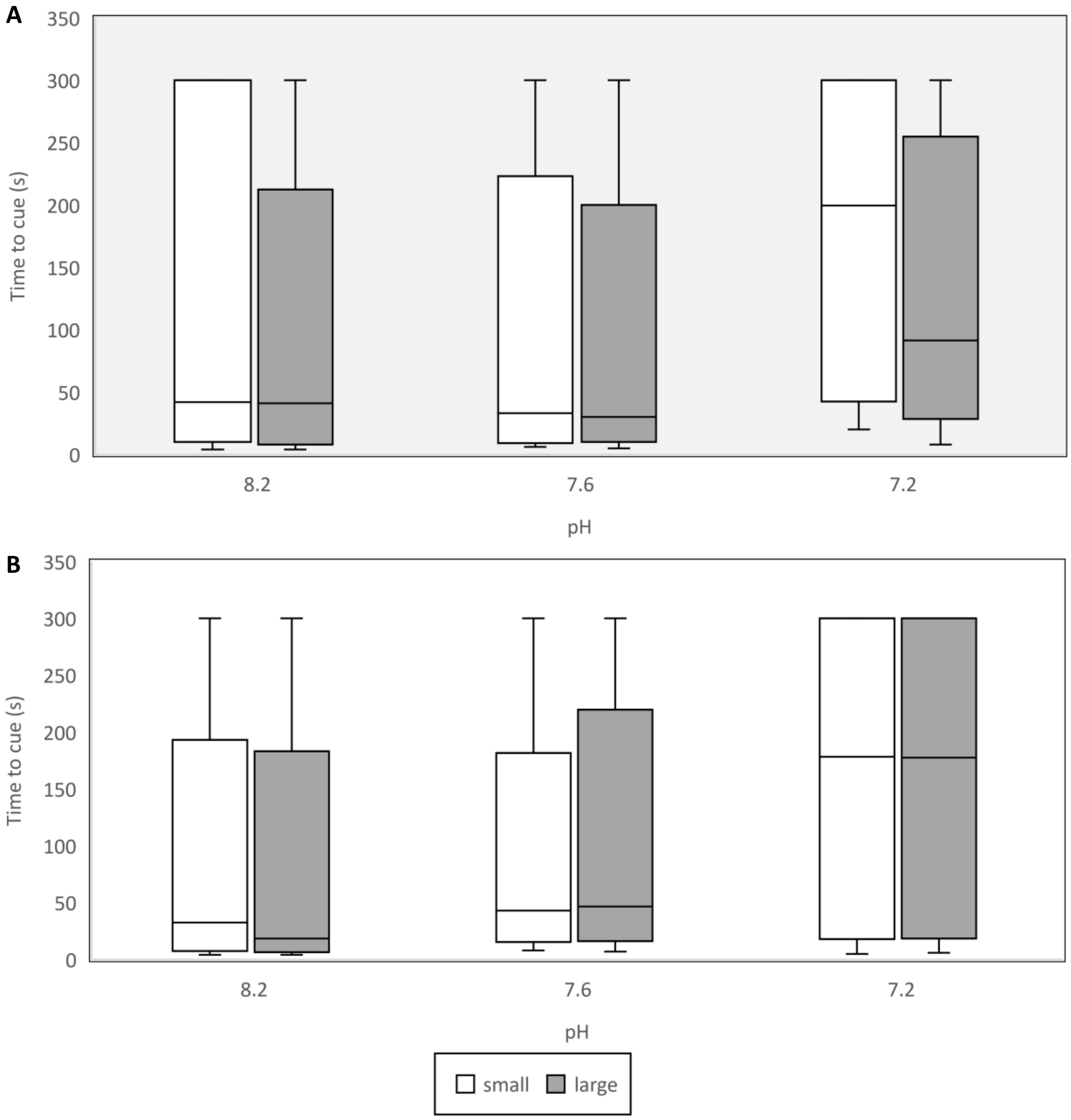


Figure 21: Time taken to reach a cue for small and large *C. maenas* individuals across three pH treatments (8.2, 7.6 and 7.2). A (light grey background) pooled results from bioassays tested containing plastic odour (PE v GSH, PE v UDP/UTP) excluding those tested against control cues, B (white background) pooled results from bioassays tested without plastic odour (GSH v UDP/UTP) excluding those tested against control cues. In each pH treatment n = 80, additional individuality data presented in Appendix Table 5 and Table 1.

4.3.3 Colour: comparison of response of red and green *C. maenas* to feeding and reproductive cues

Initial reaction of red crabs was significantly slower than green crabs in pH 7.2. Kruskal-Wallis test: Initial reaction by colour $\chi 2 = 4.1635$, df = 1, p-value = 0.0413 (Table 10). Red crabs had longer initial reaction times in extreme low pH 7.2 suggesting olfactory ability was affected more than green crabs (Figure 22). Additionally red crabs also took longer to reach a cue than green crabs in all pH conditions (Figure 23).

Table 10. Statistical output for Kruskal Wallis test, testing for individuality relating to colour and initial reaction.

Testing for individuality relating to initial reaction			
	χ 2	df	p-value
Kruskal-Wallis test: Initial reaction by colour	4.1635	1	0.0413

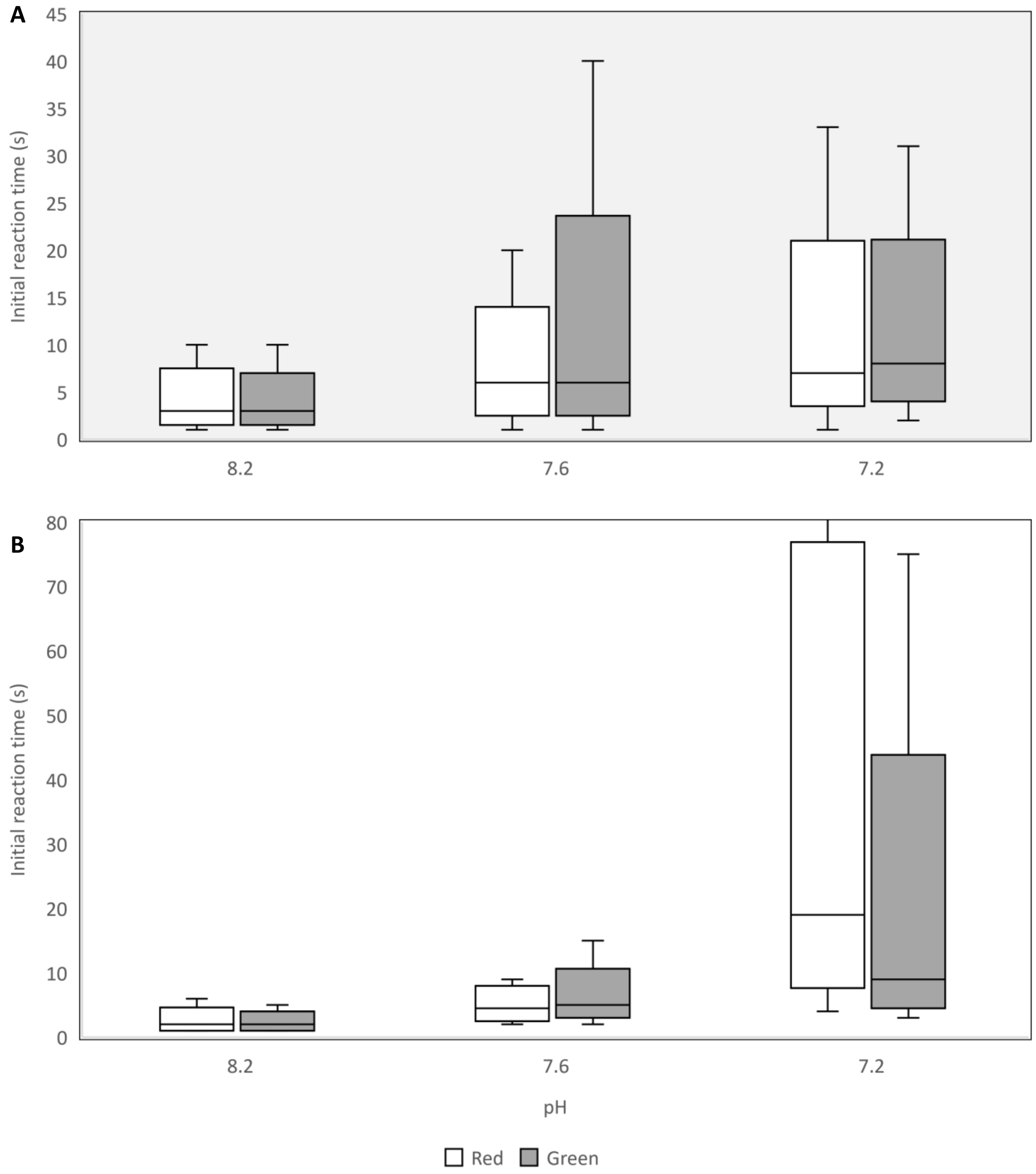


Figure 22: Initial reaction times for red and green *C. maenas* individuals across three pH treatments (8.2, 7.6 and 7.2). A (light grey background) pooled results from bioassays tested containing plastic odour (PE v GSH, PE v UDP/UTP) excluding those tested against control cues, B (white background) pooled results from bioassays tested without plastic odour (GSH v UDP/UTP) excluding those tested against control cues. In each pH treatment n = 80, additional individuality data presented in Appendix Table 5 and Table 1.

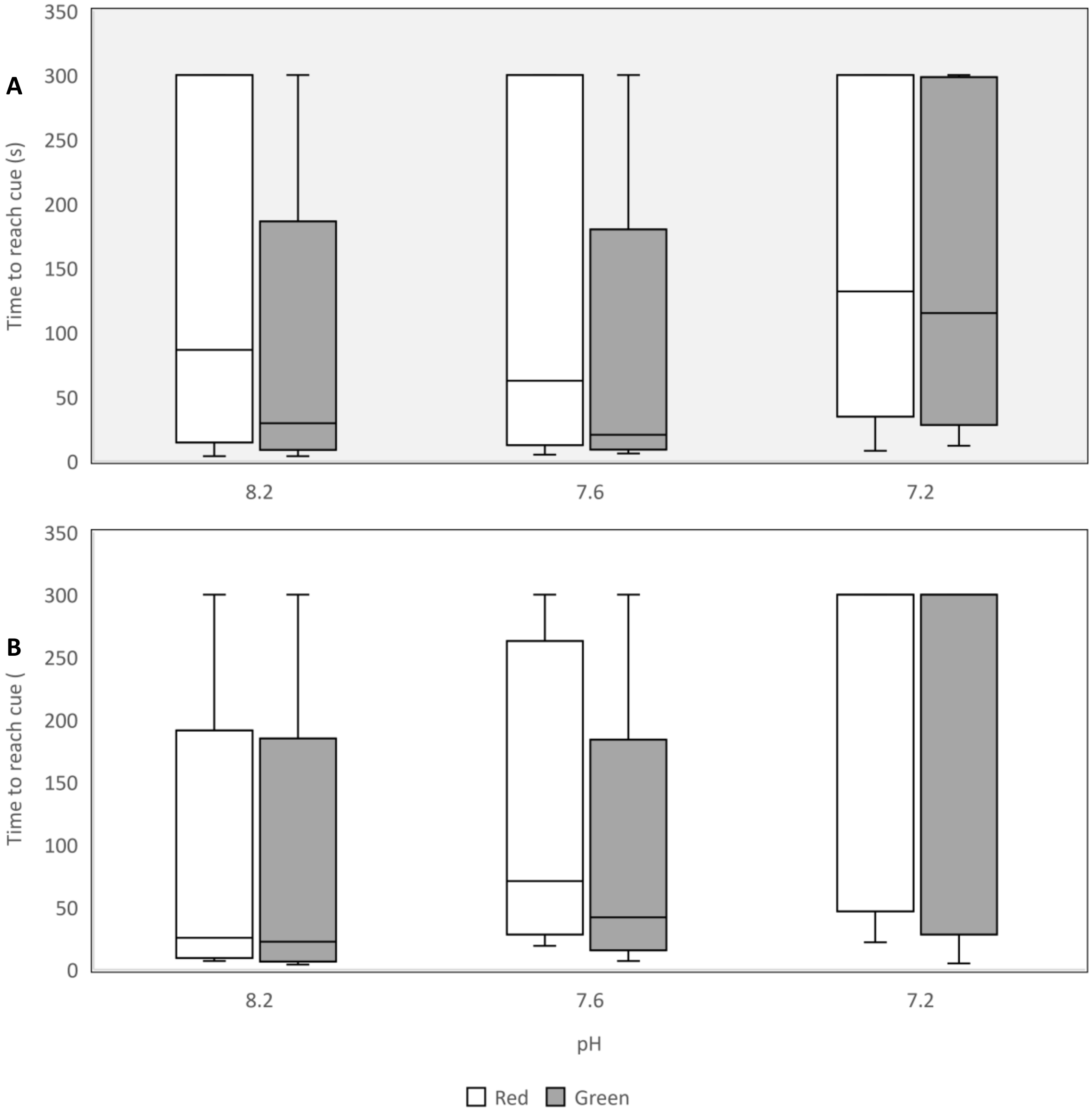


Figure 23: Time taken to reach a cue for red and green *C. maenas* individuals across three pH treatments (8.2, 7.6 and 7.2). A (light grey background) pooled results from bioassays tested containing plastic odour (PE v GSH, PE v UDP/UTP) excluding those tested against control cues, B (white background) pooled results from bioassays tested without plastic odour (GSH v UDP/UTP) excluding those tested against control cues. In each pH treatment n = 80, additional individuality data presented in Appendix Table 5 and Table 1.

4.3.4 Injury: comparison of response of injured and non-injured *C. maenas* to feeding, reproductive and plastic cues

Initial reaction was significantly impacted under extreme low pH 7.2 conditions (Figure 24 B), however there was no significant difference between reaction times in pH 8.2 and 7.6. In treatments containing plastic odour there was no significant difference between the three pH conditions (Figure 24 A). Individuals with no injuries took longer to exhibit initial reactions than those with injuries, however this was not statistically significant. Injury significantly affected the time taken to reach cue $X^2 = 8.99$, df = 1, p-value = 0.002715 (Figure 25).

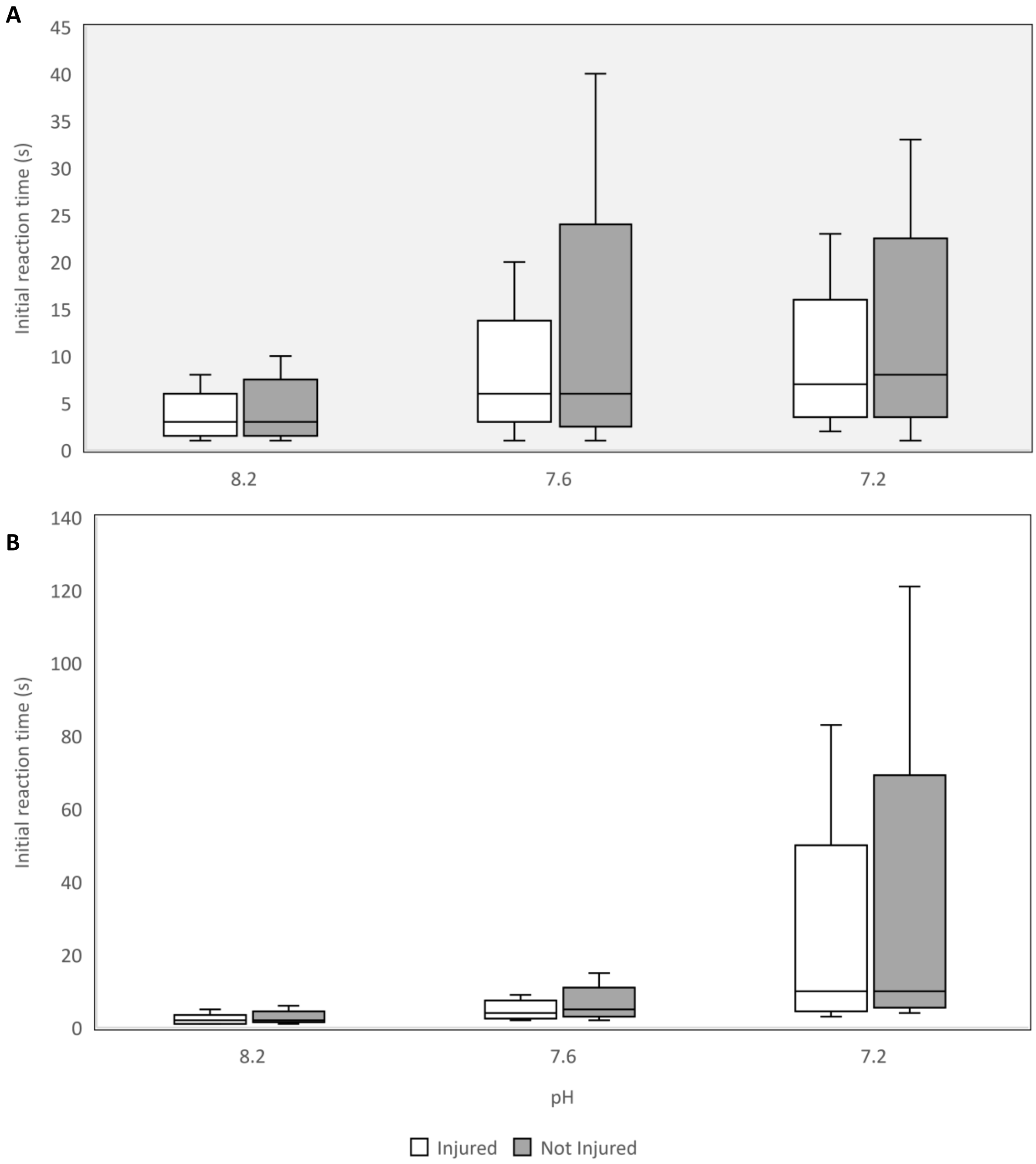


Figure 24: Initial reaction times for injured and non-injured *C. maenas* individuals across three pH treatments (8.2, 7.6 and 7.2). A (light grey background) pooled results from bioassays tested containing plastic odour (PE v GSH, PE v UDP/UTP) excluding those tested against control cues, B (white background) pooled results from bioassays tested without plastic odour (GSH v UDP/UTP) excluding those tested against control cues. In each pH treatment n = 80, additional individuality data presented in Appendix Table 5 and Table 1.

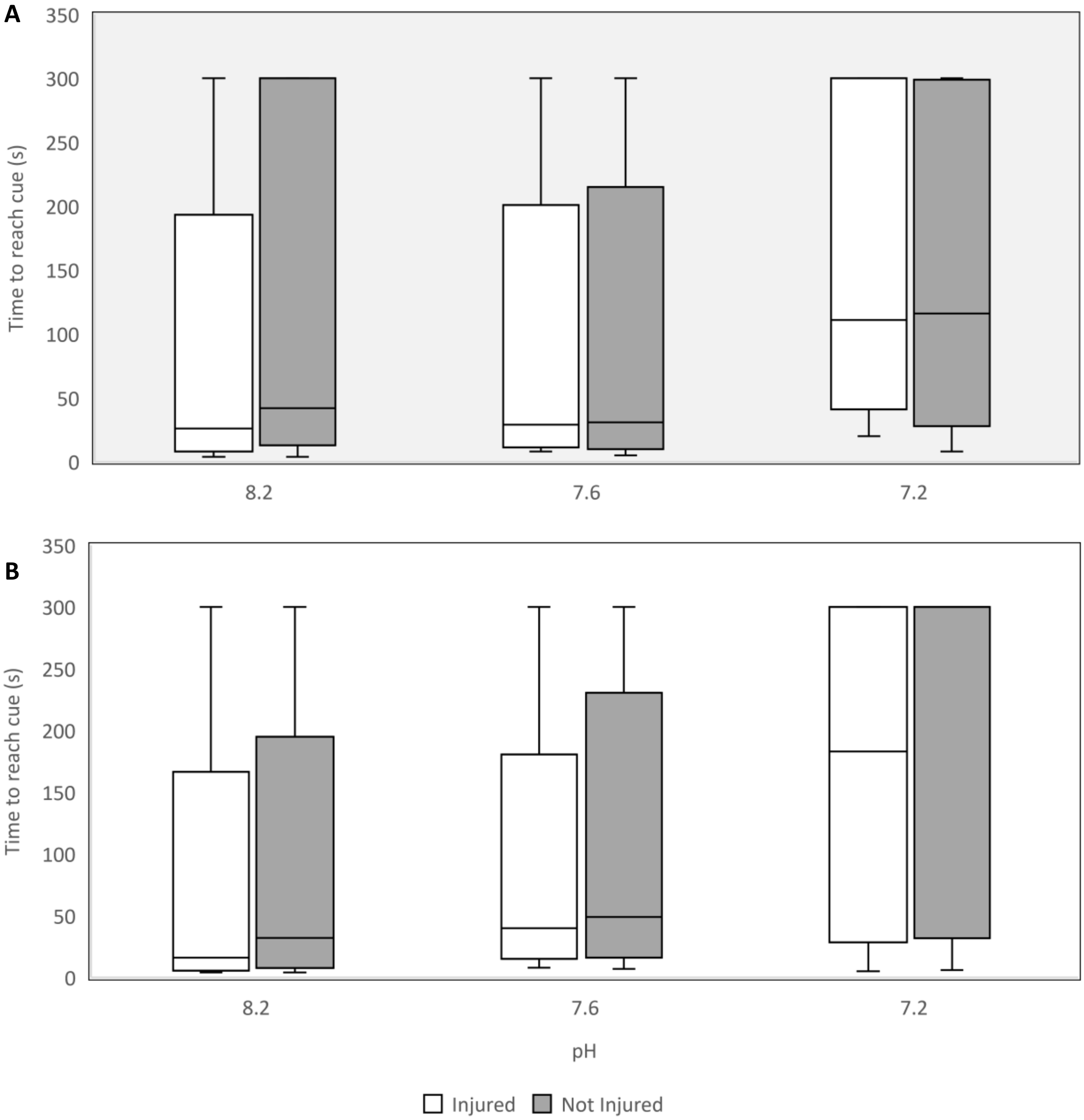


Figure 25: Time taken to reach a cue for injured and non-injured *C. maenas* individuals across three pH treatments (8.2, 7.6 and 7.2). A (light grey background) pooled results from bioassays tested containing plastic odour (PE v GSH, PE v UDP/UTP) excluding those tested against control cues, B (white background) pooled results from bioassays tested without plastic odour (GSH v UDP/UTP) excluding those tested against control cues. In each pH treatment n = 80, additional individuality data presented in Appendix Table 5 and Table 1.

4.3.5 Individuality: Overview and trendlines

Results from individuality analysis revealed similar trendlines in initial reaction time (Figure 26) and time taken to reach cue (Figure 27) across all individuality aspects studied: sex, size, colour, and injury. General trends showed initial reaction times were slower in pH 7.2 and conditions with plastic odour present also resulted in slower initial reaction times (Figure 26). Time taken to reach a cue increased in pH 7.2. Additionally, the presence of plastic odour increased time taken to reach a cue in normal pH 8.2 (Figure 27).

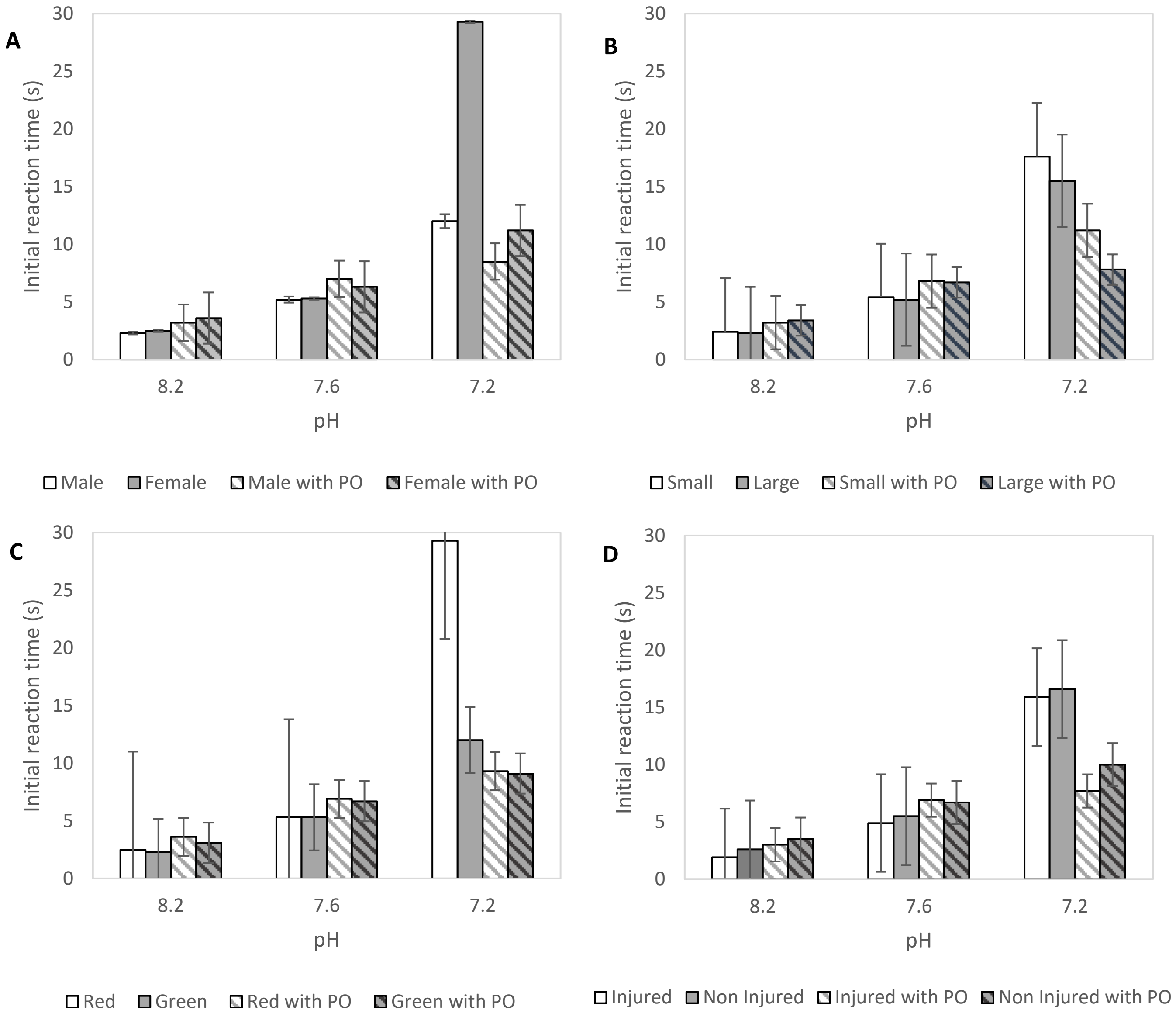


Figure 26: Comparison of initial reaction times in seconds for all aspects of individuality: Sex (A), size (B), colour (C), and Injury (D) across three pH levels in conditions without and with plastic odour. Initial reaction times with plastic odour present are indicated by diagonal banding. Results based on mean values with standard error bars.

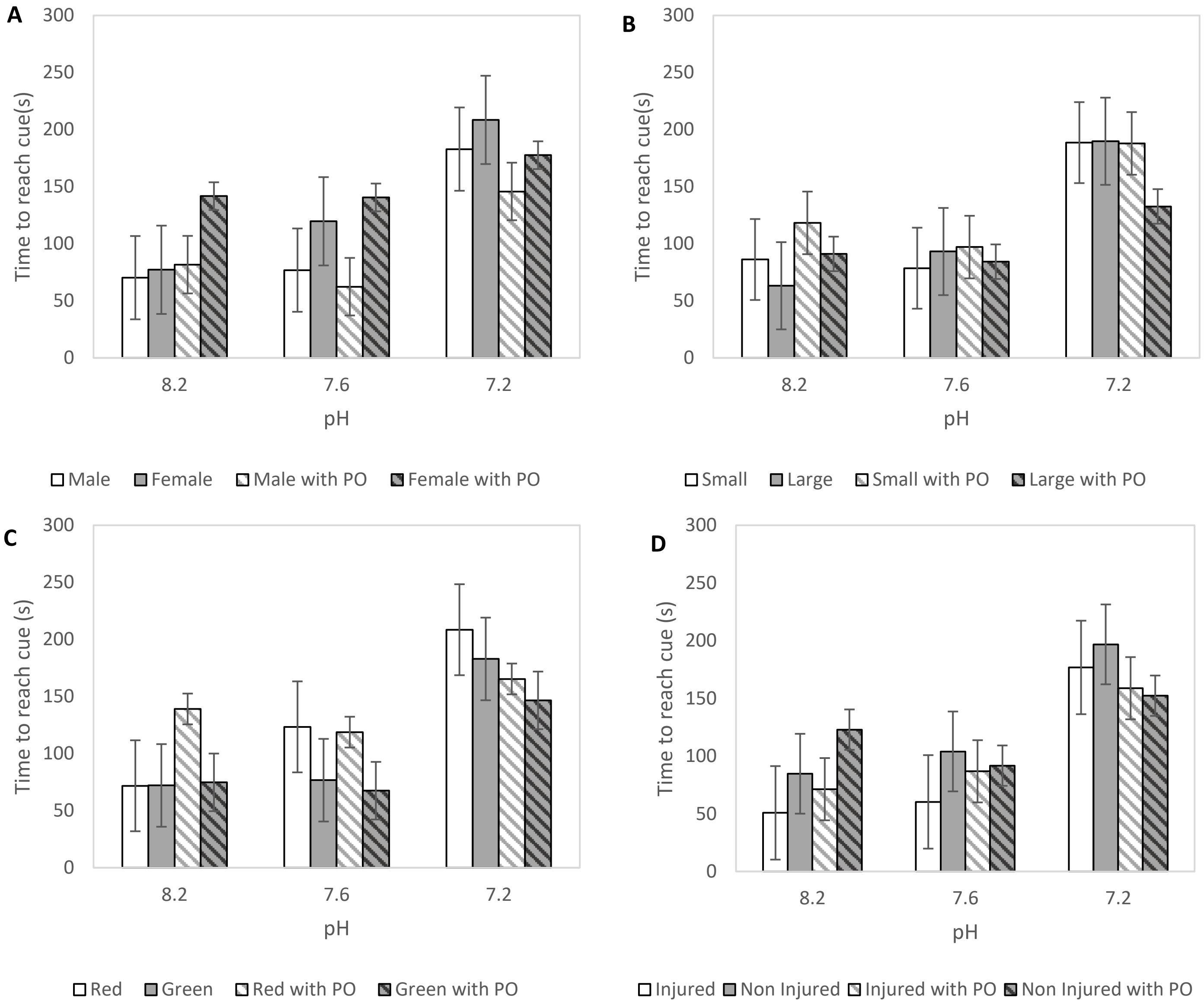


Figure 27: Comparison of time taken to reach cue in seconds for all aspects of individuality: Sex (A), size (B), colour (C), and Injury (D) across three pH levels in conditions without and with plastic odour. Initial reaction times with plastic odour present are indicated by diagonal banding. Results based on mean values with standard error bars.

5. Discussion

5.1 Response to feeding and reproductive cues under low pH

Hypothesis 1: At low pH (7.6 and 7.2) C. maenas will show a reduced response to a) feeding cue (glutathione) and b) synthetic reproductive olfactory cues (UDP/UTP) than individuals tested at normal pH 8.2.

The findings of this study confirmed that for *C. maenas*, both detection and response to feeding cues (glutathione) and synthetic reproductive olfactory cues (UDP/UTP) were significantly affected by a reduction in pH. Impairments were seen under low pH 7.6 and extremely low pH 7.2 conditions aligning with predicted changes to occur by the year 2100 (IPCC, 2014, 2019; Findlay & Turley, 2021) and potentially localised areas of extreme low pH (Hall-Spencer et al., 2008; Boatta et al., 2013; Aiuppa et al., 2021). Overall, there was a reduced response to feeding (glutathione) and sex pheromone (UDP/UTP) olfactory cues in pH 7.6 conditions and significantly lower response in pH 7.2 condition in comparison to responses in the pH 8.2 conditions (Figure 13 A and 13 B).

Initial reaction time was significantly different between pH 8.2 and 7.2 for all olfactory cues (Figure 13 A), suggesting that cue reception was impaired by extremely low pH. Examining the response to the feeding cue glutathione separately revealed that initial reaction time significantly increased when tested under pH 7.6 and 7.2. This response was also identified when examining the synthetic reproductive cue UDP/UTP, initial reaction times in both pH 7.6 and 7.2 were significantly delayed compared to pH 8.2.

In the present study, cue choices revealed that in extremely low pH 7.2 fewer individuals made it to either cue presented (Table 4) (Figure 16). The percentage of individuals that did not seek out a cue increased in extremely low pH 7.2 and more individuals remained in the far end of the tank and displayed behaviours such as burying as opposed to antennule flicking and maxilla fanning.

Time taken to reach the cue was significantly different between pH 8.2 and extremely low pH 7.2, however not between pH 8.2 and 7.6 (Figure 13 B). This suggested that low pH did not affect organism physiology and activity unless in extremely low pH. However, the increased time taken to reach the cue could also be due to impaired detection of the odour source, and therefore reduced decision making thus causing a delayed reaction time when seeking out the olfactory cue. Here the reduced response to chemical cues was presumed to be a result of impaired detection of the chemical signal rather than due to damage to physiological state or fitness levels of the individual. Many studies have described reduced olfactory mediated behaviours in marine organisms under low pH conditions, including foraging, resource assessment, and predator recognition (see Draper & Weissburg, 2019 for review).

These findings align with the current literature on ocean acidification, which predicts that low pH will trigger significant olfactory disruptions in multiple species (De La Haye et al*.,* 2011, 2012; Nilsson et al., 2012; Leduc et al*.,* 2013; Clements & Hunt 2015; Kim et al., 2016). *C. maenas* showed a significantly reduced response to both feeding and reproductive olfactory cues in low pH 7.2 compared to individuals at pH 8.2 meaning the hypothesis can be accepted. Previous studies focusing on crustacean behaviour under predicted low pH conditions have described similar results. Initial reaction, often identified by antennule flicking, was seen to reduce significantly in the freshwater crayfish (*Cambarus bartoni*) under low pH conditions, and individuals had difficulty locating a food odour (Allison et al., 1992). Kim et al*.* (2016) observed reduced antennular flicking, impaired olfactory behaviour and reduced prey detection in hermit crabs (*Pagurus tanneri*) under low pH conditions. Inability to elicit a response is generally thought to be due to failure to detect the odour due to damage to chemoreceptors or disruption in the signal pathway as highlighted in Schirrmacher et al*.* (2021) and Roggatz et al*.* (2016). A similar effect was also found in seabream (Velez et al*.,* 2019), where it was concluded that physical damage to sensory organs due to low pH, caused impaired olfactory ability. Additionally, individuals began to develop an additional thicker layer of mucus on the epithelia, presumably as a mechanism to protect sensory organs against low pH exposure (Velez et al*.,* 2019). However, crustaceans such as *C. maenas* frequently experience fluctuations in pH so are less likely to sustain serious physical damage from short term exposure. De la Haye et al*.* (2012) identified no physical damage to hermit crab antennules after a 5-day period of exposure to low pH conditions.

5.1.1 How chemical cues change under low pH

Research on behavioural responses to the alteration of cues under acidified conditions has been emphasised (Roggatz et al., 2016; Hardege et al., 2016, 2019; Nagelkerken et al., 2019; Velez et al., 2019). In low pH it is known that organic compounds including chemical olfactory cues can become structurally altered, due to mechanisms such as protonation, structural charge or tautomerisation and become less or even undetectable to the animal (Zhang & Byrne, 1996; Cripps et al., 2011 Roggatz et al*.,* 2016) when the bioactive form of a cue falls below the detection threshold. When using unidentified natural cues in experiments, it adds a level of complexity or a potential source of error or variation, as these cues can be either pH stable or unstable but are not quantifiable. The pheromone cue used in the present study is a combination of UDP and UTP (Hardege et al., 2011; Fletcher et al., 2021). These two molecules are known to alter in shape and protonation under reduced pH with an increase of the non-bioactive tautomer of UDP increasing (Hardege pers. Comm.). This may render them more difficult for *C. maenas* to detect in low pH conditions. The pheromones released by female *C. maenas* during pre-moult and post moult differ, with pre-moult urine serving as an attractant (Fletcher et al., 2021). Examining these side by side would provide a greater insight into the ecological impacts of the PE compounds. Further study should consider the use of both pre and post moult female urine to provide a comparison to the synthetic odours used in this study. As chemical cues change under low pH, using natural odour from females undergoing moulting would provide environmentally realistic insights into how behavioural responses may change.

Additionally, glutathione is a pH unstable compound between pH 7-9 and known to change protonation state under low pH conditions and therefore is not pH stable (Wang et al., 2009; Vila-Viçosa et al., 2013); this could potentially explain why detection is impacted under low pH. Roggatz et al. (2016) identified impaired functionality of the signalling tri-peptides shore crabs use for hatching and larvae irrigation behaviour under acidified conditions, presenting a plausible mechanism explaining altered behaviour such as delayed detection and response in low pH conditions. Unlike natural cues, using synthetic cues can ensure that either the chemical changes to the cues can be calculated and predicted, or that compounds are selected that remain pH stable in the pH region selected for the study. When selecting synthetic cues and identifying their uses in future applications, such as for integrated pest management of shore crabs, it is important to test the effectiveness in varying levels of pH. This ensures the cue will remain effective despite changes to pH (Porteus et al*.,* 2021).

5.2 Response to feeding and reproductive cues with exposure to plastic odour

Hypothesis 2: Individuals tested with the presence of plastic odour will show a reduced response to olfactory cues (UDP/UTP and glutathione) than individuals tested without the presence of plastic odour.

The influence of plastic odour revealed a significant difference between the responses to olfactory cues in conditions with plastic odour compared to those without. In pH 8.2, initial reaction time was significantly delayed in conditions with plastic odour present than in conditions without (Figure 14 A and B; Figure 15 A). Additionally, here plastic odour is seen to affect the response to sexual and feeding odour cues, with reduced efficiency of cue detection. This may suggest that plastic odour impairs cue reception or, more likely, emits a stronger chemical signal, which may be more persistent or mask other olfactory cues. The time taken for individuals to reach the cue with the presence of plastic odour was not significantly different to treatments without plastic odour (Figure 14 C and D; Figure 15 B). This suggests that plastic odour does not affect physiology of *C. maenas* individuals, as once a cue was detected the individual was able to source the odour regardless of plastic odour being present or not.

In the present study cue choices revealed that individual response to the synthetic pheromone cue did not change with the presence of plastic odour. In normal pH 8.2 with no plastic odour present, 55% of individuals chose the pheromone cue (Table 3) and, similarly, 50% of individuals chose the pheromone cue when plastic odour was present (Table 5). This suggests that plastic odour did not influence the choice made by individuals.

Furthermore, the same observation was made regarding the feeding cue glutathione. In treatments without plastic odour, 25% of individuals chose the feeding cue (Table 5), and

37.5% chose the feeding cue when plastic odour was present (Table 6), indicating very little difference in choice made with or without plastic odour. However, it is important to note that the plastic odour cue was favoured over the feeding cue in normal pH 8.2, and from informal observations, *Carcinus* individuals were seen to elicit feeding behaviours towards the plastic odour cues. This observation is supported by the growing literature surrounding info-chemicals and odours emitted by plastic particles that are often mistaken as food sources or acting as attractants (Allen et al., 2017; Savoca et al*.,* 2016, 2017; Potocka et al*.,* 2019; Greenshields et al., 2021).

Another unexpected finding, as shown in Table 4, was that at pH 8.2 a higher percentage of individuals interacted or favoured the control cue over the food cue GSH, this may be due to the feeding conditions throughout captivity or could point to the Y chamber not working fully effectively. Studies have criticised the use of Y shaped apparatus due to lack of realistic conditions such as non-turbulent flow (Steinberg et al., 1992; Hay, 1996; Finelli, 2000; Weissburg et al., 2002). Additionally, organisms are artificially constrained and may not respond to cues (Zimmer-Faust et al., 1996; Weissburg et al., 2002) limiting the accuracy of any behaviours exhibited. Identifying responses to choices made between two odour cues requires controlled environmental conditions which are unobtainable in the field. The foraging behaviour of invertebrates such as decapod crustaceans has been successfully examined through studies where cues have been applied in turbulent but controlled conditions (Finelli, 2000; Spooner et al., 2007).

Additionally, during this study, as animals were held for an extended period in artificial conditions, behavioural responses may have been inaccurate compared to natural scenarios under future environment predictions. Furthermore, throughout this study animals were fed a mono diet of cooked mussel flesh. Not only is this chemically different to the live mussels *C. maenas* would consume in the field, there may have also been habitation to the cues, whereby individuals learn that cooked mussel is the main source of food provided. These conditions during long term captivity are likely to affect both behaviour and olfactory sensitivity, particularly towards the food cue GSH, as this may not have been recognised as a usual food source. Studies should consider conducting experiments in the field or using 'conditioned water from live mussels' as positive cue, it would be expected that there would be an even greater impact of the plastic compounds under these conditions as the positive cue is likely to be 'weaker' than mussel juice.

Immense amounts of plastic are produced globally for the manufacturing of consumer products due to its lightweight, durable, and inexpensive qualities. However, it is these same convenient attributes of plastics, which make them detrimental to the environment. The findings of the present study contribute to the accumulating literature on the effects of marine plastic pollution. Mounting data suggest that plastics may have ecotoxicological impacts on marine ecosystems and species through the release of harmful chemicals (Wu and Seebacher, 2020; Kumar et al., 2021; Liu et al., 2022). Studies on numerous marine organisms have shown that growth (Besseling et al., 2014), immunity (Liu et al., 2019; Tang et al., 2020), food consumption (Sussarellu et al., 2016; Foley et al., 2018), reproduction (Sussarellu et al., 2016), and survivorship (Naidoo & Glassom, 2019) are adversely affected by exposure to microplastics (Foley et al., 2018), however, the effects of plastic odour on organism behavioural response is yet to be fully investigated. A recent study by Uguen et al. (2022) studied the effect of plastic leachates on the behavioural response of the blue mussel (*Mytilus edulis*). When exposed to plastic leachates, mussels displayed reduced movement compared to control conditions (Uguen et al., 2022).

Previous studies have focused on the physiological impacts of chemical additives released by plastics in the marine environment, the most detrimental being those with endocrine disruptive potential (Lithner et al. 2011). Most plasticizers appear to act by interfering with the functioning of various hormone systems. The toxic effects of exposure to the plastic additive BPA have been studied and have shown to cause adverse effects on growth (Laufer et al., 2013; Naveira et al., 2021), immunity (Tang et al., 2020), fertilization (Rhee & Rhee, 2016), embryonic development (Balbi et al., 2016), and metabolism (Huang et al., 2018; Mu et al., 2018). BPA leaching is a concern at marine sites where plastic waste has accumulated, as BPA leaches more rapidly in marine systems than freshwater (Sajiki & Yonekubo, 2003; Crain et al., 2007). Due to its omnipresence in the marine environment, organisms may face constant exposure to BPA or during vulnerable life stages. The effect of BPA appears to vary considerably among related species. Studies observed biological effects at environmentally relevant exposures, and it appears that some invertebrates may be hypersensitive to BPA exposure, such as molluscs, crustaceans, insect larvae, and marine copepods.

Ingestion of plastic debris has been recorded in multiple species (Laist, 1987; Teuten et al., 2007; Savoca et al., 2016, 2021; Wójcik-Fudalewska et al., 2016), and recently identified in the crustacean species the American lobster (*Homarus americanus*) (Potocka et al*.,* 2019). When consumed, microplastics containing plasticizers such as bisphenol A (BPA) and phthalates have been found to affect hatching success and the development and reproduction of offspring in amphibians, crustaceans, and insects (Oehlmann et al. 2009).

5.2.1 Info-chemicals

It is known that plastic debris can acquire a chemical signature from biofouling algae and bacteria in the marine environment (Lobelle & Cunliffe, 2011; Savoca et al*.,* 2016). Savoca et al*.* (2017) highlighted that the chemical signature emitted by aged plastic debris induces foraging behaviour in northern anchovy (*Engraulis mordax*) and supports previous findings suggesting that consumption of plastic material may be due to chemosensory mechanisms (Savoca et al*.,* 2016; Greenshields et al., 2021). Plastic additives such as oleamide may be perceived as a feeding cue by marine species potentially increasing the consumption of microplastics. Hermit crabs displayed an attraction to low concentrations of oleamide and elicited a feeding response (Greenshields et al., 2021). Studies on crustacean interactions with marine plastic debris revealed that the Atlantic ghost crab (*Ocypode quadrata*) was unable to distinguish between marine debris and natural food sources (Costa et al*.,* 2019). Interactions were seen with all debris types that were mixed with potential food odours, one of which being algae (*E. brasiliensis, D. hanleyanus*). However, it was noted that plastic straws were interacted with three times less than all other debris types. Fewer interactions occurred between the crabs and debris as prey availability was increased, suggesting that as natural food availability declines, the risk of interaction and ingestion of marine debris by ghost crabs increases (Costa et al*.,* 2019).

5.2.2 Conclusion

The results from the present study on *C. maenas* did not find plastic to influence the behavioural response to the feeding cue glutathione, however, informal observations and preliminary tests revealed individuals showing an attraction to the plastic odour, particularly males. The plastic odour in this study was created from virgin unaged plastic pellets, so there would be no biofouling or chemical signature that could be mistaken as a food odour as suggested by Savoca et al. (2016). This would suggest that the attraction shown towards the plastic odour cue was perhaps due to visual stimulus of the filter paper and less of an olfactory attraction to the plastic odour. The polyethylene (PE) plastic odour used in this study may have contained a concoction of chemicals resulting in adverse behavioural effects and eliciting a feeding response by *C. maenas*, however it would be a challenge to distinguish the exact chemical or mixture of chemicals causing this response. Additionally, there is a possibility that many novel compounds could be attractive to crabs previously maintained in tanks and fed a single component diet, as in this study. Future studies should aim to include a variety of natural food sources in animal husbandry procedures. To further investigate the effects of plastic odour on response to chemical cues, it would be beneficial to study the effects of a single chemical additive known to affect organism behaviour, such as BPA or PCB concentrations found in PE pellets.

5.3 Combined effects of plastic and low pH

Hypothesis 3: At low pH individuals tested with the presence of plastic odour will show a more reduced response to olfactory cues (UDP/UTP and glutathione) than individuals tested with the presence of plastic odour at normal pH.

Investigating the combined effects of plastic odour and low pH revealed that there were significant differences in the response of individuals tested at normal pH 8.2 with plastic odour present and those tested in treatments of combined low pH (7.6 and 7.2) with plastic odour present (Figure 14 A, B, Figure 15).

Initial reaction time was significantly slower in conditions with plastic odour at low pH 7.6, compared to conditions with plastic odour at normal pH 8.2. This was also the case when the pH was reduced to extremely low pH 7.2 with the presence of plastic odour (Figure 14 A, B). These findings indicate that the combined effects of low pH and plastic odour impaired detection and altered the response to olfactory cues (UDP/UTP and glutathione) to a greater extent than the singular effects of low pH or plastic odour.

The time taken to reach the cue was significantly different between treatments with plastic odour at 8.2 compared to 7.2, however was not significant from 8.2 to 7.6 (Figure 14 C, D). Based on these results it can be concluded that the combination of low pH and plastic odour does not affect *C. maenas* physiology over the short-term time frame of this study, and instead that this response is due to impaired cue detection mainly caused by extremely low pH causing individuals to take longer to locate and thus reach the cue.

In the present study on *C. maenas,* individuals' initial reaction times in extremely low pH 7.2 conditions with the additional presence of plastic odour were significantly slower than those only exposed to extremely low pH 7.2 without any plastic odour present (Figure 14 A, B) (figure 15 A). Although model predictions for oceanic pH do not suggest pH will drop to as low as pH 7.2 globally, there is still a likelihood that certain localised areas will experience extreme low pH conditions. In coastal areas the average nightly pH is predicted to drop to around pH 7.4, thus low pH will likely be frequent at night by the year 2100 which could impact species feeding rhythms (Bertolini et al., 2021).

Although these results imply that the combined effects of low pH and plastic odour influence the response to olfactory cues, further study is required to understand at a greater depth the processes happening within this interaction. It would be beneficial if future studies tested the response to these olfactory cues in the field by using slow-release cellulose gel cues, as described in Rising et al. (2022). It is unclear as to whether this impaired response is due to both stressors acting in tandem, or if extremely low pH is the driving influence of the reduced response.

5.3.1 The effect of combined stressors

A review by Gissi et al. (2021) summarised that climate change generally intensifies the effects of anthropogenic stressors at species level. However, the combined effects of climate change and anthropogenic stress can vary among and within ecosystems depending on the environmental conditions or organisms involved (Gissi et al., 2021 Review).

With the aim of understanding the ways in which climate change and anthropogenic stressors may affect marine environments in tandem, potential impacts and interactions between species, communities and ecosystems have been explored (Bartolino et al., 2014; Gissi et al., 2021). The combined effect of climate-induced coral bleaching and reduced calcification driven by elevated seawater temperatures, with reducing sedimentation and nutrient pollution from anthropogenic land use, were found to reduce the ability of coral reefs to recover from natural environmental disturbances such as storms (Bruno et al., 2007). Similarly, the detrimental impacts of intensive fishing on predator populations in the Southern Ocean was found to be intensified by climate change stress, resulting in lower reproduction success (Ainley & Blight, 2009). Le Bris et al. (2018) found ocean warming had an intensifying effect in combination with the effects of the fishery on the American lobster (*Homarus americanus*), leading to the collapse of the Southern New England lobster fishery. However, fishery management measures targeting the preservation of large reproductive individuals, in combination with lower predation, was found to reduce the adverse effects of ocean warming at a lobster fishery in the Gulf of Maine (Le Bris et al., 2018). Sarà et al., (2018) investigated the impacts of multiple anthropogenic stressors on the invasion trajectory of the non-indigenous Lessepsian mussel (*Brachidontes pharaonis*) in the Mediterranean Sea. Climate change was expected to have a generally intensifying effect, however distribution varied, with the greatest impacts caused by nutrient inputs of chlorophyll-a (from urbanization), salinity and sea surface temperature (tropicalization) (Sarà et al., 2018).

Climate change in combination with other anthropogenic stressors was found to have both intensifying and mitigating effects on seabird species depending on distribution and latitude range (Rivalan et al., 2010; Burthe et al., 2014), seasonality (Rolland et al., 2008; Burthe et al., 2014), life history traits (Rolland et al., 2008; Pardo et al., 2017) and community behaviour (Burthe et al., 2014). Two colonies of Caribbean corals (*Siderastrea sidereal* and *Pseudodiploria strigosa*) located across the Belize Mesoamerican Barrier Reef System, were analysed for growth. Skeletal extension rates over the past century showed a higher decline in nearshore colonies compared to off-shore colonies, this decline was instigated by the combined effects of long-term ocean warming and exposure to increased land-based anthropogenic pollution (Baumann et al., 2019). In only one case, the combined effects of climate change and anthropogenic stressors were seen to have a mitigating effect. Anthropogenic stressor-induced decline of species stock was reduced as warmer temperatures lead to faster growth and earlier maturation, allowing the population of Atlantic cod (*Gadus morhua*) to potentially sustain higher fishing rates (Wang et al., 2014). However, when accounting for the combined effects of temperature and acidification with the current fishing effort, the risk of collapse for the Atlantic cod stock of the Western Baltic significantly increased, counteracting the potential positive effects of warming as a stressor acting in isolation (Voss et al., 2019).

5.3.2 Water chemistry affecting plastic chemicals

An often-overlooked aspect of global climate change is how it can affect organic pollutants in the marine environment. Changing water conditions associated with climate change, such as water temperature, pH, and salinity, may affect the environmental fate, transport, bioavailability, bioaccumulation, and toxicity of pollutants, which are already present in the marine environment.

Plastics can add significant additional stress effects to an already stressed ecosystem. The stable chemical bonds in these polymer macro-molecules not only make them resilient, but also impervious to natural degradation (Shaw & Sahni, 2014), meaning plastic debris only photodegrades into smaller fragments, the majority of which accumulate and persist in ocean gyres found in the Atlantic, Pacific, and Indian oceans (Zbyszewski & Corcoran, 2011).

Low-density polyethylene microplastic particles were identified to be a persistent source releasing Bisphenol A (BPA), as mentioned above (section 5.2, page 101), into aquatic environments. BPA has been found to persist longer in seawater than in freshwater without degradation (Ying & Kookana, 2003; Kang et al., 2005) and high concentrations have been found adsorbed to sediments (Bolz et al. 2001; Heemken et al. 2001; Fromme et al. 2002). Most organic pollutants have ionizable groups, which affect their form and charge in water thus affecting degradability (Wang et al., 2015; Zhang et al., 2018). Climate change affects various ocean conditions such as salinity, temperature, and pH which are known to have an effect on the partitioning of BPA between a water phase and an organic phase and influence sorption and desorption behaviour (Borrirukwisitsak et al., 2012); and the adsorption process and aggregation behaviour of microplastics (Li et al., 2018; Liu et al., 2019; Zhou et al., 2020). Higher solubility of chemicals leads to higher concentrations in the water phase, and this results in greater bioavailability and mobility of plastic derived chemical additives in seawater, which may lead to exposure for species previously unaffected. This effect has been observed in the marine polychaete (*Platynereis dumerilii*), where the solubility of the pheromone uric acid was seen to decrease with pH and uric acid was found to be less water soluble in lower pH environments (Halabe & Sperling, 1994; Hardege et al., 1998; Zeeck et al., 1998; Bagnati et al., 1999; Breithaupt & Hardege, 2012).

Microplastics have a large hydrophobic surface area meaning these particles often become heavily contaminated with anthropogenic environmental pollutants harbouring concentrations up to six times higher than the ambient seawater (Mato et al., 2001; Teuten et al., 2009; Andrady, 2011; Hirai et al., 2011). Examples of pollutants include hydrophobic pollutants such as polycyclic aromatic hydrocarbons (PAHs), poly chlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs), and metals. The majority of these substances are considered to be harmful to organisms, and can act as mutagens, carcinogens and endocrine disruptors (Teuten et al. 2009; Zbyszewski & Corcoran, 2011; Rochman et al., 2013). The International Pellet Watch project analysed plastic pellets collected by volunteers from beaches worldwide for sorbed pollutants. PE pellets were found to have PCB concentrations up to 600 ng/g pellet and dichloro-diphenyl-trichloroethane (DDT) concentrations up to 300 ng/g (Ogata et al., 2009). PCBs have been found to have negative effects on the immune system, nervous system, endocrine system, and reproductive system and in some cases cause cancers in marine animals or (EPA, 2013).

5.3.3 Bioaccumulation within food webs

Ingestion of plastic fragments is common due to their diminutive size and availability, thus increasing the bioaccumulation of the chemicals found within the plastic or chemicals absorbed onto the plastics from the external environment (Teuten et al., 2009; Rochman et al., 2014).

Plastic biomagnification in crustaceans has been researched under laboratory conditions. Trophic level transfer of microplastics was observed in shore crabs (*Carcinus maenas*) that were fed on mussels (*Mytilus edulis*) having previously been fed with 0.5 µm fluorescent polystyrene microspheres (Farrell & Nelson, 2013). The numbers of microspheres in the haemolymph of the crabs were observed over a 21-day period, exhibiting the highest number of microspheres after 24 h with almost a complete removal after 21 days. A second study similarly observing trophic transfer from mussels (*Mytilus edulis*) to crabs (*Carcinus maenas*) found polystyrene microspheres could be retained in the foregut of the crabs up to 14 days after the ingestion of mussels that had been fed with 8 – 10 µm microspheres (Watts et al., 2014). Rochman et al. (2014) studied the bioaccumulation of hazardous chemical ingredients associated with plastic litter in myctophid fish in the South Atlantic Ocean. The fish caught in high plastic density sampling sites demonstrated a significantly higher concentration of PBDEs in their tissues, but lower concentrations were recorded in water samples. This indicates that PCB concentrations originated from the plastic as an additive substance and were not sorbed from ambient seawater (Rochman et al., 2014).

5.3.4 Conclusion

The most prominent gaps in our current knowledge on the impacts of plastic associated chemicals on organisms are the lack of data for long-term exposures to environmentally relevant concentrations and their ecotoxicity when part of complex mixtures. It would be beneficial to study the effects of plastic chemicals over an extended study period, as potential carcinogenic or mutagenic effects would only present themselves over a longer timeframe such as several months or even years. Studying the response to food and pheromone cues after continuous exposure to plastic odour compared to individuals that have not been exposed to plastic odour may present effects of long-term exposure. It would also be beneficial to study the physiological impacts as well as the behavioural responses, as additives from virgin plastic have been found to affect organism physiology and reproductive success. Furthermore, studying the effects over generations with a focus on reproductive success and juvenile growth and development would be a valuable contribution to this research and provide an insight into potential effects on *C. maenas* populations.

5.4 Individuality

Hypothesis 4: Individuals will have variability in cue detection and response relating to size, sex, colour, and injury.

5.4.1 Sex

No significant difference was found in initial reaction time or time taken to reach the cue between males and females (Figure 18, Figure 19). Response time to cues is expected to remain similar as the physiological differences between male and female *Carcinus* are very marginal. As expected, males had a stronger response to the female reproductive cue and lower response to the feeding cue glutathione (Appendix Figure 1.). This aligns with previous research findings by Hayden et al. (2007). As testing was conducted over the summer reproductive months, seasonality influences male shore crabs' response to pheromone cues (Fletcher & Hardege, 2008, 2009; Hardege, 2011). Mating season for shore crabs occurs between the months of April and October (Hardege et al., 2002), with populations of male *Carcinus* in the UK showing the greatest response to female sex pheromones in the month of July (Crothers 1967; Hardege et al., 2002). Hayden et al. (2007) reported significantly reduced feeding responses in males during the summer months' reproductive season, this shift in preference is assumed to reduce cannibalism towards moulting soft-shelled females.

Additionally, male crabs generally showed a higher response rate to olfactory cues in low pH conditions compared to females. Richardson et al. (2021) suggested that *C. maenas* males may be better acclimated to future climate change conditions due to physiological or morphological differences between males and females.

Informal observations from preliminary static tank experiments, and results of cue choices implied that there may be a sex dependent response to plastic odour cues. Males showed a much higher attraction to the plastic odour cues than females (Appendix Figure 1.). There are recognised differences between male and female foraging behaviour, males being more aggressive in their approach. Additionally, there may even be mechanical differences between make and female foraging behaviour and diet choice due to claw form and size (Elner, 1980; Spooner et al., 2007).

Mincarelli et al. (2021) investigated the responses of blue mussels (*Mytilus* spp.) to climate change induced temperature rise and exposure to DEHP (Di-2-ethylhexyl phthalate) the common softener of polyvinyl chloride (PVC) (Erythropel et al., 2014) as separate and combined stressors. The study identified differences in the response of males and females to high temperature treatments. Males displayed advanced development in sexual maturity; however, a delay was observed in females. Sex-specific differences in energy allocation and metabolic status could influence the susceptibility of either sex to thermal stress, with the potential to disrupt breeding synchrony, which in turn could alter the structure of populations and food webs (Bedulina et al., 2017). Additionally, high temperature appeared to accentuate the toxic effect of DEHP, with a higher sensitivity of males observed under combined environmental stressors, which may suggest a more resilient status of developing females under prolonged stress conditions (Mincarelli et al., 2021). Although these conclusions from Mincarelli et al. (2021) differ from the findings of the present study on *C. maenas,* there is a relevance which contributes to the understanding of the effect of plastic pollution in the context of global climate change and how this may affect future marine communities.

5.4.2 Size

There was a significant difference in initial reaction time between small and large sized individuals (Figure 20 B). On average, smaller individuals took longer before performing antennule flicking motions.

Additionally, there was a significant difference between the choice of cue and the size of the individual, with larger crabs choosing the pheromone cue more and smaller crabs opting for the feeding cue as preference. This can be explained as a difference in sexual maturity, as smaller individuals are often still sexually immature and therefore would not pursue a female. Instead, smaller individuals show more interest in feeding cues as seen here. In the current study size was split into two categories: small and large, this was done by calculating the average size of all individuals and all those below the average were categorised as small, and those over categorised as large. Crabs used in this study were housed together in groups meaning fights between individuals were inevitable. *C. maenas* show variations in behavioural response to different stimuli in relation to recent social interactions, with dominant and submissive male individuals recognising their status within the hierarchy after a fight (Fletcher & Hardege, 2009; Jiménez-Morales et al., 2018). Crabs of a higher social status, usually larger crabs, win most fights and therefore will present a stronger response towards the female pheromone cue than smaller 'submissive' crabs with lower social status (Fletcher & Hardege, 2009). Size here is shown to have significant variation on response to cues (Figure 20 B), which may be due to smaller crabs having not yet reached sexual maturity. By nature, females are also often smaller than males and so many fell into the smaller category, this may have also influenced results of cue choices as it is known that females show less interest in female sex pheromones than males (Hayden et al*.,* 2007).

In crustaceans larger body size is generally associated with higher fitness, greater reproductive success and increased survival in both males and females (Blueweiss et al., 1978; Speakman, 2005; Woodward et al., 2005; Kelley et al., 2015). Crustaceans of a larger size are likely to possess larger antennules, which is assumed to have a positive correlation with cue detection speed (Devine & Atema, 1982). Hence, larger individuals may be better equipped to adapt to future low pH projections. However, a discussion in Widdicombe and Spicer (2008) suggested that an organism's sensitivity to acidification would be related to body size with larger organisms more sensitive than smaller ones over an evolutionary timescale. Additionally, in polychaetes, it has been observed that individuals kept at low pH are on average smaller than individuals kept in current oceanic pH conditions (Batten & Bamber, 1996; Sokołowski et al*.,* 2020).

5.4.3 Colour

Compared to green individuals, red crabs showed delayed initial reaction times in extreme low pH 7.2, suggesting that olfactory ability of red colour morphs was affected more than green in extremely low pH (Figure 22). Additionally red crabs also took longer to reach the cue compared to green crabs, this was seen in all pH conditions (8.2, 7.6, and 7.2) (figure 23).

Previous studies on variation within colour morphs of *C. maenas* have highlighted interesting differences that align with the findings of the present study. Red morphotypes were observed to have a lower tolerance to high salinity and are particularly susceptible to low salinity variations (Reid & Aldrich, 1989; Styrishave et al*.,* 2004; Monteiro et al*.,* 2021). Additionally, red crabs were found to have higher rates of mortality than green crabs when exposed to prolonged environmental hypoxia (Reid & Aldrich, 1989; McGaw & Naylor, 1992).

This theory is further supported by Styrishave et al*.* (2004) who found red crabs to have higher reproductive success than green crabs, although at the expense of a lower physiological tolerance. Green crabs were shown to be more proficient in coping with changes in the surrounding environment. This increased tolerance is not only observed regarding natural habitat variations, but green coloured shore crabs also appear to be more resilient to variations caused by anthropogenic pollution (Styrishave et al*.,* 2004).

These previous findings and comparisons of physiology between green and red colour morphs of *C. maenas* support the results of the present study and reinforce observations on distribution and ecology (Abelló et al., 1997). Baeta et al*.* (2005) and Monteiro (2019) studied the shore crab population in the Mondego estuary, Portugal, and noted the green morphotype was located predominantly in the intertidal areas of the Pranto River. Green colour morphs are able to withstand and exploit turbulent estuarine habitats where salinity and temperature fluctuates daily due to tidal action. This allows for colonisation of new areas minimising interspecific competition (Monteiro et al*.,* 2021).

5.4.4 Injury

Interestingly, results showed that individuals with no injuries took longer to exhibit initial reactions than those with injuries, although this was not statistically significant (Figure 24). This may be due to other factors such as dominance hierarchies, which suggest that losers of fights are less likely to pursue a female pheromone odour than a winner (Fletcher & Hardege, 2009). Additionally, factors such as size may have played a role in this result. Most individuals sustaining injuries were larger and therefore sexually mature, meaning their responses to pheromone odours would have been stronger than those smaller immature individuals with no injuries.

Results showed that injury significantly affects the time taken to reach cue (Figure 25). This result is unsurprising as many individuals that were classified as injured had missing legs or claws. Obvious damage and loss of appendages, puts these individuals in a compromised position and physiological disadvantage. Although these individuals may have been able to detect an odour cue, inevitably it took them longer than those with no injury.

5.4.5 Conclusions

Similar trends in initial reaction time and time taken to reach the cue were observed throughout all the individuality parameters investigated. In normal pH 8.2 individual differences (size, sex, colour, injury) had little to no effect on initial reaction time. However, as pH was reduced all individuality aspects showed delayed initial reaction times suggesting impairment regardless of the individual characteristic of the animal. Furthermore, a similar trend was seen with the addition of plastic odour, initial reaction times became longer as pH decreased, although not significantly (Figure 26). There was no obvious trendline for time to reach cue, but this lack of trend was observed across all individuality parameters (Figure 27).

Individual variation in behaviour can be influenced by social situations, where the behavioural response of an individual is determined by the interactions and responses of other conspecifics (Dall et al., 2004; McNamara & Houston., 2009; Anthes et al., 2010; Schuett et al., 2010; Wolf et al., 2011). When animals interact socially, they observe other individuals' behaviour in order to make better decisions. Long-term holding will change behaviour of crabs- particularly if they are held together as occurred in this study. Regular animal collections and using fresh animals would be a better option when carrying out behavioural studies of this sort.

One of the key variables for individuality is the moult status of the crabs. Pre-moult crabs do not feed for some days prior to moult (Williams, 1982; Waiho et al., 2015), and post-moult require some days to harden prior to foraging (Cameron, 1989; Turner et al., 2003). This may have influenced the results as individuals undergoing or preparing for moulting are often less active and have reduced response to feeding cues. Additionally, red crabs in terminal moult will have deteriorating condition and therefore likely to be less active compared to crabs in earlier moult stages (Greco et al., 2014; Campos et al., 2022).

Studies often aim to select similar sized organisms in similar physiological conditions to minimize differences in biological responses. However as highlighted by Dall and Griffith (2014), a single population-representative may not be biologically meaningful as it fails to include the full-range of individual-level patterns of behavioural variation that is necessary to effectively test theoretical predictions. Studies are best to test groups of animals in sufficient numbers and then compare against similar batches of variants, as this method also introduces significant variables particularly if animal variations are mixed and representation of specific forms within tests is low. Additionally, factors such as pre-adaptation to local conditions and differences between populations may well explain differences in results achieved in separate studies.

Despite the results from this study indicating that individuals differ in their response to cues, this result can only be extrapolated to a single population, since all the animals used in this study were collected from a single population. Although individuality in olfactory response exists even within a population, it would have been more beneficial to obtain data from additional populations. Particular areas of interest would be examining populations inhabiting environments where pH conditions are stable, or individuals that stem from warmer or colder climates will allow to achieve a more rounded picture of species-wide individuality. Studies have shown that different ecosystems and pre-adaptation have an impact on responses under stress (Ferreira, 2021; Quijada-Rodriguez, 2021; Vajedsamiei et al., 2021).

Studies have explored the impact of ocean acidification on *C. maenas* and have suggested the species is already likely to be adapted to natural fluctuations in temperature, salinity, and light intensity due to their natural environmental conditions. As *C. maenas* occupy the harsh intertidal zone, many become isolated in shallower waters so naturally experience wide ranges of pH and temperature, meaning the species is highly tolerant and eurythermal. In the natural environment conditions are constantly changing for example with the tides. Static pH does not exist in the marine environment due to a multitude of interacting factors (Schmidt & Psenner, 1992; Hofmann et al., 2011; Cornwall et al., 2013). In the future there will be a greater range of fluctuation and overall lower mean pH, so it is vital to recognise the variability of pH now and what is to be expected to occur in predicted future scenarios.

Species inhabiting coastal regions, naturally experience greater spatial and temporal fluctuations in carbonate chemistry due to the complex interactions that occur at the land ocean atmosphere interface (Gattuso et al., 1998; Takahashi et al., 2002; Takahashi et al., 2009; Waldbusser & Salisbury, 2014; Kapsenberg et al., 2015; McNeil & Sasse, 2016; DeVries et al., 2017). Dilution of coastal waters due to terrestrial freshwater input decreases salinity and therefore alkalinity, resulting in a stronger shift of carbonate chemistry at a given seawater pCO_2. Moreover, nutrient enrichment of coastal waters via riverine discharge and land runoff can alter rates of primary production in coastal areas which may lead to changes in carbonate chemistry at diurnal and/or seasonal time scales (Drupp et al., 2011;

Waldbusser & Salisbury, 2014; Wahl et al., 2018; Hopwood et al., 2020; Santos et al., 2021). The species inhabiting these environments may be more resilient to shifts in oceanic pH caused by ocean acidification (Fassbender et al., 2016; Vargas et al., 2017).

6. Conclusions

Global climate change is regarded as a key driver of change in marine environments affecting resilience, functionality, and associated ecosystem services (Doney et al., 2012; Smale et al., 2019). Climate change is manifested by sea temperature rise and ocean acidification, sea level rise, and the intensification of extreme weather events, impacting marine biodiversity dynamics at species, community, and ecosystem levels (IPCC, 2019).

Cases of extreme and localised low pH can occur due to events such as leaks from volcanic seeps (Hall-Spencer et al., 2008; Cigliano et al., 2010; Crook et al., 2012), periodic upwellings of CO_2 enriched sea water (Feely et al., 2008; Thomsen et al., 2012), or leaks from geological CO_2 storage sites (Seibel & Walsh, 2001; Hawkins, 2004). Global model predictions have made it clear that overall ocean pH is likely to decrease by the end of the century. At the same time, more than 95% of the world's oceans are currently exposed to numerous other local stressors stemming from anthropogenic activities (Halpern et al., 2008, 2015). Concerns of plastic pollution in the marine environment are growing, with recurrent observations of debris ingestion in crustaceans and associated detrimental effects. Despite the knowledge we currently have on these topics, the behavioural effects of combined stressors are severely under researched. With current predictions into future ocean conditions, organisms are likely to endure multiple changes simultaneously (Solomon et al., 2009). Studies incorporating multiple stressors and natural variability will substantially increase predictive power in regard to understanding crustacean behaviour and associated ecological functioning under projected future ocean conditions.

Few studies within this field of research focus on medium or long-term projections, restricting potential management strategies to mitigate future changes at a global or regional scale. The effects of climate change can be reflected in the future through distribution shifts, phenotypic plasticity, local adaptation, and evolution (Rilov et al., 2019).

Although this study attempted to accurately replicate predicted conditions for intertidal environments of the coastal species *C. maenas*, as previously discussed, short-term laboratory studies cannot accurately simulate variation that occurs within the natural environment. As *C. maenas* is an intertidal species, individuals may have expressed varied response due to plasticity and acclimation abilities (Rilov et al., 2019).

To further this research and develop the potential suggestion for adaptation of the study species, I would recommend analysing endpoints such as food consumption by monitoring amount consumed (grams) from a pre-weighed amount of food of individuals housed in pH 8.2 and individuals housed in pH 7.2 by removing, drying and weighing left over food after a set period of time i.e. 2 minutes, as well as analysing growth rates and active time searching for food, to gain a deeper understanding of potential changes in their behaviour. Additional studies should explore longer exposure periods through housing individuals for up to a month at multiple pH levels (7.2, 7.6 and 8.2) to assess acclimation, plasticity, and genetic adaptation in the event of environmental change. Future research should also focus on transgenerational and multigenerational studies to quantify tolerance levels of organisms to ocean acidification (Clements & Hunt, 2015). Studies should aim to include eco-evolutionary processes when predicting the combined effects of anthropogenic stressors and climate change. Additionally, future research should aim to include a variety of stressors and ranges when predicting the combined effects of anthropogenic stressors and climate change. Salinity ranges below 4 and above 52% PSU, temperature ranges below 0 and above 30°C, and aquatic pollutants such as metal and plastic pollution are known to affect behavioural responses of *C. maenas* (Berrill & Arsenault, 1982; Yamada & Gillespie, 2008; Appelhans et al., 2012; Rodrigues & Pardal, 2014; Macdonald et al., 2018; Damasceno et al., 2021; Nancollas & McGaw, 2021) and are key areas that could be explored in further research as potential interacting stressors. It is important to consider the synergistic effect of environmental factors in combination as stress caused by one abnormal variable can result in diminished survival when combined with exposure to other irregular variables (Young and Elliott, 2019).

It is widely acknowledged that understanding the response of organisms to the combined effects of climate change and local anthropogenic stressors is crucial to ecosystem management efforts, however the research itself is challenging due to the complexity of the interactions among climate change, anthropogenic stressors, and the ecosystem components (Niiranen et al., 2013; Jutterström et al., 2014). Building insight into the effects of these interactions, will hopefully contribute to successful management actions and mitigation strategies (Gissi et al., 2019; Rilov et al., 2020).

The findings of this current study do however fit with the growing body of research surrounding behavioural changes under low pH conditions and showed that *C. maenas* displayed individual population-level variation, and therefore may be plausible for long term adaptation to low pH environments. The extensive tolerance ranges of *C. maenas*, owing to its success as a globally invasive species, may provide a model for successful endurance and survival in a changing environment (Tepolt & Somero, 2014).

7. Reference list

Aagaard, A. (1996) In situ variation in heart rate of the shore crab Carcinus maenas in relation to environmental factors and physiological condition. *Marine Biology*, 125(4), 765-772.

Aagaard, A., Warman, C.G. & Depledge, M.H. (1995) Tidal and seasonal changes in the temporal and spatial distribution of foraging Carcinus maenas in the weakly tidal littoral zone of Kerteminde Fjord, Denmark. *Marine Ecology Progress Series*, 122, 165-172.

Abelló, P., Aagaard, A., Warman, C.G. & Depledge, M.H. (1997) Spatial variability in the population structure of the shore crab Carcinus maenas (Crustacea: Brachyura) in a shallow-water, weakly tidal fjord. *Marine Ecology Progress Series*, 147, 97-103.

Abello, P., Warman, C.G., Reid, D.G. & Naylor, E. (1994) Chela loss in the shore crab Carcinus maenas (Crustacea: Brachyura) and its effect on mating success. *Marine biology*, 121(2), 247-252.

Adamo, N., Al-Ansari, N. & Sissakian, V. (2021) Review of Climate Change Impacts on Human Environment: Past, Present and Future Projections: Review of Climate Change Impacts on Human Environment: Past, Present and Future Projections. *engineering*, 13(11), 605-630.

Adiyodi, R.G. (1985) Reproduction and its control. *The biology of Crustacea*, 9, 147-215.

Aeschlimann, P.B., Häberli, M.A., Reusch, T.B.H., Boehm, T. & Milinski, M. (2003) Female sticklebacks Gasterosteus aculeatus use self-reference to optimize MHC allele number during mate selection. *Behavioral Ecology and Sociobiology*, 54(2), 119-126.

Ainley, D.G. & Blight, L.K. (2009) Ecological repercussions of historical fish extraction from the Southern Ocean. *Fish and Fisheries*, 10(1), 13-38.

Aiuppa, A., Hall-Spencer, J.M., Milazzo, M., Turco, G., Caliro, S. & Di Napoli, R. (2021) Volcanic CO2 seep geochemistry and use in understanding ocean acidification. *Biogeochemistry*, 152(1), 93-115.

Alabi, O.A., Ologbonjaye, K.I., Awosolu, O. & Alalade, O.E. (2019) Public and environmental health effects of plastic wastes disposal: a review. *Journal of Toxicology and Risk Assessment*, 5(021), 1-13.

Albright, R. & Langdon, C. (2011) Ocean acidification impacts multiple early life history processes of the Caribbean coral Porites astreoides. *Global change biology*, 17(7), 2478-2487.

Alimi, O.S., Farner Budarz, J., Hernandez, L.M. & Tufenkji, N. (2018) Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. *Environmental science & technology*, *52*(4), 1704-1724.

Allen, A. S., Seymour, A. C. & Rittschof, D. (2017) Chemoreception drives plastic consumption in a hard coral. *Marine Pollution Bulletin*, 124, 198−205.

Allen, A.S., Seymour, A.C. & Rittschof, D. (2017) Chemoreception drives plastic consumption in a hard coral. *Marine Pollution Bulletin*, 124(1), 198-205.

Allison, V., Dunham, D.W. & Harvey, H.H. (1992) Low pH alters response to food in the crayfish Cambarus bartoni*. Canadian Journal of Zoology*, 70(12), 2416-2420.

Allwood, J.M., Cullen, J.M. & Milford, R.L. (2010). Options for achieving a 50% cut in industrial carbon emissions by 2050. *Environmental science & technology*, 44(6), 1888–1894.

Ameyaw-Akumfi, C. & Hughes, R.N. (1987) Behaviour of Carcinus maenas feeding on large Mytilus edulis. How do they assess the optimal diet? *Marine Ecology Progress Series*, 38(3), 213-216.

Aminot, Y., Lanctôt, C., Bednarz, V., Robson, W.J., Taylor, A., Ferrier-Pagès, C., Metian, M. & Tolosa, I. (2020) Leaching of flame-retardants from polystyrene debris: bioaccumulation and potential effects on coral. *Marine pollution bulletin*, 151, 110862.

Andersson, A.J. & Gledhill, D. (2013) Ocean acidification and coral reefs: effects on breakdown, dissolution, and net ecosystem calcification. *Annual review of marine science*, *5*, 321-348.

Andrade, H., Glüge, J., Herzke, D., Ashta, N.M., Nayagar, S.M. & Scheringer, M. (2021) Oceanic long-range transport of organic additives present in plastic products: an overview. *Environmental Sciences Europe*, 33(1), 1-14.

Andrady, A.L. (2011) Microplastics in the marine environment. *Marine pollution bulletin*, 62(8),1596-1605.

Annamalai, J. & Namasivayam, V. (2017) Determination of effect of pH and storage temperature on leaching of phthalate esters from plastic containers by ultrasound-assisted dispersive liquid–liquid micro-extraction. *Journal of Food Measurement and Characterization*, 11(4), 2222-2232.

Anthes, N., Bergmüller, R., Blanckenhorn, W., Brockmann, H.J., Fichtel, C., Fromhage, L., Frommen, J., Goymann, W., Heinze, J., Hirschenhauser, K. & Hofer, H. (2010) Animal behaviour: evolution and mechanisms. Springer Science & Business Media.

Appelhans, Y.S., Thomsen, J., Pansch, C., Melzner, F. & Wahl, M. (2012) Sour times: seawater acidification effects on growth, feeding behaviour and acid–base status of Asterias rubens and Carcinus maenas. *Marine Ecology Progress Series*, 459, 85-98.

Archer, D., Eby, M., Brovkin, V., Ridgwell, A., Cao, L., Mikolajewicz, U., Caldeira, K., Matsumoto, K., Munhoven, G., Montenegro, A. & Tokos, K. (2009) Atmospheric lifetime of fossil fuel carbon dioxide. *Annual review of earth and planetary sciences*, 37.

Arnberg, M. (2016) *Combined effects of ocean acidification, ocean warming and oil spill on aspects of development of marine invertebrates*. Doctoral dissertation. The University of Plymouth. Available online: https://ethos.bl.uk/OrderDetails.do?uin=uk.bl.ethos.692916 [accessed 4/5/2022].

Ashur, M.M., Johnston, N.K. & Dixson, D.L. (2017) Impacts of ocean acidification on sensory function in marine organisms. *Integrative and Comparative Biology*, 57(1), 63-80.

Audet, D., Miron, G. & Moriyasu, M. (2008) Biological characteristics of a newly established green crab (Carcinus maenas) population in the southern Gulf of St. Lawrence, Canada. *Journal of Shellfish Research*, 27(2), 427-441.

Auta, H.S., Emenike, C.U. & Fauziah, S.H. (2017) Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. *Environment international*, 102,165-176.

Ayompe, L.M., Schaafsma, M. & Egoh, B.N. (2021) Towards sustainable palm oil production: The positive and negative impacts on ecosystem services and human wellbeing. *Journal of cleaner production*, 278, 123914.

Azani, N., Ghaffar, M.A., Suhaimi, H., Azra, M.N., Hassan, M.M., Jung, L.H. & Rasdi, N.W. (2021) The impacts of climate change on plankton as live food: A review. In IOP Conference Series: *Earth and Environmental Science*, 869,012005. IOP Publishing.

Bacastow, R., & C. D. Keeling (1973) Atmospheric carbon dioxide and radio-carbon in the natural carbon cycle. II: Changes from A. D. 1700 to 2070 as deduced from a geochemical model. *Brookhaven Symposia in Biology*, 30, 86-135.

Baeta, A., Cabral, H.N., Neto, J.M., Marques, J.C. & Pardal, M.A. (2005) Biology, population dynamics and secondary production of the green crab Carcinus maenas (L.) in a temperate estuary. *Estuarine, Coastal and Shelf Science*, 65(1-2), 43-52.

Bagnati, M., Perugini, C., Cau, C., Bordone, R., Albano, E. & Bellomo, G. (1999) When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: a study using uric acid. *Biochemical Journal*, 340(1), 143-152.

Bahr, K.D., Rodgers, K.S. & Jokiel, P.L. (2018) Ocean warming drives decline in coral metabolism while acidification highlights species-specific responses. *Marine Biology Research*, 14(9-10), 924-935.

Balbi, T., Franzellitti, S., Fabbri, R., Montagna, M., Fabbri, E. & Canesi, L. (2016) Impact of bisphenol A (BPA) on early embryo development in the marine mussel Mytilus galloprovincialis: effects on gene transcription. *Environmental pollution*, 218, 996-1004.

Bamber, S.D. & Depledge, M.H. (1997) Responses of shore crabs to physiological challenges following exposure to selected environmental contaminants. *Aquatic Toxicology*, 40(1), 79-92.

Bandow, N., Will, V., Wachtendorf, V. & Simon, F.G. (2017) Contaminant release from aged microplastic. *Environmental Chemistry*, *14*(6), 394-405.

Bang, D.Y., Kyung, M., Kim, M.J., Jung, B.Y., Cho, M.C., Choi, S.M., Kim, Y.W., Lim, S.K., Lim, D.S., Won, A.J. & Kwack, S.J. (2012) Human risk assessment of endocrine-disrupting chemicals derived from plastic food containers. *Comprehensive Reviews in Food Science and Food Safety*, *11*(5), 453-470.

Barany, S., Nagy, M. & Skvarla, J. (2012) Electrokinetic potential of polystyrene particles in polyelectrolyte and polyelectrolyte mixtures solutions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 413, 200-207.

Barki, A., Goren, M. & Karplus, I. (1991) Morphotype related dominance hierarchies in males of Macrobrachium rosenbergii (Crustacea, Palaemonidae). *Behaviour*, 117(3-4), 145-160.

Barnes, D.K. (2002) Invasions by marine life on plastic debris. *Nature*, 416(6883), 808-809.

Barnes, D.K., Galgani, F., Thompson, R.C. & Barlaz, M. (2009) Accumulation and fragmentation of plastic debris in global environments. *Philosophical transactions of the royal society B: biological sciences*, 364(1526), 1985-1998.

Bartolino, V., Margonski, P., Lindegren, M., Linderholm, H.W., Cardinale, M., Rayner, D., Wennhage, H. & Casini, M. (2014) Forecasting fish stock dynamics under climate change: B altic herring (Clupea harengus) as a case study. *Fisheries Oceanography*, 23(3), 258-269.

Bartsch, H. & Montesano, R. (1975) Mutagenic and carcinogenic effects of vinyl chloride. *Mutation Research/Reviews in Genetic Toxicology*, 32(2), 93-113.

Bates, N.R., Best, M.H.P., Neely, K., Garley, R., Dickson, A.G. & Johnson, R.J. (2012) Detecting anthropogenic carbon dioxide uptake and ocean acidification in the North Atlantic Ocean. *Biogeosciences*, 9(7), 2509-2522.

Batten, S.D. & Bamber, R.N. (1996) The effects of acidified seawater on the polychaete Nereis virens Sars, 1835. *Marine pollution bulletin*, 32(3), 283-287.

Baudrimont, M., Arini, A., Guégan, C., Venel, Z., Gigault, J., Pedrono, B., Prunier, J., Maurice, L., Ter Halle, A. & Feurtet-Mazel, A. (2020) Ecotoxicity of polyethylene nanoplastics from the North Atlantic oceanic gyre on freshwater and marine organisms (microalgae and filter-feeding bivalves). *Environmental Science and Pollution Research*, 27(4), 3746-3755.

Baumann, J.H., Ries, J.B., Rippe, J.P., Courtney, T.A., Aichelman, H.E., Westfield, I. & Castillo, K.D. (2019) Nearshore coral growth declining on the Mesoamerican Barrier Reef System. *Global change biology*, 25(11), 3932-3945.

Bedulina, D., Meyer, M. F., Gurkov, A., Kondratjeva, E., Baduev, B., Gusdorf, R., & Timofeyev, M. A. (2017) Intersexual differences of heat shock response between two amphipods

(*Eulimnogammarus verrucosus* and *Eulimnogammarus cyaneus*) in Lake Baikal. *PeerJ*, 5, 2864.

Bell, A.M., Henderson, L. & Huntingford, F.A. (2010) Behavioral and respiratory responses to stressors in multiple populations of three-spined sticklebacks that differ in predation pressure. *Journal of Comparative Physiology B*, 180(2), 211-220.

Bellis, S.L., Kass-Simon, G., & Rhoads, D.E. (1992) Partial characterization and detergent solubilization of the putative glutathione chemoreceptor from hydra. *Biochemistry*, *31*(40), 9838-9843.

Berrill, M. & Arsenault, M. (1982) Mating behavior of the green shore crab Carcinus maenas. *Bulletin of marine science*, 32(2), 632-638.

Bertolini, C., Rubinetti, S., Umgiesser, G., Witbaard, R., Bouma, T.J., Rubino, A. & Pastres, R. (2021) How to cope in heterogeneous coastal environments: Spatio-temporally endogenous circadian rhythm of valve gaping by mussels. *Science of the Total Environment*, 768, 145085.

Besseling, E., Wang, B., Lürling, M. & Koelmans, A.A. (2014) Nanoplastic affects growth of S. obliquus and reproduction of D. magna. *Environmental science & technology*, 48(20), 12336-12343.

Besseling, E., Wegner, A., Foekema, E.M., Van Den Heuvel-Greve, M.J. & Koelmans, A.A. (2013) Effects of microplastic on fitness and PCB bioaccumulation by the lugworm Arenicola marina (L.). *Environmental science & technology*, 47(1), 593-600.

Bhuiyan, K.A., Rodríguez, B.M., Pires, A., Riba, I., Dellvals, Á., Freitas, R. & Conradi, M. (2021) Experimental evidence of uncertain future of the keystone ragworm Hediste diversicolor (OF Müller, 1776) under climate change conditions. *Science of The Total Environment*, *750*, 142031.

Björnsdotter, M. (2015) *Leaching of Residual Monomers, Oligomers and Additives from Polyethylene, Polypropylene, Polyvinyl Chloride, High-density Polyethylene and Polystyrene Virgin Plastics.* Bachelor thesis. The University of Örebro. Available online: https://www.diva-portal.org/smash/get/diva2:855478/FULLTEXT01.pdf [Accessed 12/4/2022]

Blueweiss, L., Fox, H., Kudzma, V., Nakashima, D., Peters, R. & Sams, S. (1978) Relationships between body size and some life history parameters. *Oecologia*, 37(2), 257-272.

Boatta, F., D'Alessandro, W., Gagliano, A.L., Liotta, M., Milazzo, M., Rodolfo-Metalpa, R., Hall-Spencer, J.M. & Parello, F. (2013) Geochemical survey of Levante Bay, Vulcano Island (Italy), a natural laboratory for the study of ocean acidification. *Marine Pollution Bulletin*, 73(2), 485-494.

Bolz, U., Hagenmaier, H. & Körner, W. (2001) Phenolic xenoestrogens in surface water, sediments, and sewage sludge from Baden-Württemberg, south-west Germany. *Environmental Pollution*, 115(2), 291-301.

Bommarco, R., Vico, G. & Hallin, S. (2018) Exploiting ecosystem services in agriculture for increased food security. *Global Food Security*, 17, 57-63.

Bonner, J.T. (1988) *The evolution of complexity by means of natural selection*. Princeton University Press.

Booth, A.M. & Sørensen, L. (2020) Microplastic fate and impacts in the environment. *Handbook of Microplastics in the Environment*, 1-24.

Borrirukwisitsak, S., Keenan, H.E. & Gauchotte-Lindsay, C. (2012) Effects of salinity, pH and temperature on the octanol-water partition coefficient of bisphenol A. *International Journal of Environmental Science and Development*, 3(5), 460.

Bošnir, J., Puntarić, D., Škes, I., Klarić, M., Šimić, S. & Zorić, I. (2003) Migration of phthalates from plastic products to model solutions. *Collegium antropologicum*, 27(1), 23-30.

Botterell, Z.L., Beaumont, N., Cole, M., Hopkins, F.E., Steinke, M., Thompson, R.C. & Lindeque, P.K. (2020) Bioavailability of microplastics to marine zooplankton: Effect of shape and infochemicals. *Environmental Science & Technology*, 54(19), 12024-12033.

Boucher, J. & Friot, D. (2017) *Primary microplastics in the oceans: a global evaluation of sources* (Vol. 43). Gland, Switzerland: Iucn. Available online: https://holdnorgerent.no/wp-content/uploads/2020/03/IUCN-report-Primary-microplastics-in-the-oceans.pdf [Accessed 07/05/2022]

Bouraï, L., Logez, M., Laplace-Treyture, C. & Argillier, C. (2020) How do eutrophication and temperature interact to shape the community structures of phytoplankton and fish in lakes? *Water*, *12*(3), 779.

Bouwmeester, H., Hollman, P.C.H. & Peters, R.J.B. (2015) Potential Health Impact of Environmentally Released Micro- and Nanoplastics in the Human Food Production Chain: Experiences from Nanotoxicology. *Environmental Science & Technology,* 49(15), 8932-8947.

Bove, C.B., Whitehead, R.F. & Szmant, A.M. (2020) Responses of coral gastrovascular cavity pH during light and dark incubations to reduced seawater pH suggest species-specific responses to the effects of ocean acidification on calcification. *Coral Reefs*, 39(6), 1675-1691.

Bozinovic, F., Bastías, D.A., Boher, F., Clavijo-Baquet, S., Estay, S.A. & Angilletta Jr, M.J. (2011) The mean and variance of environmental temperature interact to determine physiological tolerance and fitness. *Physiological and Biochemical Zoology*, 84(6), 543-552.

Branch, T.A., DeJoseph, B.M., Ray, L.J. & Wagner, C.A. (2013). Impacts of ocean acidification on marine seafood. *Trends in ecology & evolution*, 28(3), 178-186.

Brandl, S.J., Johansen, J.L., Casey, J.M., Tornabene, L., Morais, R.A. & Burt, J.A. (2020) Extreme environmental conditions reduce coral reef fish biodiversity and productivity. *Nature communications*, 11(1), 1-14.

Brandt-Rauf, P.W., Li, Y., Long, C., Monaco, R., Kovvali, G. & Marion, M.J. (2012) Plastics and carcinogenesis: The example of vinyl chloride. *Journal of carcinogenesis*, 11.

Braun, D., Cherdron, H., Rehahn, M., Ritter, H. & Voit, B. (2005) *Polymer Synthesis: Theory and Practice: Fundamentals, Methods, Experiments*. Heidelberg, Germany: Springer.

Breithaupt, T. & Hardege, J.D. (2012) Pheromones mediating sex and dominance in aquatic animals. *Chemical Ecology in Aquatic Systems.*39-56.

Brems, A., Dewil, R., Baeyens, J. & Zhang, R. (2013) Gasification of plastic waste as waste-to-energy or waste-to-syngas recovery route. *Natural Science*, 5(6), 695-704.

Briffa, M., de la Haye, K. & Munday, P.L. (2012) High CO_2 and marine animal behaviour: potential mechanisms and ecological consequences. *Marine pollution bulletin*, 64(8), 1519-1528.

Broecker, W.S., Li, Y.H. & Peng, T.H. (1971) Carbon dioxide: Man's unseen artifact. In Hood H.W. (ed) *Impingement of Man on the Oceans*. John Wiley & Sons, Inc, 287 – 324.

Browman, H.I. (2017) Towards a broader perspective on ocean acidification research. *ICES Journal of Marine Science*, 74(4), 889-894.

Brown, G.E. (2003) Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish*. 4, 227–234.

Brown, G.E., Chivers, D.P. & Smith, R.J.F. (1997) Differential learning rates of chemical versus visual cues of a northern pike by fathead minnows in a natural habitat. *Environmental Biology of Fishes*, 49(1), 89-96.

Brown, J.H. & Maurer, B.A. (1986) Body size, ecological dominance and Cope's rule. *Nature,* 324(6094), 248-250.

Browne, M.A. (2015) Sources and pathways of microplastics to habitats. *Marine anthropogenic litter*, 229-244.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T. & Thompson, R. (2011) Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environmental science & technology*, *45*(21), 9175-9179.

Bruno, J.F. & Selig, E.R. (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. *PLoS one*, 2(8), 711.

Brydson, J.A. (1999) *Plastics materials*. Elsevier.

Bublitz, R., Sainte-Marie, B., Hardege, J., Fletcher, N., Newcomb-Hodgetts, C. & Smith, M. (2008) Interspecific activity of the sex pheromone of the European shore crab (Carcinus maenas). *Behaviour*, 145(10), 1465-1478.

Burden, C.T., Stow, A.J., Hoggard, S.J., Coleman, M.A. & Bishop, M.J. (2014) Genetic structure of Carcinus maenas in southeast Australia. *Marine Ecology Progress Series*, 500, 139-147.

Burney, D.A. & Flannery, T.F. (2005) Fifty millennia of catastrophic extinctions after human contact. *Trends in Ecology & Evolution*, 20(7), 395-401.

Burns, E.E. & Boxall, A.B. (2018) Microplastics in the aquatic environment: Evidence for or against adverse impacts and major knowledge gaps. *Environmental toxicology and chemistry*, *37*(11), 2776-2796.

Burrell, T.J., Maas, E.W., Hulston, D.A., Law, C.S. & Zhang, Y.Z. (2015) Bacterial abundance, processes and diversity responses to acidification at a coastal CO2 vent. *Microbiology Letters*, 362(18).

Burthe, S.J., Wanless, S., Newell, M.A., Butler, A. & Daunt, F. (2014) Assessing the vulnerability of the marine bird community in the western North Sea to climate change and other anthropogenic impacts. *Marine Ecology Progress Series*, 507, 277-295.

Byrne, M. (2012) Global change ecotoxicology: identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. *Marine environmental research*, 76, 3-15.

Byrne, M., Lamare, M., Winter, D., Dworjanyn, S.A. & Uthicke, S. (2013) The stunting effect of a high CO_2 ocean on calcification and development in sea urchin larvae, a synthesis from the tropics to the poles. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1627).

Cadée, G.C. (2002) Seabirds and floating plastic debris. *Marine Pollution Bulletin*, 44(11), 1294-1295.

Caldeira, K. & Wickett, M.E. (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research: Oceans*, *110*(C9).

Calosi, P., Bilton, D.T. & Spicer, J.I. (2008) Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biology letters*, 4(1), 99-102.

Calosi, P., Rastrick, S.P., Lombardi, C., de Guzman, H.J., Davidson, L., Jahnke, M., Giangrande, A., Hardege, J.D., Schulze, A., Spicer, J.I. & Gambi, M.C. (2013) Adaptation and acclimatization to ocean acidification in marine ectotherms: an in situ transplant experiment with polychaetes at a shallow CO_2 vent system. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1627).

Calosi, P., Rastrick, S.P., Lombardi, C., de Guzman, H.J., Davidson, L., Jahnke, M., Giangrande, A., Hardege, J.D., Schulze, A., Spicer, J.I. & Gambi, M.C. (2013) Adaptation and acclimatization to ocean acidification in marine ectotherms: an in situ transplant experiment with polychaetes at a shallow CO2 vent system. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1627), 20120444.

Cameron, J.N. (1989) Post-moult calcification in the blue crab, Callinectes sapidus: timing and mechanism. *Journal of Experimental Biology*, *143*(1), 285-304.

Campos, J., Ribas, F., Bio, A., Freitas, V., Souza, A.T. & van der Veer, H.W. (2022) Body condition and energy content of the shore crab Carcinus maenas L. in a temperate coastal system: the cost of barnacle epibiosis. *Biofouling*, *38*(8), 764-777.

Canadell, J.G., Le Quéré, C., Raupach, M.R., Field, C.B., Buitenhuis, E.T., Ciais, P., Conway, T.J., Gillett, N.P., Houghton, R.A. & Marland, G. (2007) Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. *Proceedings of the national academy of sciences*, 104(47), 18866-18870.

Carlton, J.T. & Cohen, A.N. (2003) Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs Carcinus maenas and C. aestuarii. *Journal of Biogeography*, *30*(12), 1809-1820.

Carrier-Belleau, C., Drolet, D., McKindsey, C.W. & Archambault, P. (2021) Environmental stressors, complex interactions and marine benthic communities' responses. *Scientific reports*, 11(1), 1-14.

Carrier-Belleau, C., Drolet, D., McKindsey, C.W. & Archambault, P. (2021) Environmental stressors, complex interactions and marine benthic communities' responses. *Scientific reports*, 11(1), 1-14.

Carstensen, J. & Duarte, C.M. (2019) Drivers of pH variability in coastal ecosystems. *Environmental science & technology*, 53(8), 4020-4029.

Chan, F., Barth, J.A., Blanchette, C.A., Byrne, R.H., Chavez, F., Cheriton, O., Feely, R.A., Friederich, G., Gaylord, B., Gouhier, T. & Hacker, S. (2017) Persistent spatial structuring of coastal ocean acidification in the California Current System. *Scientific Reports*, 7(1), 1-7.

Chase, I.D., Bartolomeo, C. & Dugatkin, L.A. (1994) Aggressive interactions and inter-contest interval: how long do winners keep winning? *Animal Behaviour*, 48(2), 393-400.

Chen, K.J., Chen, M.C. & Chen, T.H. (2021) Plastic ingestion by fish in the coastal waters of the Hengchun Peninsula, Taiwan: Associated with human activity but no evidence of biomagnification. *Ecotoxicology and Environmental Safety*, 213, 112056.

Chen, Q., Allgeier, A., Yin, D. & Hollert, H. (2019) Leaching of endocrine disrupting chemicals from marine microplastics and mesoplastics under common life stress conditions. *Environment international*, 130, 104938.

Cheng, W., Xie, Z., Blais, J.M., Zhang, P., Li, M., Yang, C., Huang, W., Ding, R. & Sun, L. (2013) Organophosphorus esters in the oceans and possible relation with ocean gyres. *Environmental pollution*, 180, 159-164.

Chiesa, J.J., Aguzzi, J., García, J.A., Sardà, F. & de la Iglesia, H.O. (2010) Light intensity determines temporal niche switching of behavioral activity in deep-water Nephrops norvegicus (Crustacea: Decapoda). *Journal of biological rhythms*, 25(4), 277-287.

Chivers, D.P. & Smith, R.J.F. (1998) Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. *Ecoscience*, 5(3), 338-352.

Choudalakis, G. & Gotsis, A.D. (2009) Permeability of polymer/clay nanocomposites: a review. *European polymer journal*, 45(4), 967-984.

Chown, S.L., Slabber, S., McGeoch, M.A., Janion, C. & Leinaas, H.P. (2007) Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proceedings of the Royal Society B: Biological Science*s, 274(1625), 2531-2537.

Cigliano, M., Gambi, M.C., Rodolfo-Metalpa, R., Patti, F.P. & Hall-Spencer, J.M. (2010) Effects of ocean acidification on invertebrate settlement at volcanic CO2 vents. *Marine Biology*, 157(11), 2489-2502.

Clark, H.L. (2017) *Reproductive success and factors affecting sexual selection in a fiddler crab, Uca mjoebergi.* PhD thesis. Research School of Biology, College of Medicine, Biology & Environment, The Australian National University. Available online: https://openresearch-repository.anu.edu.au/bitstream/1885/116841/1/Clark%20Thesis%202017.pdf [Accessed 13/5/2022]

Claudet, J. & Fraschetti, S. (2010) Human-driven impacts on marine habitats: a regional meta-analysis in the Mediterranean Sea. *Biological Conservation*, 143(9), 2195-2206.

Clements, J.C. & Chopin, T. (2017) Ocean acidification and marine aquaculture in North America: potential impacts and mitigation strategies. *Reviews in Aquaculture*, 9(4), 326-341.

Clements, J.C. & Hunt, H.L. (2015) Marine animal behaviour in a high CO2 ocean. *Marine Ecology Progress Series*, 536, 259-279.

Clements, J.C., Ramesh, K., Nysveen, J., Dupont, S. & Jutfelt, F. (2021) Animal size and sea water temperature, but not pH, influence a repeatable startle response behaviour in a wide-ranging marine mollusc. *Animal Behaviour*, 173, 191-205.

Cohen, A.N., Carlton, J.T. & Fountain, M.C. (1995) Introduction, dispersal and potential impacts of the green crab Carcinus maenas in San Francisco Bay, California. Marine biology, 122(2), 225-237.

Cole, M., Lindeque, P., Halsband, C. & Galloway, T.S. (2011) Microplastics as contaminants in the marine environment: a review. *Marine pollution bulletin*, *62*(12), 2588-2597.

Cominassi, L. (2019) *Combined effects of ocean acidification and warming on a large pelagic fish, the European sea bass (Dicentrarchus labrax).* Doctoral dissertation. The University of Hamburg. Available online: https://ediss.sub.uni-hamburg.de/handle/ediss/6081 [Accessed 14/5/2022]

Compton, T.J., Leathwick, J.R. & Inglis, G.J. (2010) Thermogeography predicts the potential global range of the invasive European green crab (Carcinus maenas). *Diversity and Distributions*, 16(2), 243-255.

Conradi, M., Sánchez-Moyano, J.E., Galotti, A., Jiménez-Gómez, F., Jiménez-Melero, R., Guerrero, F., Parra, G., Bonnail, E. & DelValls, T.Á. (2019) CO2 leakage simulation: Effects of the decreasing pH to the survival and reproduction of two crustacean species. *Marine pollution bulletin*, 143, 33-41.

Constable, A.J., Melbourne-Thomas, J., Corney, S.P., Arrigo, K.R., Barbraud, C., Barnes, D.K., Bindoff, N.L., Boyd, P.W., Brandt, A., Costa, D.P., & Davidson, A.T. (2014) Climate change and Southern Ocean ecosystems I: how changes in physical habitats directly affect marine biota. *Global change biology*, 20(10), 3004-3025.

Cook, T.R., Lescroel, A., Cherel, Y., Kato, A. & Bost, C.A. (2013) Can foraging ecology drive the evolution of body size in a diving endotherm?. *PLoS One*, 8(2), 56297.

Cordier, M., Poitelon, T. & Hecq, W. (2019) The shared environmental responsibility principle: new developments applied to the case of marine ecosystems. *Economic Systems Research*, 31(2), 228-247.

Cornwall, C.E., Hepburn, C.D., McGraw, C.M., Currie, K.I., Pilditch, C.A., Hunter, K.A., Boyd, P.W. & Hurd, C.L. (2013) Diurnal fluctuations in seawater pH influence the response of a calcifying macroalga to ocean acidification. *Proceedings of the Royal Society B: Biological Sciences*, 280(1772), 20132201.

Costa, L.L., Madureira, J.F., Di Beneditto, A.P.M. & Zalmon, I.R. (2019) Interaction of the Atlantic ghost crab with marine debris: Evidence from an in situ experimental approach. *Marine pollution bulletin*, 140, 603-609.

Cothran, R.D., Monahan, P.J. & Relyea, R.A. (2021) Antipredator behaviour affected by prey condition, food availability and pH-mediated info-disruption. *Animal Behaviour*, *171*, 111-118.

Coyle, R., Hardiman, G. & O'Driscoll, K. (2020) Microplastics in the marine environment: A review of their sources, distribution processes, uptake and exchange in ecosystems. *Case Studies in Chemical and Environmental Engineering*, 2, 100010.

Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, J.Á., Irigoien, X. & Duarte, C.M. (2015) Plastic accumulation in the Mediterranean Sea. *PloS one*, 10(4).

Crain, C.M., Kroeker, K. & Halpern, B.S. (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology letters*, 11(12), 1304-1315.

Crain, D.A., Eriksen, M., Iguchi, T., Jobling, S., Laufer, H., LeBlanc, G.A. & Guillette Jr, L.J. (2007) An ecological assessment of bisphenol-A: evidence from comparative biology. *Reproductive toxicology*, 24(2), 225-239.

Crippa, M., Guizzardi, D., Pisoni, E., Solazzo, E., Guion, A., Muntean, M., Florczyk, A., Schiavina, M., Melchiorri, M. & Hutfilter, A.F. (2021) Global anthropogenic emissions in urban areas: patterns, trends, and challenges. *Environmental Research Letters*, 16(7), 074033.

Crompton, T. R. (2007) *Additive migration from plastics into foods: a guide for analytical chemists*. Shrewsbury: Smithers Rapra Technology Publishing Ltd.

Crook, E.D., Potts, D., Rebolledo-Vieyra, M., Hernandez, L. & Paytan, A. (2012) Calcifying coral abundance near low-pH springs: implications for future ocean acidification. *Coral Reefs*, 31(1), 239-245.

Crothers J. H. (1967) The biology of the shore crab Carcinus maenas (L.): The background anatomy, growth and life history. *Field Studies.* 2, 407–434.

Crothers, J. H. (1968). The biology of the shore crab Carcinus maenas (L.) 2. The life of the adult crab. *Field Studies,* 2(5), 579-614.

Curry, A.E. (2020) *Effects of Multiple Stressors on the Development and Performance of Decapod Crustaceans*. PhD thesis. Bangor University. Available online: https://research.bangor.ac.uk/portal/files/27646053/ACURRY_PhD_2020.pdf [Accessed 15/5/2022]

Cuzin-Roudy, J. & Buchholz, F. (1999) Ovarian development and spawning in relation to the moult cycle in Northern krill, Meganyctiphanes norvegica (Crustacea: Euphausiacea), along a climatic gradient. *Marine Biology*, 133(2), 267-281.

da Costa, J.P., Santos, P.S., Duarte, A.C. & Rocha-Santos, T. (2016) (Nano) plastics in the environment–sources, fates and effects. *Science of the Total Environment*, 566, 15-26.

Dall, S.R. & Griffith, S.C. (2014) An empiricist guide to animal personality variation in ecology and evolution. *Frontiers in Ecology and Evolution*, 2, 3.

Dall, S.R., Bell, A.M., Bolnick, D.I. & Ratnieks, F.L. (2012) An evolutionary ecology of individual differences. *Ecology letters*, 15(10),1189-1198.

Dall, S.R., Houston, A.I. & McNamara, J.M. (2004) The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology letters*, 7(8), 734-739.

Damasceno, J.M., Rato, L.D., Simões, T., Morão, I.F., Meireles, G., Novais, S.C. & Lemos, M.F. (2021) Exposure to the insecticide sulfoxaflor affects behaviour and biomarkers responses of Carcinus maenas (Crustacea: Decapoda). *Biology*, 10(12), 1234.

Danso, D., Chow, J. & Streit, W.R. (2019) Plastics: environmental and biotechnological perspectives on microbial degradation. *Applied and environmental microbiology*, 85(19), 01095-19.

Darling, J.A., Bagley, M.J., Roman, J.O.E., Tepolt, C.K. & Geller, J.B. (2008) Genetic patterns across multiple introductions of the globally invasive crab genus Carcinus. *Molecular Ecology*, *17*(23), 4992-5007.

Darnerud, P. O. (2003) Toxic effects of brominated flame retardants in man and in wildlife. *Environment International*, 29(6), 841–853.

Davidson, L. (2013) *Ocean acidification and its effects upon fitness in nereidid polychaetes.* PhD thesis, University of Hull. Available online: https://ethos.bl.uk/OrderDetails.do?uin=uk.bl.ethos.612642 [Accessed 14/5/2022]

Daw, N.D., Kakade, S. & Dayan, P. (2002) Opponent interactions between serotonin and dopamine. *Neural networks*, 15(4-6), 603-616.

De La Haye, K. (2012) The behavioural consequences of reduced sea water pH in decapod crustaceans.

De la Haye, K.L., Spicer, J.I., Widdicombe, S. & Briffa, M. (2011) Reduced sea water pH disrupts resource assessment and decision making in the hermit crab Pagurus bernhardus. *Animal Behaviour*, 82(3), 495-501.

De Rivera, C.E., Hitchcock, N.G., Teck, S.J., Steves, B.P., Hines, A.H. & Ruiz, G.M. (2007) Larval development rate predicts range expansion of an introduced crab. *Marine Biology*, 150(6), 1275-1288.

DeBose J.L., Lema S.C. & Nevitt G.A. (2008) Dimethylsulfoniopropionate as a foraging cue for reef fishes. *Science.* 319, 1356.

Delaeter, C., Spilmont, N., Bouchet, V.M. & Seuront, L. (2022) Plastic leachates: Bridging the gap between a conspicuous pollution and its pernicious effects on marine life. *Science of The Total Environment*, 154091.

Delaney, D.G., Sperling, C.D., Adams, C.S. & Leung, B. (2008) Marine invasive species: validation of citizen science and implications for national monitoring networks. *Biological Invasions*, 10(1), 117-128.

DeMatteo, R., Keith, M.M., Brophy, J.T., Wordsworth, A., Watterson, A.E., Beck, M., Ford, A.R., Gilbertson, M., Pharityal, J., Rootham, M. & Scott, D.N. (2013) Chemical exposures of women workers in the plastics industry with particular reference to breast cancer and reproductive hazards. *New Solutions: A Journal of Environmental and Occupational Health Policy*, 22(4), 427-448.

DeMatteo, R., Keith, M.M., Brophy, J.T., Wordsworth, A., Watterson, A.E., Beck, M., Ford, A.R., Gilbertson, M., Pharityal, J., Rootham, M. & Scott, D.N. (2013) Chemical exposures of women workers in the plastics industry with particular reference to breast cancer and reproductive hazards. *New Solutions: A Journal of Environmental and Occupational Health Policy*, 22(4), 427-448.

Derby, C. & Schmidt, M. (2017) *Crustacean Olfaction. Oxford research encyclopedia of neuroscience*. New York: Oxford University Press.

Derby, C.D. & Sorensen, P.W. (2008) Neural processing, perception, and behavioral responses to natural chemical stimuli by fish and crustaceans. *Journal of chemical ecology*, 34(7), 898-914.

Devine, B.M., Munday, P.L. & Jones, G.P. (2012) Homing ability of adult cardinalfish is affected by elevated carbon dioxide. *Oecologia*, 168(1), 269-276.

Devine, D.V. & Atema, J., (1982) Function of chemoreceptor organs in spatial orientation of the lobster, Homarus americanus: differences and overlap. *The Biological Bulletin*, 163(1), 144-153.

DeVries, T., Holzer, M. & Primeau, F. (2017) Recent increase in oceanic carbon uptake driven by weaker upper-ocean overturning. *Nature*, 542(7640), 215-218.

Díaz-Castañeda, V., Cox, T.E., Gazeau, F., Fitzer, S., Delille, J., Alliouane, S. & Gattuso, J.P. (2019) Ocean acidification affects calcareous tube growth in adults and reared offspring of serpulid polychaetes. *Journal of Experimental Biology*, 222(13), 196543.

Diepens, N.J. & Koelmans, A.A. (2018) Accumulation of plastic debris and associated contaminants in aquatic food webs. *Environmental Science & Technology*, *52*(15), 8510-8520.

Dissanayake A., Galloway T. S. & Jones M. B. (2011) Seasonal differences in the physiology of Carcinus maenas (Crustacea: Decapoda) from estuaries with varying levels of anthropogenic contamination, *Estuarine, Coastal and Shelf Science*, 93(4), 320-327.

Dissanayake, A. & Ishimatsu, A. (2011) Synergistic effects of elevated CO2 and temperature on the metabolic scope and activity in a shallow-water coastal decapod (Metapenaeus joyneri; Crustacea: Penaeidae). *ICES Journal of Marine Science*, 68(6), 1147-1154.

Dissanayake, A., Piggott, C., Baldwin, C. & Sloman, K.A. (2010) Elucidating cellular and behavioural effects of contaminant impact (polycyclic aromatic hydrocarbons, PAHs) in both laboratory-exposed and field-collected shore crabs, Carcinus maenas (Crustacea: Decapoda). *Marine environmental research*, 70(5), 368-373.

Dixson D.L., Jennings A.R., Atema J, & Munday P.L. (2014) Odor tracking in sharks is reduced under future ocean acidification conditions. *Global Change Biology*. 21, 1454–1462.

Dixson, D.L., Munday, P.L. & Jones, G.P. (2010) Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology letters*, *13*(1), 68-75.

Dodd, L.F., Grabowski, J.H., Piehler, M.F., Westfield, I. & Ries, J.B. (2015) Ocean acidification impairs crab foraging behaviour. *Proceedings of the Royal Society B: Biological Sciences*, 282(1810), 20150333.

Domenici, P., Allan, B., McCormick, M.I. & Munday, P.L. (2012) Elevated carbon dioxide affects behavioural lateralization in a coral reef fish. *Biology letters*, 8(1), 78-81.

Doney, S.C., Busch, D.S., Cooley, S.R. & Kroeker, K.J. (2020) The impacts of ocean acidification on marine ecosystems and reliant human communities. *Annual Review of Environment and Resources*, 45, 83-112.

Doney, S.C., Fabry, V.J., Feely, R.A. & Kleypas, J.A. (2009) Ocean acidification: the other CO2 problem. *Annual review of marine science*, 1, 169-192.

Doney, S.C., Ruckelshaus, M., Duffy, J.E., Barry, J.P., Chan, F., English, C.A., Galindo, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlton, N. & Polovina, J. (2012) Climate change impacts on marine ecosystems. *Annual Review of Marine Science*, 4, 11-37.

Dowaidar, A.M., El-Shahawi, M.S. & Ashour, I. (2007) Adsorption of polycyclic aromatic hydrocarbons onto activated carbon from non-aqueous media: 1. the influence of the organic solvent polarity. *Separation Science and Technology*, 42(16), 3609-3622.

Draper A.M., & Weissburg M.J. (2019) Impacts of global warming and elevated CO2 on sensory behavior in predator-prey interactions: a review and synthesis. *Frontiers in Ecology and Evolution*, 7(72).

Drupp, P., De Carlo, E.H., Mackenzie, F.T., Bienfang, P. & Sabine, C.L. (2011) Nutrient inputs, phytoplankton response, and CO2 variations in a semi-enclosed subtropical embayment, Kaneohe Bay, Hawaii. *Aquatic Geochemistry*, 17(4), 473-498.

Dube, M.A. & Salehpour, S. (2014) Applying the principles of green chemistry to polymer production technology. *Macromolecular Reaction Engineering*, 8(1), 7-28.

Dulvy, N.K., Pacoureau, N., Rigby, C.L., Pollom, R.A., Jabado, R.W., Ebert, D.A., Finucci, B., Pollock, C.M., Cheok, J., Derrick, D.H. & Herman, K.B. (2021) Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology*, 31(21), 4773-4787.

Duquette, A. (2016) *Calcium carbonate composition and other measures of vulnerability of the skeletons and shells of echinoderms and gastropods challenged by ocean acidification or climate warming.* PhD thesis. The University of Alabama.

Edelaar, P., Siepielski, A.M. & Clobert, J. (2008) Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. *Evolution: International Journal of Organic Evolution*, 62(10), 2462-2472.

Edgell, T.C. & Hollander, J. (2011) The evolutionary ecology of European green crab, Carcinus maenas, in North America. *In In the Wrong Place-Alien Marine Crustaceans: Distribution, Biology and Impacts*). Dordrecht: Springer, 641-659.

Edwards, D.H., Herberholz, J. & Nelson, R.J. (2005) Crustacean models of aggression. *The biology of aggression*, 38-61.

Ekerholm, M. & Hallberg, E. (2005) Primer and short-range releaser pheromone properties of premolt female urine from the shore crab Carcinus maenas. *Journal of chemical ecology*, 31(8), 1845-1864.

Eldøy, S.H., Bordeleau, X., Lawrence, M.J., Thorstad, E.B., Finstad, A.G., Whoriskey, F.G., Crossin, G.T., Cooke, S.J., Aarestrup, K., Rønning, L. & Sjursen, A.D. (2021) The effects of nutritional state, sex and body size on the marine migration behaviour of sea trout. *Marine Ecology Progress Series*, 665, 185-200.

Ellis, E.C., Fuller, D.Q., Kaplan, J.O. & Lutters, W.G. (2013) Dating the Anthropocene: Towards an empirical global history of human transformation of the terrestrial biosphere Dating the Anthropocene. *Elementa: Science of the Anthropocene*, 1.

Ellis, E.C., Kaplan, J.O., Fuller, D.Q., Vavrus, S., Klein Goldewijk, K. & Verburg, P.H. (2013) Used planet: A global history. Proceedings of the National Academy of Sciences, 110(20), 7978-7985.

Ellis, J.I., Jamil, T., Anlauf, H., Coker, D.J., Curdia, J., Hewitt, J., Jones, B.H., Krokos, G., Kürten, B., Hariprasad, D. & Roth, F. (2019) Multiple stressor effects on coral reef ecosystems. *Global change biology*, *25*(12), 4131-4146.

El-Shahawi, M.S., Hamza, A., Bashammakh, A.S. & Al-Saggaf, W.T. (2010) An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. *Talanta*, 80(5), 1587-1597.

El-Shahawi, M.S., Hamza, A., Bashammakh, A.S. & Al-Saggaf, W.T. (2010) An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. *Talanta*, 80(5), 1587-1597.

Erbe, C. (2012) *Effects of underwater noise on marine mammals. In The effects of noise on aquatic life*. New York: Springer.

Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G. & Reisser, J. (2014) Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PloS one*, 9(12), 111913.

Eriksson, B.K., van der Heide, T., van de Koppel, J., Piersma, T., van der Veer, H.W. & Olff, H. (2010) Major changes in the ecology of the Wadden Sea: human impacts, ecosystem engineering and sediment dynamics. *Ecosystems*, 13(5), 752-764.

Erythropel, H. C., Maric, M., Nicell, J. A., Leask, R. L., & Yargeau, V. (2014) Leaching of the plasticizer di(2-ethylhexyl)phthalate (DEHP) from plastic containers and the question of human exposure. *Applied microbiology and biotechnology*, 98(24), 9967–9981.

Espinel-Velasco, N., Hoffmann, L., Agüera, A., Byrne, M., Dupont, S., Uthicke, S., Webster, N.S. & Lamare, M. (2018) Effects of ocean acidification on the settlement and metamorphosis of marine invertebrate and fish larvae: a review. *Marine Ecology Progress Series*, 606, 237-257.

Espinel-Velasco, N., Tobias-Hünefeldt, S.P., Karelitz, S., Hoffmann, L.J., Morales, S.E. & Lamare, M.D. (2021) Reduced seawater pH alters marine biofilms with impacts for marine polychaete larval settlement. *Marine Environmental Research*, 167, 105291.

Falkowski, P., Scholes, R.J., Boyle, E.E.A., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Högberg, P., Linder, S. & Mackenzie, F.T. (2000) The global carbon cycle: a test of our knowledge of earth as a system. *science*, 290(5490), 291-296.

Fangue, N.A., Hofmeister, M. & Schulte, P.M. (2006) Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, Fundulus heteroclitus. *Journal of Experimental Biology*, 209(15), 2859-2872.

Farine, D.R., Montiglio, P.O. & Spiegel, O. (2015) From individuals to groups and back: the evolutionary implications of group phenotypic composition. *Trends in ecology & evolution*, 30(10), 609-621.

Farrell, P. & Nelson, K. (2013) Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). *Environmental pollution*, 177, 1-3.

Fassbender, A.J., Sabine, C.L. & Feifel, K.M. (2016) Consideration of coastal carbonate chemistry in understanding biological calcification. *Geophysical Research Letters*, 43(9), 4467-4476.

Fauvelle, V., Garel, M., Tamburini, C., Nerini, D., Castro-Jiménez, J., Schmidt, N., Paluselli, A., Fahs, A., Papillon, L., Booth, A.M. & Sempéré, R. (2021) Organic additive release from plastic to seawater is lower under deep-sea conditions. *Nature Communications*, 12(1), 1-8.

Feely, R.A., Doney, S.C. & Cooley, S.R. (2009) Ocean acidification: Present conditions and future changes in a high-CO_2 world. *Oceanography*, 22(4), 36-47.

Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D. & Hales, B. (2008) Evidence for upwelling of corrosive" acidified" water onto the continental shelf. *science*, 320(5882), 1490-1492.

Feely, R.A., Sabine, C.L., Lee, K., Berelson, W., Kleypas, J., Fabry, V.J. & Millero, F.J. (2004) Impact of anthropogenic CO2 on the CaCO3 system in the oceans. *Science*, 305(5682), 362-366.

Feely, R.A., Sabine, C.L., Takahashi, T. & Wanninkhof, R. (2001) Uptake And Storage Of Carbon Dioxide In The Ocean: The Global CO~ 2 Survey. *Oceanography*, 14(4), 18–32.

Ferrari M.C.O., Dixson D.L., Munday P.L., McCormick M.I., Meekan M.G., Sih A. & Chivers D.P. (2011) Intrageneric variation in antipredator responses of coral reef fishes affected by ocean acidification: implications for climate change projections on marine communities. *Global Change Biology*, 17, 2980 – 2986.

Ferrari M.C.O., McCormick M.I., Munday P.L., Meekan M.G., Dixson D.L., Lonnstedt O. & Chivers D.P. (2011) Putting prey and predator into the CO2 equation – qualitative and quantitative effects of ocean acidification on predator–prey interactions. *Ecology Letters,* 14, 1143 – 1148.

Ferrari, M.C.O., Wisenden, B.D. & Chivers, D.P. (2010) Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*. 88, 698- 724.

Ferreira, P., Fonte, E., Soares, M.E., Carvalho, F. & Guilhermino, L. (2016). Effects of multi-stressors on juveniles of the marine fish Pomatoschistus microps: gold nanoparticles, microplastics and temperature. *Aquatic Toxicology*, 170, 89-103.

Ferreira, S.I.R. (2021). Future ocean warming and acidification: Simultaneous impacts on the green crab Carcinus maenas. MSc thesis. de University of Porto. Available online: https://repositorio-aberto.up.pt/bitstream/10216/139216/2/525720.pdf [Accessed 26/6/2022].

Fielder, D.R. & Lee, C.L. (1983) Agonistic behaviour and the development of dominance hierarchies in the freshwater prawn, Macrobrachium australiense Holthuis, 1950 (Crustacea: Palaemonidae). *Behaviour*, 83(1-2), 1-16.

Figuerola, B., Hancock, A.M., Bax, N., Cummings, V.J., Downey, R., Griffiths, H.J., Smith, J. & Stark, J.S. (2020) A review and meta-analysis of potential impacts of ocean acidification on marine calcifiers from the Southern Ocean. *Frontiers in Marine Science*, 8, 24.

Filiciotto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Arizza, V., de Vincenzi, G., Grammauta, R., Mazzola, S. & Buscaino, G. (2016) Underwater noise from boats: Measurement of its influence on the behaviour and biochemistry of the common prawn (Palaemon serratus, Pennant 1777). *Journal of Experimental Marine Biology and Ecology*, 478, 24-33.

Findlay, H.S. & Turley, C. (2021) Ocean acidification and climate change. *Climate Change*. 251-279.

Finelli, C.M. (2000) Velocity and concentration distributions in turbulent odor plumes in the presence of vegetation mimics: a flume study. *Marine Ecology Progress Series*, *207*, 297-309.

Fischer, E.M. & Knutti, R. (2015) Anthropogenic contribution to global occurrence of heavy-precipitation and high-temperature extremes. *Nature climate change*, 5(6), 560-564.

Fleming, J.R. (2005) *Historical perspectives on climate change*. Oxford: Oxford University Press.

Fletcher, N. & Hardege, J. D. (2009) the cost of conflict: antagonistic encounters influence responses to chemical signals in the shore crab, Carcinus maenas. *Animal Behaviour.* 77, 357-361.

Fletcher, N., Terschak, J.A., Bartels-Hardege, H.D., Bublitz, R., Schirrmacher, P. & Hardege, J.D. (2021) A pheromone bouquet controls the reproductive behaviour of the male shore crab, Carcinus maenas. *Aquatic Ecology*, 1-9.

Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J. & Koelmans, A.A. (2013) Plastic in north sea fish. *Environmental science & technology*, 47(15), 8818-8824.

Foley, C.J., Feiner, Z.S., Malinich, T.D. & Höök, T.O. (2018) A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Science of the total environment*, 631, 550-559.

Foo, S.A. & Byrne, M. (2016) Acclimatization and adaptive capacity of marine species in a changing ocean. *Advances in marine biology*, 74, 69-116.

Ford, H.V., Jones, N.H., Davies, A.J., Godley, B.J., Jambeck, J.R., Napper, I.E., Suckling, C.C., Williams, G.J., Woodall, L.C. & Koldewey, H.J. (2022) The fundamental links between climate change and marine plastic pollution. *Science of the Total Environment*, 806, 150392.

Forward Jr, R.B. (1987) Larval release rhythms of decapod crustaceans: an overview. *Bulletin of Marine Science*, 41(2), 165-176.

Fossi, M.C., Casini, S., Savelli, C., Corbelli, C., Franchi, E., Mattei, N., Sanchez-Hernandez, J.C., Corsi, I., Bamber, S. & Depledge, M.H. (2000) Biomarker responses at different levels of biological organisation in crabs (Carcinus aestuarii) experimentally exposed to benzo (α) pyrene. *Chemosphere*, 40(8), 861-874.

Fromme, H., Küchler, T., Otto, T., Pilz, K., Müller, J. & Wenzel, A. (2002) Occurrence of phthalates and bisphenol A and F in the environment. *Water research*, 36(6), 1429-1438.

Galic, N., Sullivan, L.L., Grimm, V. & Forbes, V.E. (2018) When things don't add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing. *Ecology Letters*, *21*(4), 568-577.

Gallardo, B., Aldridge, D.C., González-Moreno, P., Pergl, J., Pizarro, M., Pyšek, P., Thuiller, W., Yesson, C. & Vilà, M. (2017) Protected areas offer refuge from invasive species spreading under climate change. *Global change biology*, 23(12), 5331-5343.

Gao, K., Beardall, J., Häder, D.P., Hall-Spencer, J.M., Gao, G. & Hutchins, D.A. (2019) Effects of ocean acidification on marine photosynthetic organisms under the concurrent influences of warming, UV radiation, and deoxygenation. *Frontiers in Marine Science*, 6, 322.

Gao, P., Yue, S. & Chen, H. (2021) Carbon emission efficiency of China's industry sectors: From the perspective of embodied carbon emissions. *Journal of cleaner production*, 283, 124655.

Garcia de Leaniz, C., Fleming, I.A., Einum, S., Verspoor, E., Jordan, W.C., Consuegra, S., Aubin-Horth, N., Lajus, D., Letcher, B.H., Youngson, A.F. & Webb, J.H. (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. *Biological reviews*, 82(2), 173-211.

Garcia-Soto, C., Cheng, L., Caesar, L., Schmidtko, S., Jewett, E.B., Cheripka, A., Rigor, I., Caballero, A., Chiba, S., Báez, J.C. & Zielinski, T. (2021) An overview of ocean climate change indicators: Sea surface temperature, ocean heat content, ocean pH, dissolved oxygen concentration, arctic sea ice extent, thickness and volume, sea level and strength of the AMOC (Atlantic Meridional Overturning Circulation). *Frontiers in Marine Science*.

Gattuso, J.-P., Allemand, D., & Frankignoulle, M. (1999) Interactions between the carbon and carbonate cycles at organism and community levels in coral reefs: A review on processes, rates and environmental control, *American Journal of Zoology*, 39, 160 – 183.

Gattuso, J.P., Frankignoulle, M., Bourge, I., Romaine, S. & Buddemeier, R.W. (1998) Effect of calcium carbonate saturation of seawater on coral calcification. *Global and Planetary Change,* 18(1-2), 37-46.

Gaylor, M.O., Harvey, E., & Hale, R.C. (2013) Polybrominated diphenyl ether (PBDE) accumulation by earthworms (Eisenia fetida) exposed to biosolids-, polyurethane foam microparticle-, and penta-BDE-amended soils. *Environmental science & technology*, 47(23), 13831-13839.

Gewert, B., Plassmann, M.M. & MacLeod, M. (2015) Pathways for degradation of plastic polymers floating in the marine environment. *Environmental Science: Processes & Impacts*, 17(9), 1513-1521.

Gibert, P., Hill, M., Pascual, M., Plantamp, C., Terblanche, J.S., Yassin, A. & Sgrò, C.M. (2016) Drosophila as models to understand the adaptive process during invasion. *Biological invasions*, 18(4), 1089-1103.

Gibson, R., Atkinson, R., Gordon, J., Smith, I. & Hughes, D. (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanography Marine Biology Annual Review*, 49, 1-42.

Gilpin, J.A. & Chadwick, N.E. (2020) Social behavior of the Pederson cleaner shrimp Ancylomenes pedersoni (Chace, 1958)(Decapoda: Caridea: Palemonidae) in the Caribbean Sea: size-and gender-based dominance hierarchy. *The Journal of Crustacean Biology*, 40(1), 24-36.

GISD Global Invasive Species Database (2021) *100 of the World's Worst Invasive Alien Species.* Available online: http://www.iucngisd.org/gisd/100_worst.php [Accessed 25/03/2021].

Gissi, E., Manea, E., Mazaris, A.D., Fraschetti, S., Almpanidou, V., Bevilacqua, S., Coll, M., Guarnieri, G., Lloret-Lloret, E., Pascual, M. & Petza, D. (2021) A review of the combined effects of climate change and other local human stressors on the marine environment. *Science of the Total Environment*, 755, 142564.

Giulivo, M., de Alda, M.L., Capri, E. & Barceló, D. (2016) Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. *Environmental research*, 151, 251-264.

Glaspie, C.N., Longmire, K. & Seitz, R.D. (2017) Acidification alters predator-prey interactions of blue crab Callinectes sapidus and soft-shell clam Mya arenaria. *Journal of Experimental Marine Biology and Ecology*, 489, 58-65.

Glass, C.W. & Huntingford, F.A. (1988) Initiation and resolution of fights between swimming crabs (Liocarcinus depurator). *Ethology*, 77(3), 237-249.

Goessmann, C., Hemelrijk, C. & Huber, R. (2000) The formation and maintenance of crayfish hierarchies: behavioral and self-structuring properties. *Behavioral Ecology and Sociobiology*, 48(6), 418-428.

Greco, G., Faimali, M. & Davenport, J. (2014) Factors influencing the deterioration of the carapace surface during the moult cycle of Carcinus maenas (Linnaeus, 1758). *Contributions to Zoology*, *83*(3), 167-175.

Greenshields, J., Schirrmacher, P. & Hardege, J.D. (2021) Plastic additive oleamide elicits hyperactivity in hermit crabs. *Marine Pollution Bulletin*, 169, 112533.

Gregory, M.R. (2009) Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1526), 2013-2025.

Greiner, D.M. (2021) *Fermentation of Carcinus Maenas as a Method of Waste Stream Valorization*. MSc thesis. The University of Maine.

Groh, K.J., Backhaus, T., Carney-Almroth, B., Geueke, B., Inostroza, P.A., Lennquist, A., Leslie, H.A., Maffini, M., Slunge, D., Trasande, L. & Warhurst, A.M. (2019) Overview of known plastic packaging-associated chemicals and their hazards. *Science of the total environment*, 651, 3253-3268.

Grosholz, E.D. & Ruiz, G.M. (1996) Predicting the impact of introduced marine species: lessons from the multiple invasions of the European green crab Carcinus maenas. *Biological Conservation*, 78(1-2), 59-66.

Gunaalan, K., Fabbri, E. & Capolupo, M. (2020) The hidden threat of plastic leachates: A critical review on their impacts on aquatic organisms. *Water Research*, 184, 116170.

Guy, E.D. & Levine, N.S. (2001) GIS modeling and analysis of Ohio’s CO2 budget: mitigation CO2 emissions through reforestation. *Ohio Journal of Science (Ohio Academy of Science*), 101(3), 34-41.

Häder, D.P. & Gao, K. (2015) Interactions of anthropogenic stress factors on marine phytoplankton. *Frontiers in environmental science*, 3, 14.

Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E. & Purnell, P. (2018) An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *Journal of hazardous materials*, 344, 179-199.

Halabe, A. & Sperling, O. (1994) Uric acid nephrolithiasis. *Mineral and electrolyte metabolism*, 20(6), 424-431.

Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M.,Rowley, S.J., Tedesco, D. & Buia, M. (2008) Volcanic carbon dioxide vents show eco-system effects of ocean acidification. *Nature* 454(7200), 96–99.

Halpern, B.S., Frazier, M., Potapenko, J., Casey, K.S., Koenig, K., Longo, C., Lowndes, J.S., Rockwood, R.C., Selig, E.R., Selkoe, K.A. & Walbridge, S. (2015) Spatial and temporal changes in cumulative human impacts on the world’s ocean. *Nature communications*, *6*(1), 1-7.

Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., d'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E. & Fujita, R. (2008) A global map of human impact on marine ecosystems. *science*, 319(5865), 948-952.

Hardege, J. D., Rotchell, J., Terschak, J. & Greenway, G. (2011) Analytical challenges and the development of biomarkers to measure and monitor the effects of ocean acidification. *Trends in analytical chemistry*, 30, 1320-1326.

Hardege, J.D., Bartels-Hardege, H., Müller, C.T. & Beckmann, M. (2004) Peptide pheromones in female Nereis succinea. *Peptides*, 25(9), 1517-1522.

Hardege, J.D., Bartels-Hardege, H.D., Fletcher, N., Terschak, J.A., Harley, M., Smith, M.A., Davidson, L., Hayden, D., Müller, C.T., Lorch, M. & Welham, K. (2011) Identification of a female sex pheromone in Carcinus maenas. *Marine Ecology Progress Series*, 436, 177-189.

Hardege, J.D., Müller, C.T., Beckmann, M., BartelShardege, H.D. & Bentley, M.G. (1998) Timing of reproduction in marine polychaetes: The role of sex pheromones. *Ecoscience*, 5(3), 395-404.

Harrould-Kolieb, E., Huelsenbeck, M. & Selz, V. (2010) Ocean Acidification: The Untold Stories. *Oceana*.

Hart, R.C. & Bychek, E.A. (2011) Body size in freshwater planktonic crustaceans: an overview of extrinsic determinants and modifying influences of biotic interactions. *Hydrobiologia*, 668(1), 61-108.

Harvey, B.P., Gwynn-Jones, D. & Moore, P.J. (2013) Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecology and evolution*, 3(4), 1016-1030.

Hasan, S.S., Zhen, L., Miah, M.G., Ahamed, T. & Samie, A. (2020) Impact of land use change on ecosystem services: A review. *Environmental Development*, 34, 100527.

Hawkins, D.G. (2004) No exit: thinking about leakage from geologic carbon storage sites. *Energy*, 29(9-10), 1571-1578.

Hay, M.E. & Kubanek, J. (2002) Community and ecosystem level consequences of chemical cues in the plankton. *Journal of chemical ecology*, 28(10), 2001-2016.

Hay, M.E. (2009) Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems. *Annual review of marine science*, 1, 193-212.

Hayden, D., Jennings, A., Müller, C., Pascoe, D., Bublitz, R., Webb, H., Breithaupt, T., Watkins, L. & Hardege, J. (2007) Sex-specific mediation of foraging in the shore crab, Carcinus maenas. *Hormones and behaviour*, *52*(2), 162-168.

Hays, G.C., Richardson, A.J. & Robinson, C. (2005) Climate change and marine plankton. *Trends in ecology & evolution*, 20(6), 337-344.

Hayward, P.J. & Ryland, J.S. (2017) *Handbook of the marine fauna of North-West Europe.* Oxford: Oxford University Press.

Heemken, O.P., Reincke, H., Stachel, B. & Theobald, N. (2001) The occurrence of xenoestrogens in the Elbe river and the North Sea. *Chemosphere*, 45(3), 245-259.

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P. & Duflos, G. (2017) Occurrence and effects of plastic additives on marine environments and organisms: a review. *Chemosphere*, 182, 781-793.

Heudorf, U., Mersch-Sundermann, V. & Angerer, J. (2007). Phthalates: Toxicology and exposure. *International Journal of Hygiene and Environmental Health*, 210(5), 623–634.

Heuschele, J. & Candolin, U. (2007) An increase in pH boosts olfactory communication in sticklebacks. *Biology Letters*, 3(4), 411-413.

Hidalgo, F.J., Baron, P.J. & Orensanz, J.M.L. (2005) A prediction come true: the green crab invades the Patagonian coast. *Biological Invasions*, 7(3), 547-552.

Hiltner, A., Liu, R.Y.F., Hu, Y.S. & Baer, E. (2005) Oxygen transport as a solid-state structure probe for polymeric materials: A review. *Journal of Polymer Science Part B: Polymer Physics*, 43(9), 1047-1063.

Hines, A.H. (1982) Allometric constraints and variables of reproductive effort in brachyuran crabs. *Marine Biology*, 69(3), 309-320.

Hines, A.H. (1982) Coexistence in a kelp forest: size, population dynamics, and resource partitioning in a guild of spider crabs (Brachyura, Majidae). *Ecological monographs*, 52(2), 179-198.

Hines, A.H. (1988) Fecundity and reproductive output in two species of deep-sea crabs, Geryon fenneri and G. quinquedens (Decapoda: Brachyura). *Journal of Crustacean Biology*, 8(4), 557-562.

Hirai, H., Takada, H., Ogata, Y., Yamashita, R., Mizukawa, K., Saha, M., Kwan, C., Moore, C., Gray, H., Laursen, D. & Zettler, E.R. (2011) Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. *Marine pollution bulletin*, 62(8), 1683-1692.

Hoegh-Guldberg, O. & Bruno, J.F. (2010) The impact of climate change on the world's marine ecosystems. *Science*, *328*(5985), 1523-1528.

Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., Steneck, R.S., Greenfield, P., Gomez, E., Harvell, C.D., Sale, P.F., Edwards, A.J., Caldeira, K. & Knowlton, N. (2007) Coral reefs under rapid climate change and ocean acidification. *science*, 318(5857), 1737-1742.

Hoegh-Guldberg, O., Poloczanska, E.S., Skirving, W. & Dove, S. (2017) Coral reef ecosystems under climate change and ocean acidification. *Frontiers in Marine Science*, 4, 158.

Hoet, P.H., Brüske-Hohlfeld, I. & Salata, O.V. (2004) Nanoparticles–known and unknown health risks. *Journal of nanobiotechnology*, 2(1), 1-15.

Hofmann, G.E., Barry, J.P., Edmunds, P.J., Gates, R.D., Hutchins, D.A., Klinger, T. & Sewell, M.A. (2010) The effect of ocean acidification on calcifying organisms in marine ecosystems: an organism-to-ecosystem perspective. *Annual review of ecology, evolution, and systematics,* 41, 127-147.

Hofmann, G.E., Smith, J.E., Johnson, K.S., Send, U., Levin, L.A., Micheli, F., Paytan, A., Price, N.N., Peterson, B., Takeshita, Y. & Matson, P.G. (2011) High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PloS one*, 6(12), 28983.

Hönisch, B., Ridgwell, A., Schmidt, D.N., Thomas, E., Gibbs, S.J., Sluijs, A., Zeebe, R., Kump, L., Martindale, R.C., Greene, S.E. & Kiessling, W. (2012) The geological record of ocean acidification. *science*, 335(6072), 1058-1063.

Hopwood, M.J., Carroll, D., Dunse, T., Hodson, A., Holding, J.M., Iriarte, J.L., Ribeiro, S., Achterberg, E.P., Cantoni, C., Carlson, D.F. & Chierici, M. (2020) How does glacier discharge affect marine biogeochemistry and primary production in the Arctic?. *The Cryosphere*, 14(4), 1347-1383.

Houghton, R.A. (1991) Tropical deforestation and atmospheric carbon dioxide. *Tropical forests and climate*. 99-118.

https://doi.org/10.1016/j.marpolbul.2019.01.039.

Huang, Q., Liu, Y., Chen, Y., Fang, C., Chi, Y., Zhu, H., Lin, Y., Ye, G. & Dong, S. (2018) New insights into the metabolism and toxicity of bisphenol A on marine fish under long-term exposure. *Environmental Pollution*, 242, 914-921.

Huber, R. & Schroeder, L. (2001) Fight strategies differ with size and allometric growth of claws in crayfish, Orconectes rusticus. *Behaviour,* 138(11-12), 1437-1449.

Huber, R., Daws, A.G., Tuttle, S.A. & Panksepp, J.B. (2002) Quantitative behavioral techniques for the study of crustacean aggression. *The crustacean nervous system*. 186-201).

Huff, J. & Infante, P.F. (2011) Styrene exposure and risk of cancer. *Mutagenesis*, 26(5), 583-584.

Hung, Y.T. & Skerl, P. (2020) Carbon Dioxide Emission and Mitigation. In HANDBOOK OF ENVIRONMENT AND WASTE MANAGEMENT: Acid Rain and Greenhouse Gas *Pollution Control*. 237-280.

Hunter, E. & Naylor, E. (1993) Intertidal migration by the shore crab Carcinus maenas. *Marine Ecology-Progress Series,* 101, 131-131.

Huntingford, F.A., Taylor, A.C., Smith, I.P. & Thorpe, K.E. (1995) Behavioural and physiological studies of aggression in swimming crabs. *Journal of Experimental Marine Biology and Ecology*, 193(1-2), 21-39.

IPCC (2014) Climate change 2014: impacts, adaptation, and vulnerability. Volume I: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge and New York: Cambridge University Press.

IPCC (2019) IPCC Special Report on the Ocean and Cryosphere in a Changing Climate. Cambridge: Cambridge University Press. Available online: https://doi.org/10.1017/9781009157964. [Accessed 23/7/2021]

IPCC (2021) Intergovernmental Panel on Climate Change. Available online: https://www.ipcc.ch/ 2021/08/09/ar6-wg1-20210809-pr/ [Accessed 24/7/2021]

Jachowski, R.L. (1974) Agonistic Behavior of the Blue Crab, Callinect Us Sapid Us Rathbun. *Behaviour,* 50(3-4), 232-253.

Jackson, R.B., Canadell, J.G., Le Quéré, C., Andrew, R.M., Korsbakken, J.I., Peters, G.P. & Nakicenovic, N. (2016) Reaching peak emissions. *Nature Climate Change*, *6*(1), 7-10.

Jackson, R.B., Le Quéré, C., Andrew, R.M., Canadell, J.G., Peters, G.P., Roy, J. & Wu, L. (2017) Warning signs for stabilizing global CO2 emissions. *Environmental Research Letters*, *12*(11), 110202.

Jacobsen, H.P. & Stabell, O.B. (2004) Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signalling and predator labelling in the avoidance response of a marine gastropod. *Oikos*, 104(1), 43-50.

Jahnke, A., Arp, H.P.H., Escher, B.I., Gewert, B., Gorokhova, E., Kühnel, D., Ogonowski, M., Potthoff, A., Rummel, C., Schmitt-Jansen, M. & Toorman, E. (2017) Reducing uncertainty and confronting ignorance about the possible impacts of weathering plastic in the marine environment. *Environmental Science & Technology Letters*, *4*(3), 85-90.

Jayasiri, H.B., Purushothaman, C.S. & Vennila, A. (2013) Quantitative analysis of plastic debris on recreational beaches in Mumbai, India. *Marine pollution bulletin*, 77(1-2), 107-112.

Jewett, L. & Romanou, A. (2017) Ocean acidification and other ocean changes. *Climate science special report: fourth national climate assessment*, 1, 364-392.

Jiménez-Morales, N., Mendoza-Ángeles, K., Porras-Villalobos, M., Ibarra-Coronado, E., Roldán-Roldán, G. & Hernández-Falcón, J. (2018) Who is the boss? Individual recognition memory and social hierarchy formation in crayfish. *Neurobiology of learning and memory*, 147, 79-89.

Jin, P., Hutchins, D.A. & Gao, K. (2020) The impacts of ocean acidification on marine food quality and its potential food chain consequences. *Frontiers in Marine Science*, 7, 543979.

Johnson, A.A., Shokhirev, M.N. & Shoshitaishvili, B. (2019) Revamping the evolutionary theories of aging. *Ageing research reviews*, 55, 100947.

Johnson, M., Katoh, E. & Breithaupt, T. (2008) Fighting behaviour and the role of urinary signals in dominance assessment of Norway lobsters, Nephrops norvegicus. *Behaviour,* 145(10), 1447-1464.

Joo, S.H., Liang, Y., Kim, M., Byun, J. & Choi, H. (2021) Microplastics with adsorbed contaminants: Mechanisms and Treatment. *Environmental Challenges*, 3, 100042.

Jorgenson, A.K., Fiske, S., Hubacek, K., Li, J., McGovern, T., Rick, T., Schor, J.B., Solecki, W., York, R. & Zycherman, A. (2019) Social science perspectives on drivers of and responses to global climate change. *Wiley Interdisciplinary Reviews: Climate Change*, 10(1), 554.

Jutterström, S., Andersson, H.C., Omstedt, A. & Malmaeus, J.M. (2014) Multiple stressors threatening the future of the Baltic Sea–Kattegat marine ecosystem: Implications for policy and management actions. *Marine pollution bulletin*, 86(1-2), 468-480.

Kabir, M.S., Zhao, R., Zhang, L., Wang, H. & Luster-Teasley, S. (2022) Sources, Occurrence, and Removal of Microplastic/Nanoplastic in landfill leachate: A Comprehensive Review. *ChemRxiv*. Cambridge: Cambridge Open Engage.

Kaiser, M.J., Hughes, R.N. & Reid, D.G. (1990) Chelal morphometry, prey-size selection and aggressive competition in green and red forms of Carcinus maenas (L.). *Journal of experimental marine biology and ecology*, 140(1-2), 121-134.

Kang, J.H., Kondo, F. & Katayama, Y. (2006) Human exposure to bisphenol A. *Toxicology*, 226(2-3), 79-89.

Kapsenberg, L., Kelley, A.L., Shaw, E.C., Martz, T.R. & Hofmann, G.E. (2015) Near-shore Antarctic pH variability has implications for the design of ocean acidification experiments. *Scientific Reports*, 5(1), 1-10.

Karl, T.R. & Trenberth, K.E. (2003) Modern global climate change. *science*, 302(5651), 1719-1723.

Katzenberger, T.D. (2015) *Assessing the biological effects of exposure to microplastics in the three-spined stickleback (Gasterosteus aculeatus)(Linnaeus 1758)*. PhD thesis. University of York.

Kawecki, D., Scheeder, P.R. & Nowack, B. (2018) Probabilistic material flow analysis of seven commodity plastics in Europe. *Environmental science & technology*, 52(17), 9874-9888.

Keenan, H., Borrirukwisitsak, S., Gauchotte-Lindsay, C., & Cormack, P. (2013) *Effect of climate change on the environmental fate, bioavailability, bioaccumulation and toxicity of* *bisphenol A in the marine environment*. 329-334. Paper presented at 2nd Asian Conference on Civil, Material and Environmental Sciences, Osaka, Japan.

Kelley, A. (2013) *The Effect of Temperature on Phenotypes of the Invasive European Green Crab: Physiologic Mechanisms that Facilitate Invasion Success.* PhD thesis. Portland State University.

Kelley, A.L., de Rivera, C.E., Grosholz, E.D., Ruiz, G.M., Yamada, S.B. & Gillespie, G. (2015) Thermogeographic variation in body size of Carcinus maenas, the European green crab. *Marine Biology*, *162*(8), 1625-1635.

Kelley, J. L. & Magurran, A. E. (2003) Learned predator recognition and antipredator responses in fishes. *Fish*. 4, 216–226.

Kim, T.W., Taylor, J., Lovera, C. & Barry, J.P. (2016) CO2-driven decrease in pH disrupts olfactory behaviour and increases individual variation in deep-sea hermit crabs. *ICES Journal of Marine Science*, *73*(3), 613-619.

Kirtman, B., Power, S.B., Adedoyin, A.J., Boer, G.J., Bojariu, R., Camilloni, I., Doblas-Reyes, F., Fiore, A.M., Kimoto, M., Meehl, G. & Prather, M. (2013) Near-term climate change: projections and predictability. *Climate Change: The Physical Science Basis. IPCC Working Group I Contribution to AR5.* pp.953-1028.

Klassen, G.J. & Locke, A. (2007) A biological synopsis of the European green crab, Carcinus maenas. *Canadian Manuscript Report of Fisheries and Aquatic Sciences* No. 2818, 1-75).

Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J.-P., Langdon, C. & Opdyke B. N. (1999) Geochemical consequences of increased atmospheric CO2 on coral reefs, *Science*, 284, 118 – 120.

Koelmans, A.A., Bakir, A., Burton, G.A. & Janssen, C.R. (2016) Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. *Environmental science & technology*, *50*(7), 3315-3326.

Koelmans, A.A., Besseling, E. & Foekema, E.M. (2014) Leaching of plastic additives to marine organisms. *Environmental pollution*, *187*, 49-54.

Koolhaas, J.M., De Boer, S.F., Coppens, C.M. & Buwalda, B. (2010) Neuroendocrinology of coping styles: towards understanding the biology of individual variation. *Frontiers in neuroendocrinology*, 31(3), 307-321.

Krång, A.S. & Rosenqvist, G. (2006) Effects of manganese on chemically induced food search behaviour of the Norway lobster, Nephrops norvegicus (L.). *Aquatic toxicology*, 78(3), 284-291.

Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M. & Gattuso, J.P. (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global change biology*, *19*(6), 1884-1896.

Kroeker, K.J., Sanford, E., Jellison, B.M. & Gaylord, B. (2014) Predicting the effects of ocean acidification on predator-prey interactions: a conceptual framework based on coastal molluscs. *The Biological Bulletin*, 226(3), 211-222.

Kulkarni, R. & Galande, S. (2014) Measuring glutathione-induced feeding response in Hydra. *Journal of Visualized Experiments*, (93), 52178.

Kumar, M., Chen, H., Sarsaiya, S., Qin, S., Liu, H., Awasthi, M.K., Kumar, S., Singh, L., Zhang, Z., Bolan, N.S. & Pandey, A. (2021) Current research trends on micro-and nano-plastics as an emerging threat to global environment: a review. *Journal of Hazardous Materials*, 409, 124967.

Kurihara, H., Shimode, S. & Shirayama, Y. (2004), Sub-lethal effects of elevated concentration of CO2 on planktonic copepods and sea urchins, *Oceanography*, 60, 743 – 750.

Kyrikou, I. & Briassoulis, D. (2007) Biodegradation of agricultural plastic films: a critical review. *Journal of Polymers and the Environment*, *15*(2), 125-150.

Laist, D.W. (1987) Overview of the biological effects of lost and discarded plastic debris in the marine environment. *Marine pollution bulletin*, 18(6), 319-326.

Laist, D.W. (1997) Impacts of marine debris: entanglement of marine life in marine debris including a comprehensive list of species with entanglement and ingestion records. *Marine debris.* 99-139.

Lamb, A. & Hanby, B.P. (2005) *Marine life of the Pacific Northwest: a photographic encyclopedia of invertebrates, seaweeds and selected fishes*. Harbour Publishing.

Landrigan, P.J., Stegeman, J.J., Fleming, L.E., Allemand, D., Anderson, D.M., Backer, L.C., Brucker-Davis, F., Chevalier, N., Corra, L., Czerucka, D. & Bottein, M.Y.D. (2020) Human health and ocean pollution. *Annals of global health*, 86(1).

Landschützer, P., Gruber, N., Bakker, D.C., Stemmler, I. & Six, K.D. (2018) Strengthening seasonal marine CO 2 variations due to increasing atmospheric CO 2. *Nature Climate Change*, *8*(2), 146-150.

Lane, A.C., Mukherjee, J., Chan, V. & Thiyagarajan, V. (2013) Decreased pH does not alter metamorphosis but compromises juvenile calcification of the tube worm Hydroides elegans. *Marine Biology*, 160(8), 1983-1993.

Langdon, C., Broecker, W. S., Hammond, D. E., Glenn, E., Fitzsimmons, K., Nelson, S. G., Peng, T.-S., Hajdas, I. & Bonani, G. (2003) Effect of elevated CO2 on the community metabolism of an experimental coral reef, *Global Biogeochemical Cycles*, 17(1), 1011, Langhamer, O., Holand, H. & Rosenqvist, G. (2016) Effects of an offshore wind farm (OWF) on the common shore crab Carcinus maenas: Tagging pilot experiments in the Lillgrund offshore wind farm (Sweden). *PloS one*, 11(10), 0165096.

Laufer, H., Chen, M., Baclaski, B., Bobbitt, J.M., Stuart, J.D., Zuo, Y. & Jacobs, M.W. (2013) Multiple factors in marine environments affecting lobster survival, development, and growth, with emphasis on alkylphenols: a perspective. *Canadian journal of fisheries and aquatic sciences*, 70(11), 1588-1600.

Lauvset, S.K., Carter, B.R., Pérez, F.F., Jiang, L.Q., Feely, R.A., Velo, A. & Olsen, A. (2020) Processes driving global interior ocean pH distribution. *Global Biogeochemical Cycles*, 34(1).

Le Bris, A., Mills, K.E., Wahle, R.A., Chen, Y., Alexander, M.A., Allyn, A.J., Schuetz, J.G., Scott, J.D. & Pershing, A.J. (2018) Climate vulnerability and resilience in the most valuable North American fishery. *Proceedings of the National Academy of Sciences*, 115(8), 1831-1836.

Le Bris, A., Mills, K.E., Wahle, R.A., Chen, Y., Alexander, M.A., Allyn, A.J., Schuetz, J.G., Scott, J.D. & Pershing, A.J. (2018) Climate vulnerability and resilience in the most valuable North American fishery. *Proceedings of the National Academy of Sciences*, 115(8), 1831-1836.

Le Quéré, C., Andrew, R.M., Canadell, J.G., Sitch, S., Korsbakken, J.I., Peters, G.P., Manning, A.C., Boden, T.A., Tans, P.P., Houghton, R.A. & Keeling, R.F. (2016) Global carbon budget 2016. *Earth System Science Data*, *8*(2), 605-649.

Le Quéré, C., Andrew, R.M., Friedlingstein, P., Sitch, S., Pongratz, J., Manning, A.C., Korsbakken, J.I., Peters, G.P., Canadell, J.G., Jackson, R.B. & Boden, T.A. (2018) Global carbon budget 2017. *Earth System Science Data*, 10(1), 405-448.

Leduc, A.O., Munday, P.L., Brown, G.E. & Ferrari, M.C. (2013) Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: a synthesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1627), 20120447.

Lee, K.T., Jivoff, P. & Bishop, R.E. (2005) A low cost, reliable method for quantifying coloration in Carcinus maenas (Linnaeus, 1758)(Decapoda, Brachyura). *Crustaceana*, 579-590.

Lee, S.Y. & Seed, R. (1992) Ecological implications of cheliped size in crabs: some data from Carcinus maenas and Liocarcinus holsatus. *Marine Ecology Progress Series*, 151-160.

Lefèvre, G., Čerović, L., Milonjić, S., Fédoroff, M., Finne, J. & Jaubertie, A. (2009) Determination of isoelectric points of metals and metallic alloys by adhesion of latex particles. *Journal of colloid and interface science*, 337(2), 449-455.

Lehner, R., Weder, C., Petri-Fink, A. & Rothen-Rutishauser, B. (2019) Emergence of Nanoplastic in the Environment and Possible Impact on Human Health. *Environmental Science & Technology*, 53, 1748–1765.

Leignel, V.S.J.H., Stillman, J.H., Baringou, S., Thabet, R. & Metais, I. (2014) Overview on the European green crab Carcinus spp.(Portunidae, Decapoda), one of the most famous marine invaders and ecotoxicological models. *Environmental Science and Pollution Research*, 21(15), 9129-9144.

Leis, J.M. (2018) Paradigm lost: Ocean acidification will overturn the concept of larval-fish biophysical dispersal. *Frontiers in Marine Science*, 5, 47.

Lenhoff, H.M. (2018) The discovery of the GSH receptor in Hydra and its evolutionary significance. In *Glutathione in the nervous system*. CRC Press. 25-43.

Lenz, M., da Gama, B.A., Gerner, N.V., Gobin, J., Gröner, F., Harry, A., Jenkins, S.R., Kraufvelin, P., Mummelthei, C., Sareyka, J. & Xavier, E.A. (2011) Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: results from a globally replicated study. *Environmental research*, 111(7), 943-952.

Levin, L.A. & Le Bris, N., (2015) The deep ocean under climate change. *Science*, *350*(6262), 766-768.

Lewejohann, L., Reinhard, C., Schrewe, A., Brandewiede, J., Haemisch, A., Görtz, N., Schachner, M. & Sachser, N. (2006) Environmental bias? Effects of housing conditions, laboratory environment and experimenter on behavioral tests. Genes, *Brain and Behavior*, 5(1), 64-72.

Li, B., Lan, Z., Wang, L., Sun, H., Yao, Y., Zhang, K. & Zhu, L. (2019) The release and earthworm bioaccumulation of endogenous hexabromocyclododecanes (HBCDDs) from expanded polystyrene foam microparticles. *Environmental Pollution*, *255*, 113163.

Li, S., Liu, H., Gao, R., Abdurahman, A., Dai, J. & Zeng, F. (2018) Aggregation kinetics of microplastics in aquatic environment: complex roles of electrolytes, pH, and natural organic matter. *Environmental pollution*, 237, 126-132.

Lindstedt, K.J. (1971) Biphasic feeding response in a sea anemone: control by asparagine and glutathione. *Science*, 173(3994), 333-334.

Lithner, D., Larsson, Å., & Dave, G. (2011) Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Science of The Total Environment*, 409(18), 3309–3324.

Lithner, D., Nordensvan, I. & Dave, G. (2012) Comparative acute toxicity of leachates from plastic products made of polypropylene, polyethylene, PVC, acrylonitrile–butadiene–styrene, and epoxy to Daphnia magna. *Environmental Science and Pollution Research*, 19(5), 1763-1772.

Liu, H., Liu, K., Fu, H., Ji, R. & Qu, X. (2020) Sunlight mediated cadmium release from colored microplastics containing cadmium pigment in aqueous phase. *Environmental Pollution*, *263*, 114484.

Liu, X., Shi, H., Xie, B., Dionysiou, D.D. & Zhao, Y. (2019) Microplastics as both a sink and a source of bisphenol A in the marine environment. *Environmental Science & Technology*, 53(17), 10188-10196.

Liu, Y., Zhang, K., Xu, S., Yan, M., Tao, D., Chen, L., Wei, Y., Wu, C., Liu, G. & Lam, P.K. (2022) Heavy metals in the "plastisphere" of marine microplastics: adsorption mechanisms and composite risk. *Gondwana research*, 108, 171-180.

Lobelle, D., & Cunliffe, M. (2011) Early microbial biofilm formation on marine plastic debris. *Marine Pollution Bulletin*. 62, 197– 200.

Lockwood, B.L. & Somero, G.N. (2011) Invasive and native blue mussels (genus Mytilus) on the California coast: the role of physiology in a biological invasion. *Journal of Experimental Marine Biology and Ecology*, 400(1-2), 167-174.

Lohrer, A.M. & Whitlatch, R.B. (2002) Relative impacts of two exotic brachyuran species on blue mussel populations in Long Island Sound. *Marine Ecology Progress Series*, 227, 135-144.

Long, W.C., Swiney, K.M., Harris, C., Page, H.N. & Foy, R.J. (2013) Effects of ocean acidification on juvenile red king crab (Paralithodes camtschaticus) and Tanner crab (Chionoecetes bairdi) growth, condition, calcification, and survival. *PloS one*, 8(4), 60959.

Loomis, W.F. (1955) Glutathione control of the specific feeding reactions of hydra. *Annals of the New York Academy of Sciences*, 62(9), 211-227.

Lowe, S., Browne, M., Boudjelas, S. & Poorter, M.D. (2000) *100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database*. The Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN).

Lundebye, A.K. & Depledge, M.H. (1998) Automated interpulse duration assessment (AIDA) in the shore crab Carcinus maenas in response to copper exposure. *Marine Biology*, 130(4), 613-620.

Lundebye, A.K. & Depledge, M.H. (1998) Molecular and physiological responses in shore crabs Carcinus maenas following exposure to copper. *Marine environmental research*, 46(1-5), 567-572.

Luo, H., Liu, C., He, D., Xu, J., Sun, J., Li, J. & Pan, X. (2022) Environmental behaviors of microplastics in aquatic systems: A systematic review on degradation, adsorption, toxicity and biofilm under aging conditions. *Journal of Hazardous Materials*, *423*, 126915.

Lürling, M. & Scheffer, M. (2007) Info-disruption: pollution and the transfer of chemical information between organisms. *Trends in Ecology & Evolution*, 22(7), 374-379.

Lürling, M. (2012) Infodisruption: pollutants interfering with the natural chemical information conveyance in aquatic systems. *Chemical ecology in aquatic systems*, 250-271.

Lusher, A. (2015) *Microplastics in the marine environment: distribution, interactions and effects.* Marine anthropogenic litter. Cambridge: Springer.

Lyons, L.J., O'Riordan, R.M., Cross, T.F. & Culloty, S.C. (2012) Reproductive biology of the shore crab Carcinus maenas (Decapoda, Portunidae): a macroscopic and histological view. *Invertebrate Reproduction & Development*, 56(2), 144-156.

Lyu, K., Zhang, X. & Church, J.A. (2021) Projected Ocean warming constrained by the ocean observational record. *Nature Climate Change*, 11(10), 834-839.

Mabin, C.A., Wilson, J.R.U., Le Roux, J.J. & Robinson, T.B. (2017) Reassessing the invasion of South African waters by the European shore-crab Carcinus maenas. *African Journal of Marine Science*, 39(3), 259-267.

MacDonald, A.J., Kienzle, H.M., Drolet, D. & Hamilton, D.J. (2018) Distribution and habitat use of the invasive Carcinus maenas L.(European green crab) and the native Cancer irroratus (Say)(rock crab) in intertidal zones in the Upper Bay of Fundy, Canada. Northeastern *Naturalist*, 25(1), 161-180.

Madeira, D., Narciso, L., Cabral, H.N. & Vinagre, C. (2012) Thermal tolerance and potential impacts of climate change on coastal and estuarine organisms. *Journal of Sea Research*, 70, 32-41.

Maraveas, C. (2020) Production of sustainable and biodegradable polymers from agricultural waste. *Polymers*, *12*(5), 1127.

Marcilla, A., Garcia, S. & Garcia-Quesada, J. C. (2004) Study of the migration of PVC plasticizers. *Journal of Analytical and Applied Pyrolysis*, 71(2), 457–463.

Markic, A., Gaertner, J.C., Gaertner-Mazouni, N. & Koelmans, A.A. (2020) Plastic ingestion by marine fish in the wild. *Critical reviews in environmental science and technology*, *50*(7), 657-697.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C. & Kaminuma, T. (2001) Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environmental science & technology*, 35(2), 318-324.

McCormick, L.R., Levin, L.A. & Oesch, N.W. (2019) Vision is highly sensitive to oxygen availability in marine invertebrate larvae. *Journal of Experimental Biology*, 222(10), 200899.

McGaw, I.J., Kaiser, M.J., Naylor, E. & Hughes, R.N. (1992) Intraspecific morphological variation related to the moult-cycle in colour forms of the shore crab Carcinus maenas. *Journal of Zoology*, 228(2), 351-359.

McGowan, S., Leavitt, P.R., Hall, R.I., Anderson, N.J., Jeppesen, E. & Odgaard, B.V. (2005) Controls of algal abundance and community composition during ecosystem state change. *Ecology*, 86(8), 2200-2211.

McNamara, J.M. & Houston, A.I. (2009) Integrating function and mechanism. *Trends in ecology & evolution*, 24(12), 670-675.

McNeil, B.I. & Sasse, T.P. (2016) Future ocean hypercapnia driven by anthropogenic amplification of the natural CO2 cycle. *Nature*, 529(7586), 383-386.

Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A. & Raper, S.C. (2007) *Global climate projections*. 10.

Mei, W., Chen, G., Bao, J., Song, M., Li, Y. & Luo, C. (2020) Interactions between microplastics and organic compounds in aquatic environments: A mini review. *Science of the Total Environment*, *736*, 139472.

Melatunan, S. (2012) *Biochemical, metabolic and morphological responses of the intertidal gastropod Littorina littorea to ocean acidification and increase temperature.* PhD thesis. University of Plymouth

Melzner, F., Gutowska, M.A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., Bleich, M. & Pörtner, H.O. (2009) Physiological basis for high CO 2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?. *Biogeosciences*, 6(10), 2313-2331.

Millenium Ecosystem Assessment. (2005) Ecosystems and Human Wellbeing: Wetlands and Water. *World Resources Institute*, Washington, DC.

Miller, M.E., Hamann, M. & Kroon, F.J. (2020) Bioaccumulation and biomagnification of microplastics in marine organisms: a review and meta-analysis of current data. *PLoS One*, 15(10), 0240792.

Mincarelli, L.F., Rotchell, J.M., Chapman, E.C., Turner, A.P. & Valero, K.C.W. (2021) Consequences of combined exposure to thermal stress and the plasticiser DEHP in Mytilus spp. differ by sex. *Marine pollution bulletin*, 170, 112624.

Mishra, S., charan Rath, C. & Das, A.P. (2019) Marine microfiber pollution: a review on present status and future challenges. *Marine pollution bulletin*, 140, 188-197.

Mishra, S., Rath, C.C., Das, A.P., 2019. Marine microfiber pollution: a review on

Miura, O. (2007) Molecular genetic approaches to elucidate the ecological and evolutionary issues associated with biological invasions. *Ecological Research*, 22(6), 876-883.

Monteiro, J.N., Pinto, M., Crespo, D., Pardal, M.A. & Martinho, F. (2021) Effects of climate variability on an estuarine green crab Carcinus maenas population. *Marine Environmental Research*, 169, 105404.

Monteiro, J.N.G. (2019) *Effect of extreme climatic events on Carcinus maenas population in the Mondego estuary.* PhD thesis. Universidade de Coimbra.

Montero-Serra, I., Garrabou, J., Doak, D.F., Ledoux, J.B. & Linares, C. (2019) Marine protected areas enhance structural complexity but do not buffer the consequences of ocean warming for an overexploited precious coral. *Journal of Applied Ecology*, 56(5), 1063-1074.

Moran, E.V. & Alexander, J.M. (2014) Evolutionary responses to global change: lessons from invasive species. *Ecology Letters*, 17(5), 637-649.

Morris, S. & Taylor, A.C. (1983) Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Estuarine, Coastal and Shelf Science*, 17(3), 339-355.

Mu, X., Huang, Y., Li, X., Lei, Y., Teng, M., Li, X., Wang, C. & Li, Y. (2018) Developmental effects and estrogenicity of bisphenol A alternatives in a zebrafish embryo model. *Environmental science & technology*, 52(5), 3222-3231.

Muller, T.P.H., Iglesias-Rodriguez, D., Knorr, P., Kurihara, H., Lisle, J., Manno, C., McCoy, S., Melzner, F., Munday, P., Pörtner, H.O. & Ries, J. (2013) Arctic Ocean Acidification (AOA) Scientific Report Chapter 3. Biological responses to ocean acidification.

Munday, P.L., Dixson, D.L., Donelson, J.M., Jones, G.P., Pratchett, M.S., Devitsina, G.V. & Doving, K.B (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. P*roceedings of the National Academy of Sciences of the United States of America*. 106, 1848–1852.

Munday, P.L., Hernaman, V., Dixson, D.L. & Thorrold, S.R. (2011) Effect of ocean acidification on otolith development in larvae of a tropical marine fish. *Biogeosciences*, 8(6), 1631-1641.

Murphy, G.E. & Romanuk, T.N. (2014) A meta-analysis of declines in local species richness from human disturbances. *Ecology and evolution*, 4(1), 91-103.

Myers, R.A. & Worm, B. (2003) Rapid worldwide depletion of predatory fish communities. *Nature*, *423*(6937), 280-283.

Naganuma, K.H. & Roughgarden, J.D. (1990) Optimal Body Size in Lesser Antillean Anolis Lizards--A Mechanistic Approach. *Ecological Monographs*, 60(2), 239-256.

Nagelkerken, I. & Munday, P.L. (2016) Animal behaviour shapes the ecological effects of ocean acidification and warming moving from individual to community-level responses. *Global Change Biology*, *22*(3), 974-989.

Nagelkerken, I., Doney, S. C. & Munday, P. L. (2019) Consequences of Anthropogenic Changes in the Sensory Landscape of Marine Animals. Oceanography and Marine *Biology: An Annual Review*, 57, 229–264.

Naidoo, T. & Glassom, D. (2019) Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. *Marine pollution bulletin*, 145, 254-259.

Nancollas, S.J. & McGaw, I.J. (2021) The role of tidal acclimation on the physiological responses of the green shore crab, Carcinus maenas, to thermal stress. *Journal of Experimental Marine Biology and Ecology*, 545, 151630.

Naveira, C., Rodrigues, N., Santos, F.S., Santos, L.N. & Neves, R.A. (2021) Acute toxicity of Bisphenol A (BPA) to tropical marine and estuarine species from different trophic groups. *Environmental Pollution*, 268, 115911.

Nelson, K. (1991) Scheduling of reproduction in relation to molting and growth in malacostracan crustaceans. *Crustacean egg production,* 7, 77-116.

Niiranen, S., Yletyinen, J., Tomczak, M.T., Blenckner, T., Hjerne, O., MacKenzie, B.R., Müller-Karulis, B., Neumann, T. & Meier, H.M. (2013) Combined effects of global climate change and regional ecosystem drivers on an exploited marine food web. *Global change biology*, 19(11), 3327-3342.

Nilsson, G.E., Dixson, D.L., Domenici, P., McCormick, M.I., Sørensen, C., Watson, S.A. & Munday, P.L. (2012) Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nature Climate Change*, 2(3), 201-204.

Nogales, B., Lanfranconi, M.P., Piña-Villalonga, J.M. & Bosch, R. (2011) Anthropogenic perturbations in marine microbial communities. *FEMS Microbiology reviews*, 35(2), 275-298.

Nyamukondiwa, C., Kleynhans, E. & Terblanche, J.S. (2010) Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (Ceratitis capitata). *Ecological Entomology*, 35(5), 565-575.

Occhipinti-Ambrogi, A. (2007) Global change and marine communities: alien species and climate change. *Marine pollution bulletin*, *55*(7-9), 342-352.

O'Connor, J.J., Fobert, E.K., Besson, M., Jacob, H. & Lecchini, D. (2019) Live fast, die young: Behavioural and physiological impacts of light pollution on a marine fish during larval recruitment. *Marine pollution bulletin*, 146, 908-914.

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look, K.J. & Tyler, C.R. (2009) A critical analysis of the biological impacts of plasticizers on wildlife. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 2047-2062.

Ofori, B.Y., Stow, A.J., Baumgartner, J.B. & Beaumont, L.J. (2017) Influence of adaptive capacity on the outcome of climate change vulnerability assessment. *Scientific reports*, 7(1), 1-12.

Ogata, Y., Takada, H., Mizukawa, K., Hirai, H., Iwasa, S., Endo, S., Mato, Y., Saha, M., Okuda, K., Nakashima, A. & Murakami, M. (2009) International Pellet Watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. *Marine pollution bulletin*, 58(10), 1437-1446.

Ohsawa, K., Murata, M. & Ohshima, H. (1986) Zeta potential and surface charge density of polystyrene-latex; comparison with synaptic vesicle and brush border membrane vesicle. *Colloid and Polymer Science*, 264(12), 1005-1009.

Okamura, S., Kawaminami, T., Matsuura, H., Fusetani, N. & Goshima, S. (2018) Correction to: Behavioural assay and chemical characters of female sex pheromones in the hermit crab Pagurus filholi. Journal of Ethology. 36, 213.

Olsén, K.H. (2010) Effects of pollutants on olfactory mediated behaviors in fish and crustaceans. *Chemical communication in crustaceans*. 507-529.

Onwudili, J.A., Insura, N. & Williams, P.T. (2009) Composition of products from the pyrolysis of polyethylene and polystyrene in a closed batch reactor: Effects of temperature and residence time. *Journal of Analytical and Applied Pyrolysis*, 86(2), 293-303.

Oreskes, N., (2004) The scientific consensus on climate change. *Science*, *306*(5702), 1686-1686.

Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F. & Key, R.M. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, 437(7059), 681-686.

Paluselli, A., Fauvelle, V., Galgani, F. & Sempere, R. (2018) Phthalate release from plastic fragments and degradation in seawater. *Environmental science & technology*, *53*(1), 166-175.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G. & Ciais, P. (2011) A large and persistent carbon sink in the world’s forests. *science*, 333(6045), 988-993.

Pankhurst, N.W. & Munday, P.L. (2011) Effects of climate change on fish reproduction and early life history stages. *Marine and Freshwater Research*, 62(9), 1015-1026.

Pardo, D., Forcada, J., Wood, A.G., Tuck, G.N., Ireland, L., Pradel, R., Croxall, J.P. & Phillips, R.A. (2017) Additive effects of climate and fisheries drive ongoing declines in multiple albatross species. *Proceedings of the National Academy of Sciences,* 114(50), E10829-E10837.

Peck, L.S., Morley, S.A., Richard, J. & Clark, M.S. (2014) Acclimation and thermal tolerance in Antarctic marine ectotherms. *Journal of Experimental Biology*, 217(1), 16-22.

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., Van Der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J. & Nardin, E. (2013) Human-induced nitrogen–

phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature communications*, 4(1), 1-10.

Peters, R. H. (1983) *Ecological implications of body size*. Cambridge: Cambridge University Press.

Peters, R.H. & Wassenberg, K. (1983) The effect of body size on animal abundance. *Oecologia*, 60(1), 89-96.

Pfaller, J.B., Goforth, K.M., Gil, M.A., Savoca, M.S. & Lohmann, K.J. (2020) Odors from marine plastic debris elicit foraging behavior in sea turtles. *Current Biology*, 30(5), 213-214.

Pickering, T.R. (2011) *Predator-prey interactions between the European green crab (Carcinus maenas) and bivalves native to Prince Edward Island*. MSc thesis. University of Prince Edward Island.

Pixton, M.R., Paul, D.R. & Yampol'skii, Y. (1994) Relationships between structure and transport properties for polymers with aromatic backbones. *Polymeric gas separation membranes*, 83-154.

Pohnert, G., Steinke, M. & Tollrian, R. (2007) Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions. *Trends in Ecology & Evolution*, *22*(4), 198-204.

Poloczanska, E.S., Burrows, M.T., Brown, C.J., García Molinos, J., Halpern, B.S., Hoegh-Guldberg, O., Kappel, C.V., Moore, P.J., Richardson, A.J., Schoeman, D.S. & Sydeman, W.J. (2016) Responses of marine organisms to climate change across oceans. *Frontiers in Marine Science*, 3, 62.

Porteus, C.S., Roggatz, C.C., Velez, Z., Hardege, J.D. & Hubbard, P.C. (2021) Acidification can directly affect olfaction in marine organisms. *Journal of Experimental Biology*, 224(14),

Pörtner, H.O. (2002) Environmental and functional limits to muscular exercise and body size in marine invertebrate athletes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 133(2), 303-321.

Pörtner, H.O., Langenbuch, M. & Michaelidis, B. (2005) Synergistic effects of temperature extremes, hypoxia, and increases in CO2 on marine animals: From Earth history to global change. *Journal of Geophysical Research: Oceans*, 110(C9).

Pörtner, H.O., Langenbuch, M. & Reipschläger, A. (2004) Biological impact of elevated ocean CO 2 concentrations: lessons from animal physiology and earth history. *Journal of Oceanography*, 60(4), 705-718.

Pörtner, H.O., Roberts, D.C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E. & Weyer, N.M. (2019) The ocean and cryosphere in a changing climate. *IPCC Special Report on the Ocean and Cryosphere in a Changing Climate*.

Potocka, M., Bayer, R.C. & Potocki, M. (2019) Plastic pollution affects American lobsters, Homarus americanus. *Marine pollution bulletin*, 138, 545-548.

present status and future challenges. Mar. Pollut. Bull. 140 (2019), 188e197.

Price, N.N., Hamilton, S.L., Tootell, J.S. & Smith, J.E. (2011) Species-specific consequences of ocean acidification for the calcareous tropical green algae Halimeda. *Marine Ecology Progress Series*, 440, 67-78.

Procter, J., Hopkins, F. E., Fileman, E. S. & Lindeque, P. K. (2019) Smells good enough to eat: Dimethyl sulfide (DMS) enhances copepod ingestion of microplastics. *Marine Pollution Bulletin*, 138, 1−6.

Quijada-Rodriguez, A.R., Kuan, P.L., Sung, P.H., Hsu, M.T., Allen, G.J., Hwang, P.P., Tseng, Y.C. & Weihrauch, D. (2021) Anthropogenic CO 2-mediated freshwater acidification limits survival, calcification, metabolism, and behaviour in stress-tolerant freshwater crustaceans. *Biogeosciences*, 18(23), 6287-6300.

Rai, P.K., Sonne, C., Brown, R.J., Younis, S.A. & Kim, K.H. (2021) Adsorption of environmental contaminants on micro-and nano-scale plastic polymers and the influence of weathering processes on their adsorptive attributes. *Journal of Hazardous Materials*,127903.

Ramanathan, V. & Feng, Y. (2009) Air pollution, greenhouse gases and climate change: Global and regional perspectives. *Atmospheric environment*, *43*(1), 37-50.

Rani, M., Shim, W.J., Han, G.M., Jang, M., Al-Odaini, N.A., Song, Y.K. & Hong, S.H. (2015) Qualitative analysis of additives in plastic marine debris and its new products. *Archives of environmental contamination and toxicology*, 69(3), 352-366.

Rani, M., Shim, W.J., Jang, M., Han, G.M. & Hong, S.H. (2017) Releasing of hexabromocyclododecanes from expanded polystyrenes in seawater-field and laboratory experiments. *Chemosphere*, *185*, 798-805.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C. & Watson, A. (2005) *Ocean acidification due to increasing atmospheric carbon dioxide.* The Royal Society.

Reid, D.G. & Aldrich, J.C. (1989) Variations in response to environmental hypoxia of different colour forms of the shore crab, Carcinus maenas. *Comparative Biochemistry and Physiology Part A: Physiology*, *92*(4), 535-539.

Reid, D.G., Abelló, P., Kaiser, M.J. & Warman, C.G. (1997) Carapace colour, inter-moult duration and the behavioural and physiological ecology of the shore crab Carcinus maenas. *Estuarine, Coastal and Shelf Science*, 44(2), 203-211.

Reid, P.C., Fischer, A.C., Lewis-Brown, E., Meredith, M.P., Sparrow, M., Andersson, A.J., Antia, A., Bates, N.R., Bathmann, U., Beaugrand, G. & Brix, H. (2009) Impacts of the oceans on climate change. *Advances in marine biology*, *56*, 1-150.

Reimchen, T.E. (1991) Trout foraging failures and the evolution of body size in stickleback. *Copeia*, 1991(4), 1098-1104.

Reimer, A.A. (1972) Glutathione-proline activation of feeding behavior in the zoanthid Palythoa psammophilia Walsh & Bowers. *Journal of Experimental Marine Biology and Ecology*, 10(3), 177-181.

Reusch, T.B., HaÈberli, M.A., Aeschlimann, P.B. & Milinski, M. (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*, 414(6861), 300-302.

Rhee, Y.J. & Rhee, J.S. (2016) Bisphenol A causes mortality and reduced hatching success through increase of cell damage and dysfunction of antioxidant defense system in marine medaka embryo. *Toxicology and Environmental Health Sciences*, 8(5), 290-295.

Richardson, B., Martin, H., Bartels-Hardege, H., Fletcher, N. & Hardege, J.D. (2021) The role of changing pH on olfactory success of predator–prey interactions in green shore crabs, Carcinus maenas. *Aquatic Ecology*, 1-10.

Richardson, R.B. (2010) Ecosystem services and food security: Economic perspectives on environmental sustainability. *Sustainability*, 2(11), 3520-3548.

Riebesell, U., Zondervan, I., Rost, B., Tortell, P.D., Zeebe, R.E. & Morel, F.M. (2000) Reduced calcification of marine plankton in response to increased atmospheric CO2. *Nature*, 407(6802), 364-367.

Rilov, G., Fraschetti, S., Gissi, E., Pipitone, C., Badalamenti, F., Tamburello, L., Menini, E., Goriup, P., Mazaris, A.D., Garrabou, J. & Benedetti-Cecchi, L. (2020) A fast-moving target: achieving marine conservation goals under shifting climate and policies. *Ecological Applications*, 30(1), 02009.

Rilov, G., Mazaris, A.D., Stelzenmüller, V., Helmuth, B., Wahl, M., Guy-Haim, T., Mieszkowska, N., Ledoux, J.B. & Katsanevakis, S. (2019) Adaptive marine conservation planning in the face of climate change: What can we learn from physiological, ecological and genetic studies?. *Global Ecology and Conservation*, 17, 00566.

Rios, L.M., Jones, P.R., Moore, C. & Narayan, U.V. (2010) Quantitation of persistent organic pollutants adsorbed on plastic debris from the Northern Pacific Gyre's “eastern garbage patch”. *Journal of Environmental Monitoring*, 12(12), 2226-2236.

Ritchie, H. & Roser, M. (2018) Plastic pollution. *Our World in Data*.

Rivalan, P., Barbraud, C., Inchausti, P. & Weimerskirch, H. (2010) Combined impacts of longline fisheries and climate on the persistence of the Amsterdam Albatross Diomedia amsterdamensis. *Ibis*, 152(1), 6-18.

Roberts, C.M., O’Leary, B.C., McCauley, D.J., Cury, P.M., Duarte, C.M., Lubchenco, J., Pauly, D., Sáenz-Arroyo, A., Sumaila, U.R., Wilson, R.W. & Worm, B. (2017) Marine reserves can mitigate and promote adaptation to climate change. *Proceedings of the National Academy of Sciences*, *114*(24), 6167-6175.

Rochman, C. M. (2015) The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment. *Marine Anthropogenic Litter*,117-140.

Rochman, C. M., Browne, M. A., Halpern, B. S., Hentschel, B. T., Hoh, E., Karapanagioti, H. K., H., Rios, L. R., Takada, H., Teh, S. & Thompson, R. C. (2013) Policy: Classify plastic waste as hazardous. *Nature*, 494(7436), 169–171.

Rochman, C.M., Kurobe, T., Flores, I. & Teh, S.J. (2014) Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. *Science of the total environment*, 493, 656-661.

Rochman, C.M., Lewison, R.L., Eriksen, M., Allen, H., Cook, A.M. & Teh, S.J. (2014) Polybrominated diphenyl ethers (PBDEs) in fish tissue may be an indicator of plastic contamination in marine habitats. *Science of the total environment*, 476, 622-633.

Rodolfo-Metalpa, R., Houlbrèque, F., Tambutté, É., Boisson, F., Baggini, C., Patti, F.P., Jeffree, R., Fine, M., Foggo, A., Gattuso, J.P. & Hall-Spencer, J.M. (2011) Coral and mollusc resistance to ocean acidification adversely affected by warming. *Nature Climate Change*, 1(6), 308-312.

Rodolfo-Metalpa, R., Lombardi, C., Cocito, S., Hall-Spencer, J.M. & Gambi, M.C. (2010) Effects of ocean acidification and high temperatures on the bryozoan Myriapora truncata at natural CO2 vents. *Marine Ecology*, 31(3), 447-456.

Rodrigues, E.T. & Pardal, M.Â. (2014) The crab Carcinus maenas as a suitable experimental model in ecotoxicology. *Environment international*, 70, 158-182.

Rodrigues, E.T. & Pardal, M.Â. (2014) The crab Carcinus maenas as a suitable experimental model in ecotoxicology. *Environment international*, 70, 158-182.

Roggatz, C.C., Kenningham, N. & Bartels-Hardege, H.D. (2019) Taking current climate change research to the classroom—the “will hermit crabs go hungry in future oceans?” project. *Climate Change and the Role of Education*. 255-277.

Roggatz, C.C., Lorch, M., Hardege, J.D. & Benoit, D.M. (2016) Ocean acidification affects marine chemical communication by changing structure and function of peptide signalling molecules. *Global change biology*, *22*(12), 3914-3926.

Rolland, V., Barbraud, C. & Weimerskirch, H. (2008) Combined effects of fisheries and climate on a migratory long-lived marine predator. *Journal of Applied Ecology*, 45(1), 4-13.

Romera-Castillo, C., Pinto, M., Langer, T.M., Álvarez-Salgado, X.A. & Herndl, G.J. (2018) Dissolved organic carbon leaching from plastics stimulates microbial activity in the ocean. *Nature communications*, 9(1), 1-7.

Ropes, J.W. (1968) The feeding habits of the green crab, Carcinus maenas (L.). *Fishery Bulletin*, 67(2), 183-203.

Rovero, F., Hughes, R.N., Whiteley, N.M. & Chelazzi, G. (2000) Estimating the energetic cost of fighting in shore crabs by non-invasive monitoring of heartbeat rate. *Animal Behaviour*, 59(4), 705-713.

Ryan, P.G. (2020) The transport and fate of marine plastics in South Africa and adjacent oceans. *South African Journal of Science*, 116(5-6), 1-9.

Ryan, P.G., Connell, A.D. & Gardner, B.D. (1988) Plastic ingestion and PCBs in seabirds: is there a relationship?. *Marine pollution bulletin*, *19*(4), 174-176.

Sajiki, J. & Yonekubo, J. (2003) Leaching of bisphenol A (BPA) to seawater from polycarbonate plastic and its degradation by reactive oxygen species. *Chemosphere*, 51(1), 55-62.

Santos, I.R., Chen, X., Lecher, A.L., Sawyer, A.H., Moosdorf, N., Rodellas, V., Tamborski, J., Cho, H.M., Dimova, N., Sugimoto, R. & Bonaglia, S. (2021) Submarine groundwater discharge impacts on coastal nutrient biogeochemistry. *Nature Reviews Earth & Environment*, 2(5), 307-323.

Sarà, G., Porporato, E.M., Mangano, M.C. & Mieszkowska, N. (2018) Multiple stressors facilitate the spread of a non-indigenous bivalve in the Mediterranean Sea. *Journal of Biogeography*, 45(5), 1090-1103.

Sarà, G., Porporato, E.M., Mangano, M.C. & Mieszkowska, N. (2018) Multiple stressors facilitate the spread of a non-indigenous bivalve in the Mediterranean Sea. *Journal of Biogeography*, 45(5), 1090-1103.

Sato, T. & Goshima, S. (2007) Effects of risk of sperm competition, female size, and male size on number of ejaculated sperm in the stone crab Hapalogaster dentata. *Journal of Crustacean Biology,* 27(4), 570-575.

Sato, T. & Goshima, S. (2007) Female choice in response to risk of sperm limitation by the stone crab, Hapalogaster dentata. *Animal Behaviour*, 73(2), 331-338.

Savoca, M.S., McInturf, A.G. & Hazen, E.L. (2021) Plastic ingestion by marine fish is widespread and increasing. *Global change biology*, 27(10), 2188-2199.

Savoca, M.S., Tyson, C.W., McGill, M. & Slager, C.J. (2017) Odours from marine plastic debris induce food search behaviours in a forage fish. *Proceedings of the Royal Society B: Biological Sciences*, 284(1860), 20171000.

Savoca, M.S., Wohlfeil, M.E., Ebeler, S.E. & Nevitt, G.A. (2016) Marine plastic debris emits a keystone infochemical for olfactory foraging seabirds. *Science Advances*. 1600395.

Scharf, F.S., Juanes, F. & Rountree, R.A. (2000) Predator size-prey size relationships of marine fish predators: interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Marine Ecology Progress Series*, 208, 229-248.

Schellnhuber, H J , Cramer, W , Nakicenovic, N , Wigley, T & Yohe, G. (2006) *Avoiding Dangerous Climate Change.* Cambridge: Cambridge University Press.

Schirrmacher, P., Roggatz, C.C., Benoit, D.M. & Hardege, J.D. (2021) Ocean Acidification Amplifies the Olfactory Response to 2-Phenylethylamine: Altered Cue Reception as a Mechanistic Pathway?. *Journal of Chemical Ecology*, 1-18.

Schmidt, R. & Psenner, R. (1992) Climate changes and anthropogenic impacts as causes for pH fluctuations in remote high alpine lakes. *Documenta Istituto Italiano Idrobiologia*, 32, 31-57.

Schmolke, A., Brain, R., Thorbek, P., Perkins, D. & Forbes, V. (2017) Population modeling for pesticide risk assessment of threatened species—A case study of a terrestrial plant, Boltonia decurrens. *Environmental toxicology and chemistry*, *36*(2), 480-491.

Schneider, K.R. (2008) Heat stress in the intertidal: comparing survival and growth of an invasive and native mussel under a variety of thermal conditions. *The Biological Bulletin*, 215(3), 253-264.

Schober, P. & Vetter, T.R. (2018) Survival analysis and interpretation of time-to-event data: the tortoise and the hare. *Anesthesia and analgesia*, 127(3), 792.

Schram, J.B., Schoenrock, K.M., McClintock, J.B., Amsler, C.D. & Angus, R.A. (2014) Multiple stressor effects of near-future elevated seawater temperature and decreased pH on righting and escape behaviors of two common Antarctic gastropods. *Journal of experimental marine biology and ecology*, *457*, 90-96.

Schuett, W., Dall, S.R. & Royle, N.J. (2011) Pairs of zebra finches with similar ‘personalities’ make better parents. *Animal Behaviour*, 81(3), 609-618.

Schuett, W., Tregenza, T. & Dall, S.R. (2010) Sexual selection and animal personality. *Biological Reviews*, 85(2), 217-246.

Schuyler, Q.A., Wilcox, C., Townsend, K., Hardesty, B.D., Marshall, N.J., 2014. Mistaken identity? Visual similarities of marine debris to natural prey items of sea turtles. BMC *Ecology*. 14, 1–7.

Scott, K., Piper, A.J., Chapman, E.C. & Rochas, C.M. (2020) Review of the effects of underwater sound, vibration and electromagnetic fields on crustaceans. *Seafish Report*

Seibel, B. A., & P. J. Walsh (2001) Potential impacts of CO2 injections on deep-sea biota, *Science*, 294, 319 – 320.

Seinfeld, J.H. & Pandis, S.N. (1998) From air pollution to climate change. *Atmospheric chemistry and physics*, 1326.

Sekkelsten, G.I. (1988) Effect of handicap on mating success in male shore crabs Carcinus maenas. *Oikos*, 131-134.

Senner, N.R., Stager, M. & Cheviron, Z.A. (2018) Spatial and temporal heterogeneity in climate change limits species' dispersal capabilities and adaptive potential. *Ecography*, 41(9), 1428-1440.

Shams, M., Alam, I. & Chowdhury, I. (2020) Aggregation and stability of nanoscale plastics in aquatic environment. *Water Research*, 171, 115401.

Shaw, D.K. & Sahni, P. (2014) Plastic to oil. Journal of Mechanical and Civil Engineering, 46-48.

Sheavly, S.B. & Register, K.M. (2007) Marine debris & plastics: environmental concerns, sources, impacts and solutions. *Journal of Polymers and the Environment*, 15(4), 301-305.

Sih, A., Bell, A. & Johnson, J.C. (2004) Behavioral syndromes: an ecological and evolutionary overview. *Trends in ecology & evolution*, 19(7), 372-378.

Simčič, T. & Brancelj, A. (2007) The effect of light on oxygen consumption in two amphipod crustaceans–the hypogean Niphargus stygius and the epigean Gammarus fossarum. *Marine and Freshwater Behaviour and Physiology*, 40(2), 141-150.

Smale, D.A., Wernberg, T., Oliver, E.C., Thomsen, M., Harvey, B.P., Straub, S.C., Burrows, M.T., Alexander, L.V., Benthuysen, J.A., Donat, M.G. & Feng, M. (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change*, 9(4), 306-312.

Smith, M.R. & Myers, S.S. (2018) Impact of anthropogenic CO2 emissions on global human nutrition. *Nature Climate Change*, *8*(9), 834-839.

Smith, P., Ashmore, M.R., Black, H.I., Burgess, P.J., Evans, C.D., Quine, T.A., Thomson, A.M., Hicks, K. & Orr, H.G. (2013) The role of ecosystems and their management in regulating climate, and soil, water and air quality. *Journal of Applied Ecology*, 50(4), 812-829.

Smith, S.L., Yamanaka, Y., Pahlow, M. & Oschlies, A. (2009) Optimal uptake kinetics: physiological acclimation explains the pattern of nitrate uptake by phytoplankton in the ocean. *Marine Ecology Progress Series*, 384, 1-12.

Smith, T.G., Hammill, M.O. & Taugbøl, G. (1991) A review of the developmental, behavioural and physiological adaptations of the ringed seal, Phoca hispida, to life in the Arctic winter. *Arctic*, 124-131.

Sneddon, L.U., Huntingford, F.A. & Taylor, A.C. (1997) Weapon size versus body size as a predictor of winning in fights between shore crabs, Carcinus maenas (L.). *Behavioral Ecology and Sociobiology,* 41(4), 237-242.

Sneddon, L.U., Huntingford, F.A., Taylor, A.C. & Clare, A.S. (2003) Female sex pheromone-mediated effects on behavior and consequences of male competition in the shore crab (Carcinus maenas). *Journal of Chemical Ecology*, 29(1), 55-70.

Sogard, S.M. (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of marine science*, 60(3), 1129-1157.

Sokolova, I.M. (2013) Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integrative and comparative biology*, 53(4), 597-608.

Sokołowski, A., Brulińska, D. & Sokołowska, E. (2020) Multimarker response of the ragworm Hediste diversicolor (Polychaeta) to seawater acidification derived from potential CO2 leakage from the CCS sub-seabed storage site in the Baltic Sea. *Journal of Experimental Marine Biology and Ecology*, 530, 151433.

Solomon, S., Plattner, G.K., Knutti, R. & Friedlingstein, P. (2009) Irreversible climate change due to carbon dioxide emissions. *Proceedings of the national academy of sciences*, 106(6), 1704-1709.

Sordo, L., Santos, R., Reis, J., Shulika, A. & Silva, J. (2016) A direct CO2 control system for ocean acidification experiments: testing effects on the coralline red algae Phymatolithon lusitanicum. *Peer Journal*, 4, 2503.

Sorensen, R.M. and Jovanović, B. (2021) From nanoplastic to microplastic: A bibliometric analysis on the presence of plastic particles in the environment. *Marine Pollution Bulletin*, *163*, 111926.

Sorte, C.J., Williams, S.L. & Carlton, J.T. (2010) Marine range shifts and species introductions: comparative spread rates and community impacts. *Global Ecology and Biogeography*, 19(3), 303-316.

Speakman, J.R. (2005) Body size, energy metabolism and lifespan. *Journal of Experimental Biology*, 208(9), 1717-1730.

Spicer, J.I., Raffo, A. & Widdicombe, S. (2007) Influence of CO2-related sea water acidification on extracellular acid–base balance in the velvet swimming crab Necora puber. *Marine Biology*, 151, 1117–1125.

Spooner, E.H., Coleman, R.A. and Attrill, M.J. (2007) Sex differences in body morphology and multitrophic interactions involving the foraging behaviour of the crab Carcinus maenas. *Marine Ecology*, *28*(3), 394-403.

Stahlhut, R.W., Welshons, W.V. & Swan, S.H. (2009) Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. *Environmental health perspectives*, 117(5), 784-789.

Staniszewska, M., Graca, B., & Nehring, I. (2016) The fate of bisphenol A, 4-tert-octylphenol and 4-nonylphenol leached from plastic debris into marine water – experimental studies on biodegradation and sorption on suspended particulate matter and nano-TiO2. *Chemosphere*, 145, 535–542.

Staples, C. A., Adams, W. J., Parkerton, T. F., Gorsuch, J. W., Biddinger, G. R., & Reinert, K. H. (1997) Aquatic toxicity of eighteen phthalate esters. *Environmental Toxicology and Chemistry*, 16(5), 875–891.

Steel, C.G.H., (1980) Mechanisms of coordination between moulting and reproduction in terrestrial isopod Crustacea. *The Biological Bulletin*, 159(1), 206-218.

Stein, R.A. (1976) Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. *Canadian Journal of Zoology*, 54(2), 220-227.

Stein, W. & Harzsch, S. (2021) The Neurobiology of Ocean Change–insights from decapod crustaceans. *Zoology*, 144, 125887.

Steinberg, P.D. & van Altena, I. (1992) Tolerance of marine invertebrate herbivores to brown algal phlorotannins in temperate Australasia. *Ecological Monographs*, *62*(2), 189-222.

Stenseng, E., Braby, C.E. & Somero, G.N. (2005) Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (genus Tegula): implications for vertical zonation. *The Biological Bulletin*, 208(2), 138-144.

Stenseth, N.C. & Mysterud, A. (2002) Climate, changing phenology, and other life history traits: nonlinearity and match–mismatch to the environment. *Proceedings of the National Academy of Sciences*, 99(21), 13379-13381.

Stevens, M., Lown, A.E. & Wood, L.E. (2014) Camouflage and individual variation in shore crabs (Carcinus maenas) from different habitats. *PLoS One*, 9(12), 115586.

Stillman, J.H. & Somero, G.N. (2000) A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus Petrolisthes: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology*, 73(2), 200-208.

Stillman, J.H. (2003) Acclimation capacity underlies susceptibility to climate change. *Science*, 301(5629), 65-65.

Sturm, C.F., Pearce, T.A. & Valdés, Á. (2006) *The mollusks: a guide to their study, collection, and preservation.* American Malacologican Society. Pittsburg: Universal-Publishers.

Styrishave, B. & Andersen, O. (2000) Seasonal variations in hepatopancreas fatty acid profiles of two colour forms of shore crabs, Carcinus maenas. *Marine Biology*, 137(3), 415-422.

Styrishave, B., Rewitz, K. & Andersen, O. (2004) Frequency of moulting by shore crabs Carcinus maenas (L.) changes their colour and their success in mating and physiological performance. *Journal of Experimental Marine Biology and Ecology*, 313(2), 317-336.

Suhrhoff, T. J., & Scholz-Böttcher, B. M. (2016) Qualitative impact of salinity, UV radiation and turbulence on leaching of organic plastic additives from four common plastics — A lab experiment. *Marine Pollution Bulletin*, 102(1), 84–94.

Sun, B., Hu, Y., Cheng, H. & Tao, S. (2019) Releases of brominated flame retardants (BFRs) from microplastics in aqueous medium: Kinetics and molecular-size dependence of diffusion. *Water research*, *151*, 215-225.

Sunday, J.M., Calosi, P., Dupont, S., Munday, P.L., Stillman, J.H. & Reusch, T.B. (2014) Evolution in an acidifying ocean. *Trends in ecology & evolution*, 29(2), 117-125.

Sundin, J., Rosenqvist, G. & Berglund, A. (2013) Altered Oceanic p H Impairs Mating Propensity in a Pipefish. *Ethology*, 119(1), 86-93.

Susoy, V., Ragsdale, E.J., Kanzaki, N. & Sommer, R.J. (2015) Rapid diversification associated with a macroevolutionary pulse of developmental plasticity. *elife*, 4, 05463.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y. & Corporeau, C. (2016) Oyster reproduction is affected by exposure to polystyrene microplastics. *Proceedings of the national academy of sciences*, 113(9), 2430-2435.

Swain, S., Sawant, P.B., Chadha, N.K., Chhandaprajnadarsini, E.M. & Katare, M.B. (2020) Significance of water pH and hardness on fish biological processes: A review. *International Journal of Chemical Studies*, 8(4), 330-337.

Tai, A.P., Martin, M.V. & Heald, C.L. (2014) Threat to future global food security from climate change and ozone air pollution. *Nature Climate Change*, 4(9), 817-821.

Takahashi, T., Sutherland, S.C., Sweeney, C., Poisson, A., Metzl, N., Tilbrook, B., Bates, N., Wanninkhof, R., Feely, R.A., Sabine, C. & Olafsson, J. (2002) Global sea–air CO2 flux based on climatological surface ocean pCO2, and seasonal biological and temperature effects. *Deep Sea Research Part II: Topical Studies in Oceanography*, 49(9-10), 1601-1622.

Takahashi, T., Sutherland, S.C., Wanninkhof, R., Sweeney, C., Feely, R.A., Chipman, D.W., Hales, B., Friederich, G., Chavez, F., Sabine, C. & Watson, A. (2009) Climatological mean and decadal change in surface ocean pCO2, and net sea–air CO2 flux over the global oceans. *Deep Sea Research Part II: Topical Studies in Oceanography*, 56(8-10), 554-577.

Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M.A. & Watanuki, Y. (2013). Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. *Marine pollution bulletin*, *69*(1-2), 219-222.

Tanaka, K., Watanuki, Y., Takada, H., Ishizuka, M., Yamashita, R., Kazama, M., Hiki, N., Kashiwada, F., Mizukawa, K., Mizukawa, H. & Hyrenbach, D. (2020) In vivo accumulation of plastic-derived chemicals into seabird tissues. *Current Biology*, *30*(4), 723-728.

Tang, Y., Zhou, W., Sun, S., Du, X., Han, Y., Shi, W. & Liu, G. (2020) Immunotoxicity and neurotoxicity of bisphenol A and microplastics alone or in combination to a bivalve species, Tegillarca granosa. *Environmental Pollution*, 265, 115115.

Tepolt, C.K. & Somero, G.N. (2014) Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, Carcinus maenas. *Journal of Experimental Biology*, 217(7), 1129-1138.

Teuten, E.L., Rowland, S.J., Galloway, T.S. & Thompson, R.C. (2007) Potential for plastics to transport hydrophobic contaminants. *Environmental science & technology*, 41(22), 7759-7764.

Teuten, E.L., Saquing, J.M., Knappe, D.R., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R. & Ochi, D. (2009) Transport and release of chemicals from plastics to the environment and to wildlife. *Philosophical transactions of the royal society B: biological sciences*, *364*(1526), 2027-2045.

Therriault, T.W., Herborg, L.M., Locke, A. & McKindsey, C.W. (2008) Risk assessment for European green crab (Carcinus maenas) in Canadian waters. *Fisheries and Oceans Canada, Science*.

Thompson, S.A., Sydeman, W.J., Santora, J.A., Black, B.A., Suryan, R.M., Calambokidis, J., Peterson, W.T. & Bograd, S.J. (2012) Linking predators to seasonality of upwelling: using food web indicators and path analysis to infer trophic connections. *Progress in Oceanography*, 101(1), 106-120.

Thoré, E.S., Brendonck, L. & Pinceel, T. (2021) Neurochemical exposure disrupts sex-specific trade-offs between body length and behaviour in a freshwater crustacean. *Aquatic Toxicology*, 237, 105877.

Thresher, R., Proctor, C., Ruiz, G., Gurney, R., MacKinnon, C., Walton, W., Rodriguez, L. & Bax, N. (2003) Invasion dynamics of the European shore crab, Carcinus maenas, in Australia. *Marine Biology*, 142(5), 867-876.

Thushari, G.G.N. & Senevirathna, J.D.M. (2020) Plastic pollution in the marine environment. *Heliyon*, 6(8), 04709.

Tierney, A.J. & Atema, T. (1988) Amino acid chemoreception: Effects of pH on receptors and stimuli. *Journal of Chemical Ecology*. 14, 135–141.

Todd, P.A., Briers, R.A., Ladle, R.J. & Middleton, F. (2006) Phenotype-environment matching in the shore crab (Carcinus maenas). *Marine Biology*, 148(6), 1357-1367.

Todd, P.A., Oh, J., Loke, L.H. & Ladle, R.J. (2012) Multi-scale phenotype-substrate matching: Evidence from shore crabs (Carcinus maenas L.). *Ecological Complexity*, 12, 58-62.

Tran, M. (2015). Behavioural reactions to novel food odours by intertidal hermit crabs. *Behavioural Processes*, 113, 35-40.

Trotter, B., Ramsperger, A.F.R.M., Raab, P., Haberstroh, J. & Laforsch, C. (2019) Plastic waste interferes with chemical communication in aquatic ecosystems. *Scientific reports*, 9(1), 1-8.

Turley, C., Blackford, J., Widdicombe, S., Lowe, D., Nightingale, P.D. & Rees, A.P. (2006) Reviewing the impact of increased atmospheric CO2 on oceanic pH and the marine ecosystem. *Avoiding dangerous climate change*, 8, 65-70.

Turner, H.V., Wolcott, D.L., Wolcott, T.G. & Hines, A.H. (2003) Post-mating behavior, intramolt growth, and onset of migration to Chesapeake Bay spawning grounds by adult female blue crabs, Callinectes sapidus Rathbun. *Journal of Experimental Marine Biology and Ecology*, *295*(1), pp.107-130.

Turner, L.M., Ricevuto, E., Massa-Gallucci, A., Gambi, M.C. & Calosi, P. (2015) Energy metabolism and cellular homeostasis trade-offs provide the basis for a new type of sensitivity to ocean acidification in a marine polychaete at a high-CO2 vent: adenylate and phosphagen energy pools versus carbonic anhydrase. *The Journal of Experimental Biology*, 218(14), 2148-2151.

Uguen, M., Nicastro, K.R., Zardi, G.I., Gaudron, S.M., Spilmont, N., Akoueson, F., Duflos, G. & Seuront, L. (2022) Microplastic leachates disrupt the chemotactic and chemokinetic behaviours of an ecosystem engineer (Mytilus edulis). *Chemosphere*, 135425.

Ullah, H., Nagelkerken, I., Goldenberg, S.U. & Fordham, D.A. (2018) Climate change could drive marine food web collapse through altered trophic flows and cyanobacterial proliferation. *PLoS biology*, 16(1), 2003446.

Uthicke, S., Deshpande, N.P., Liddy, M., Patel, F., Lamare, M. & Wilkins, M.R. (2019) Little evidence of adaptation potential to ocean acidification in sea urchins living in “Future Ocean” conditions at a CO2 vent. *Ecology and evolution*, 9(17), 10004-10016.

Vainio, H. (1978) Vinyl chloride and vinyl benzene (styrene) — Metabolism, mutagenicity and carcinogenicity. *Chemico-Biological Interactions*, 22(1), 117–124.

Vajedsamiei, J., Wahl, M., Schmidt, A.L., Yazdanpanahan, M. & Pansch, C. (2021) The higher the needs, the lower the tolerance: Extreme events may select ectotherm recruits with lower metabolic demand and heat sensitivity. *Frontiers in Marine Science*, 8, 264.

van Wezel, A. P., van Vlaardingen, P., Posthumus, R., Crommentuijn, G. H., & Sijm, D. T. H. M. (2000) Environmental Risk Limits for Two Phthalates, with Special Emphasis on Endocrine Disruptive Properties. *Ecotoxicology and Environmental Safety*, 46(3), 305– 321.

Vargas, C.A., Lagos, N.A., Lardies, M.A., Duarte, C., Manríquez, P.H., Aguilera, V.M., Broitman, B., Widdicombe, S. & Dupont, S. (2017) Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. *Nature Ecology & Evolution*, 1(4), 1-7.

Velez, Z., Roggatz, C.C., Benoit, D.M., Hardege, J.D. & Hubbard, P.C. (2019) Short-and medium-term exposure to ocean acidification reduces olfactory sensitivity in gilthead seabream. *Frontiers in physiology*, 731.

Verma, R., Vinoda, K.S., Papireddy, M. & Gowda, A.N.S. (2016) Toxic pollutants from plastic waste-a review. *Procedia Environmental Sciences*, 35, 701-708.

Vila-Viçosa, D., Teixeira, V.H., Santos, H.A. & Machuqueiro, M. (2013) Conformational study of GSH and GSSG using constant-pH molecular dynamics simulations. *The Journal of Physical Chemistry B*, 117(25), 7507-7517.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. (1997) Human domination of Earth's ecosystems. *Science*, 277(5325), 494-499.

Von Schuckmann, K., Cheng, L., Palmer, M.D., Hansen, J., Tassone, C., Aich, V., Adusumilli, S., Beltrami, H., Boyer, T., Cuesta-Valero, F.J. & Desbruyères, D. (2020) Heat stored in the Earth system: where does the energy go?. *Earth System Science Data*, *12*(3), 2013-2041.

Voss, R., Quaas, M.F., Stiasny, M.H., Hänsel, M., Pinto, G.A.S.J., Lehmann, A., Reusch, T.B. & Schmidt, J.O. (2019) Ecological-economic sustainability of the Baltic cod fisheries under ocean warming and acidification. *Journal of Environmental Management*, 238, 110-118.

Wahl, M., Schneider Covachã, S., Saderne, V., Hiebenthal, C., Müller, J.D., Pansch, C. & Sawall, Y. (2018) Macroalgae may mitigate ocean acidification effects on mussel calcification by increasing pH and its fluctuations. *Limnology and Oceanography*, 63(1), 3-21.

Waiho, K., Mustaqim, M., Fazhan, H., Norfaizza, W.I.W., Megat, F.H. & Ikhwanuddin, M., 2015. Mating behaviour of the orange mud crab, Scylla olivacea: The effect of sex ratio and stocking density on mating success. *Aquaculture Reports*, *2*, 50-57.

Waldbusser, G.G. & Salisbury, J.E. (2014) Ocean acidification in the coastal zone from an organism's perspective: multiple system parameters, frequency domains, and habitats. *Annual review of marine science*, 6, 221-247.

Waldbusser, G.G., Gray, M.W., Hales, B., Langdon, C.J., Haley, B.A., Gimenez, I., Smith, S.R., Brunner, E.L. & Hutchinson, G. (2016) Slow shell building, a possible trait for resistance to the effects of acute ocean acidification. *Limnology and Oceanography*, 61(6), 1969-1983.

Waldbusser, G.G., Hales, B., Langdon, C.J., Haley, B.A., Schrader, P., Brunner, E.L., Gray, M.W., Miller, C.A. & Gimenez, I. (2015) Saturation-state sensitivity of marine bivalve larvae to ocean acidification. *Nature Climate Change*, 5(3), 273-280.

Wale, M.A., Simpson, S.D. & Radford, A.N. (2013) Noise negatively affects foraging and antipredator behaviour in shore crabs. *Animal Behaviour*, 86(1), 111-118.

Walters, C., English, K., Korman, J. & Hilborn, R. (2019) The managed decline of British Columbia's commercial salmon fishery. *Marine Policy*, 101, 25-32.

Wang, H.Y., Botsford, L.W., White, J.W., Fogarty, M.J., Juanes, F., Hastings, A., Holland, M.D. & Brander, K. (2014) Effects of temperature on life history set the sensitivity to fishing in Atlantic cod Gadus morhua. *Marine Ecology Progress Series*, 514, 217-229.

Wang, M.H., He, Y. & Sen, B. (2019) Research and management of plastic pollution in coastal environments of China. *Environmental pollution*, 248, 898-905.

Wang, T. & Wang, Y. (2020) Behavioral responses to ocean acidification in marine invertebrates: new insights and future directions. *Journal of Oceanology and Limnology*, *38*(3), 759-772.

Wang, X., Li, K., Di Yang, X., Wang, L.L. & Shen, R.F. (2009) Complexation of Al (III) with reduced glutathione in acidic aqueous solutions. *Journal of Inorganic Biochemistry*, 103(5), 657-665.

Wang, Y., Hu, M., Shin, P.K.S. & Cheung, S.G. (2010) Induction of anti-predator responses in the green-lipped mussel Perna viridis under hypoxia. *Marine Biology*, 157(4), 747-754.

Wang, Y., Zhao, H. & Zhao, G. (2015) Iron-copper bimetallic nanoparticles embedded within ordered mesoporous carbon as effective and stable heterogeneous Fenton catalyst for the degradation of organic contaminants. *Applied Catalysis B: Environmental*, 164, 396-406.

Warman, C.G., Reid, D.G. & Naylor, E. (1993) Variation in the tidal migratory behaviour and rhythmic light-responsiveness in the shore crab, Carcinus maenas. *Journal of the Marine Biological Association of the United Kingdom*, 73(2), 355-364.

Watson, S.A., Lefevre, S., McCormick, M.I., Domenici, P., Nilsson, G.E. & Munday, P.L. (2014) Marine mollusc predator-escape behaviour altered by near-future carbon dioxide levels. *Proceedings of the Royal Society B: Biological Sciences*, 281(1774), 20132377.

Watts, A.J., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler, C.R. & Galloway, T.S. (2014) Uptake and retention of microplastics by the shore crab Carcinus maenas. *Environmental science & technology*, 48(15), 8823-8830.

Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P. & Koelmans, A.A. (2012) Effects of nanopolystyrene on the feeding behavior of the blue mussel (Mytilus edulis L.). *Environmental Toxicology and Chemistry*, 31(11), 2490-2497.

Weissburg, M.J., Berkenkamp, K. & Mankin, D. (2010) Turbulent mixing inhibits discrimination of attractive vs. aversive chemicals in crabs by eroding small scale filament structure impinging on antennulary chemosensors. *Integrative and comparative biology*, 50, E188-E188.

Weissburg, M.J., Dusenbery, D.B., Ishida, H., Janata, J., Keller, T., Roberts, P.J.W. & Webster, D.R., (2002) A multidisciplinary study of spatial and temporal scales containing information in turbulent chemical plume tracking. *Environmental Fluid Mechanics*, *2*, 65-94.

Welden, N.A. (2020) The environmental impacts of plastic pollution. *Plastic Waste and Recycling*, 195-222).

Western, D., (2001) Human-modified ecosystems and future evolution. Proceedings of the *National Academy of Sciences*, 98(10), 5458-5465.

Whiteley, N.M. (2011) Physiological and ecological responses of crustaceans to ocean acidification. *Marine Ecology Progress Series*, 430, 257-271.

Whitman, D.W. & Agrawal, A.A. (2009) What is phenotypic plasticity and why is it important. *Phenotypic plasticity of insects: Mechanisms and consequences*, 1-63.

Widdicombe, S. & Spicer, J.I. (2008) Predicting the impact of ocean acidification on benthic biodiversity: what can animal physiology tell us?. *Journal of Experimental Marine Biology and Ecology*, 366(1-2), 187-197.

Willett, H.C. (1949) Long-period fluctuations of the general circulation of the atmosphere. *Journal of Meteorology*, *6*(1), 34-50.

Williams, M.J. (1982) Natural food and feeding in the commercial sand crab Portunus pelagicus Linnaeus, 1766 (Crustacea: Decapoda: Portunidae) in Moreton Bay, Queensland. *Journal of Experimental Marine Biology and Ecology*, *59*(2-3), 165-176.

Wilson, C.H., Nancollas, S.J., Rivers, M.L., Spicer, J.I. & McGaw, I.J. (2021) Effects of handling during experimental procedures on stress indices in the green shore crab, Carcinus maenas (L). *Marine and Freshwater Behaviour and Physiology*, 54(2), 65-86.

Wisenden, B.D., (2000) Olfactory assessment of predation risk in the aquatic environment. Proc. R. Soc. London. B. 355, 1205–1208

Wójcik-Fudalewska, D., Normant-Saremba, M. & Anastácio, P. (2016) Occurrence of plastic debris in the stomach of the invasive crab Eriocheir sinensis. *Marine pollution bulletin*, 113(1-2), 306-311.

Wolf, M. & Krause, J. (2014) Why personality differences matter for social functioning and social structure. *Trends in Ecology & Evolution*, 29(6), 306-308.

Wolf, M., Van Doorn, G.S. & Weissing, F.J. (2011) On the coevolution of social responsiveness and behavioural consistency. *Proceedings of the Royal Society B: Biological Sciences*, 278(1704), 440-448.

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A. & Warren, P.H. (2005) Body size in ecological networks. *Trends in ecology & evolution*, 20(7), 402-409.

Wootton, J.T., Pfister, C.A. & Forester, J.D. (2008) Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *Proceedings of the National Academy of Sciences*, 105(48), 18848-18853.

Wootton, J.T., Pfister, C.A. & Forester, J.D. (2008) Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *Proceedings of the National Academy of Sciences*, 105(48), 18848-18853.

Wright, H.O. (1968) Visual displays in brachyuran crabs: field and laboratory studies. *American Zoologist*, 8(3), 655-665.

Wright, S.L., Thompson, R.C. & Galloway, T.S. (2013) The physical impacts of microplastics on marine organisms: a review. *Environmental pollution*, *178*, 483-492.

Wu, N.C. & Seebacher, F. (2020) Effect of the plastic pollutant bisphenol A on the biology of aquatic organisms: A meta-analysis. *Global change biology*, 26(7), 3821-3833.

Würsig, B. (2017) Marine mammals of the Gulf of Mexico. *Habitats and Biota of the Gulf of Mexico: before the deepwater horizon oil spill*. 1489-1587.

Yamada, S.B & Gillespie, G.E. (2008) Will the European green crab (Carcinus maenas) persist in the Pacific Northwest?. *ICES Journal of Marine Science*, *65*(5), 725-729.

Yamashita, R., Takada, H., Fukuwaka, M.A. & Watanuki, Y. (2011) Physical and chemical effects of ingested plastic debris on short-tailed shearwaters, Puffinus tenuirostris, in the North Pacific Ocean. *Marine pollution bulletin*, 62(12), 2845-2849.

Yasuda, C.I., Otoda, M., Nakano, R., Takiya, Y. & Koga, T. (2017) Seasonal change in sexual size dimorphism of the major cheliped in the hermit crab Pagurus minutus. *Ecological research*, 32(3), 347-357.

Yeh, P.J. & Price, T.D. (2004) Adaptive phenotypic plasticity and the successful colonization of a novel environment. *The American Naturalist*, 164(4), 531-542.

Ying, G.G. & Kookana, R.S. (2003) Degradation of five selected endocrine-disrupting chemicals in seawater and marine sediment. *Environmental Science & Technology*, 37(7), 1256-1260.

Yoch, D. C. (2002) Dimethylsulfoniopropionate: Its Sources, Role in the Marine Food Web, and Biological Degradation to Dimethylsulfide. *Applied and Environmental Microbiology*, 68, 5804−5815.

Yoshino, K., Koga, T. & Oki, S. (2011) Chelipeds are the real weapon: cheliped size is a more effective determinant than body size in male–male competition for mates in a hermit crab. *Behavioral Ecology and Sociobiology*, 65(9), 1825-1832.

Young, A.M. & Elliott, J.A. (2019) Life history and population dynamics of green crabs (Carcinus maenas). *Fishes*, 5(1), 4.

Young, A.M., Elliott, J.A., Incatasciato, J.M. & Taylor, M.L. (2017) Seasonal catch, size, color, and assessment of trapping variables for the European green crab Carcinus maenas (Brachyura: Portunoidea: Carcinidae), a nonindigenous species in Massachusetts, USA. *Journal of Crustacean Biology*, *37*(5), 556-570.

Zabaniotou, A., & Kassidi, E. (2003) Life cycle assessment applied to egg packaging made from polystyrene and recycled paper. *Journal of Cleaner Production*, 11(5), 549–559.

Zapata, O. (2021) The relationship between climate conditions and consumption of bottled water: A potential link between climate change and plastic pollution. *Ecological Economics*, 187, 107090.

Zarfl, C., Fleet, D., Fries, E., Galgani, F., Gerdts, G., Hanke, G. & Matthies, M. (2011) Microplastics in oceans. *Marine pollution bulletin*, 62, 1589-1591.

Zbyszewski, M. & Corcoran, P.L. (2011) Distribution and degradation of fresh water plastic particles along the beaches of Lake Huron, Canada. *Water, Air, & Soil Pollution*, 220(1), 365-372.

Zeeck, E., Harder, T. & Beckmann, M. (1998) Uric acid: the sperm-release pheromone of the marine polychaete Platynereis dumerilii. *Journal of Chemical Ecology*, 24(1), 13-22.

Zerebecki, R.A. & Sorte, C.J. (2011) Temperature tolerance and stress proteins as mechanisms of invasive species success. *PLOS one*, 6(4), 14806.

Zettler, E. R., Mincer, T. J. & Amaral-Zettler, L. A. (2013) Life in the “plastisphere”: Microbial communities on plastic marine debris. *Environmental Science and Technology*, 47, 7137−7146.

Zhang, H. & Byrne, R.H. (1996) Spectrophotometric pH measurements of surface seawater at in-situ conditions: absorbance and protonation behavior of thymol blue. *Marine Chemistry*, 52(1), 17-25.

Zhang, K., Chen, B., Mao, J., Zhu, L. & Xing, B. (2018) Water clusters contributed to molecular interactions of ionizable organic pollutants with aromatized biochar via π-PAHB: sorption experiments and DFT calculations. *Environmental Pollution*, 240, 342-352.

Zhou, Y., Yang, Y., Liu, G., He, G. & Liu, W. (2020) Adsorption mechanism of cadmium on microplastics and their desorption behavior in sediment and gut environments: the roles of water pH, lead ions, natural organic matter and phenanthrene. *Water Research*, 184, 116209.

Zimmer-Faust, R.K., O'Neill, P.B. & Schar, D.W. (1996) The relationship between predator activity state and sensitivity to prey odor. *The Biological Bulletin*, *190*(1), 82-87.

Zimmermann, L., Dierkes, G., Ternes, T.A., Völker, C. & Wagner, M. (2019) Benchmarking the in vitro toxicity and chemical composition of plastic consumer products. *Environmental science & technology*, 53(19), 11467-11477.

8. Appendices

Table 1. Database of crab IDs and individual characteristics: sex, size, colour, injury of animals used. Dates of death and moults.

COLLECTED	DATE	CRAB ID	SEX	SIZE	WEIGHT	COLOUR	INJURED	POST MOULT	DEATH
04/06/2021	25/08/2021	1	M	7.6	92.1	G	Y		10/09/2021
04/06/2021	25/08/2021	2	M	6.9	90.4	R	N		10/09/2021
04/06/2021	11/11/2021	3	M	7.1	72	G	N		
04/06/2021	11/11/2021	4	M	7.5	89.1	R	Y		
04/06/2021	25/08/2021	5	M	6.9	71.9	R	Y		12/10/2021
04/06/2021	11/11/2021	6	M	7.5	88.4	R	N		
04/06/2021	11/11/2021	7	M	7.5	92.1	G	Y		
04/06/2021	11/11/2021	8	M	7	90.4	R	N		14/11/2021
04/06/2021	11/11/2021	9	M	7	72	G	N		
04/06/2021	11/11/2021	10	M	7.3	86	R	Y		
04/06/2021	11/11/2021	11	M	6.9	70.5	R	Y	15/10/2021	
04/06/2021	11/11/2021	12	M	7.5	89.2	R	Y		
04/06/2021	11/11/2021	13	M	5.8	48.8	G	N		
04/06/2021	11/11/2021	14	M	5.5	44.7	G	Y		
04/06/2021	11/11/2021	15	M	5.7	42.2	G	N		
04/06/2021	11/11/2021	16	M	5.5	36.6	G	Y		
04/06/2021	11/11/2021	17	M	4.5	22	G	N		
04/06/2021	11/11/2021	18	M	3.5	16.5	G	N		
04/06/2021	11/11/2021	19	M	5.5	47.2	R	N		
04/06/2021	11/11/2021	20	M	7	92	G	N		
04/06/2021	11/11/2021	21	M	8.2	93.5	G	Y		
04/06/2021	11/11/2021	22	M	7.6	83	R	N		
04/06/2021	11/11/2021	23	M	6.6	78.9	R	N		
04/06/2021	11/11/2021	24	M	6.6	68.5	G	N		
04/06/2021	11/11/2021	25	M	7	89	R	N		
04/06/2021	11/11/2021	26	M	7.3	83.8	R	Y		
04/06/2021	11/11/2021	27	M	6.7	81	R	N	10/08/2021	
04/06/2021	11/11/2021	28	M	6.8	82.5	G	N		
04/06/2021	08/11/2021	29	M	6	51.8	R	Y		
04/06/2021	11/11/2021	30	M	5.4	42.6	G	Y		
04/06/2021	11/11/2021	31	M	5	31.1	G	Y		
04/06/2021	11/11/2021	32	M	5.6	51	G	N		
04/06/2021	11/11/2021	33	M	5.5	50	G	N		
04/06/2021	05/08/2021	34	M	5.7	46	R	N		07/08/2021
04/06/2021	11/11/2021	35	M	6	47.8	G	N		
04/06/2021	11/11/2021	36	M	4.5	18.8	G	N		
04/06/2021	10/08/2021	37	M	7.5	115.5	G	N		17/08/2021
04/06/2021	11/11/2021	38	M	8.5	142.1	G	N		
04/06/2021	10/08/2021	39	M	6.5	87.5	G	Y		11/08/2021
04/06/2021	11/11/2021	40	M	6	65.7	G	Y		
04/06/2021	11/11/2021	41	M	6.5	73.3	G	Y		
04/06/2021	11/11/2021	42	M	5.4	41.7	G	N	12/08/2021	
04/06/2021	11/11/2021	43	M	6.7	90.4	G	N		
04/06/2021	11/11/2021	44	M	5.7	47.5	G	N		
04/06/2021	11/11/2021	45	M	5.5	44.7	G	Y		
04/06/2021	11/11/2021	46	M	5.6	40.2	G	N		
04/06/2021	11/11/2021	47	M	5.5	36.6	G	Y		
04/06/2021	11/11/2021	48	M	8.1	98.1	G	Y		
04/06/2021	25/08/2021	49	M	7.5	83	R	N		07/10/2021
04/06/2021	11/11/2021	50	M	6.5	78.9	R	N		
04/06/2021	11/11/2021	51	M	6.6	71.2	G	N		
04/06/2021	11/11/2021	52	M	7.2	88.3	R	N		
04/06/2021	25/08/2021	53	M	7.1	83.8	R	Y		08/10/2021
04/06/2021	11/11/2021	54	M	6.5	81	R	N		
04/06/2021	11/11/2021	55	M	6.8	80.2	G	N		17/11/2021
04/06/2021	11/11/2021	56	M	6	50.6	R	Y		
04/06/2021	11/11/2021	57	M	5.7	45.7	G	N	16/08/2021	
04/06/2021	11/11/2021	58	M	5.1	31.7	G	N		
04/06/2021	11/11/2021	59	M	5.6	48.5	R	N		
04/06/2021	10/08/2021	60	M	7	89.8	G	N		23/08/2021

COLLECTED	DATE	CRAB ID	SEX	SIZE	WEIGHT	COLOUR	INJURED	POST MOULT	DEATH
04/06/2021	11/11/2021	1	F	4.5	26.2	G	N		
04/06/2021	11/11/2021	2	F	6	57	R	N		
04/06/2021	11/11/2021	3	F	6.2	66.1	R	Y		
04/06/2021	11/11/2021	4	F	6.3	57.7	R	Y		
04/06/2021	25/08/2021	5	F	4.5	25.5	G	N		03/10/2021
04/06/2021	11/11/2021	6	F	5.6	48.8	R	Y		
04/06/2021	11/11/2021	7	F	5.2	37.7	R	N	12/08/2021	
04/06/2021	11/11/2021	8	F	5.8	37.6	R	N		
04/06/2021	11/11/2021	9	F	5.5	42.8	G	Y	18/08/2021	
04/06/2021	11/11/2021	10	F	6	53.1	R	N		17/11/2021
04/06/2021	11/11/2021	11	F	7	69.7	R	N		
04/06/2021	11/11/2021	12	F	6.5	57.2	R	N		
04/06/2021	11/11/2021	13	F	3.5	10.4	G	N		
04/06/2021	11/11/2021	14	F	5.1	28.6	R	N	04/08/2021	
04/06/2021	11/11/2021	15	F	6	52.8	R	Y		
04/06/2021	11/11/2021	16	F	5.5	42.2	R	Y		
04/06/2021	11/11/2021	17	F	6.1	52.8	R	N		
04/06/2021	11/11/2021	18	F	5.6	45	R	N		
04/06/2021	11/11/2021	19	F	6.5	62.6	R	N		
04/06/2021	11/11/2021	20	F	6.1	44.6	R	N	19/07/2021	
04/06/2021	11/11/2021	21	F	6	49	R	Y		
04/06/2021	11/11/2021	22	F	5.9	47.7	R	N		
04/06/2021	10/08/2021	23	F	5.5	38	R	N		25/08/2021
04/06/2021	11/11/2021	24	F	6	52.2	R	N		
04/06/2021	11/11/2021	25	F	4.5	19.2	R	Y		
04/06/2021	11/11/2021	26	F	6	49.1	R	N		
04/06/2021	11/11/2021	27	F	6.1	55.5	R	Y		
04/06/2021	11/11/2021	28	F	6.2	57.8	R	Y		
04/06/2021	11/11/2021	29	F	6.6	63.6	R	N		
04/06/2021	11/11/2021	30	F	4.9	32.7	R	Y	17/08/2021	
04/06/2021	11/11/2021	31	F	6.5	59	R	Y		
04/06/2021	11/11/2021	32	F	6.3	59.1	R	Y		
04/06/2021	25/08/2021	33	F	5.6	44.7	R	Y	03/08/2021	11/09/2021
04/06/2021	11/11/2021	34	F	5.5	42.8	G	Y		
04/06/2021	11/11/2021	35	F	6.1	53.1	R	N		
04/06/2021	10/08/2021	36	F	7	69.7	R	N		15/08/2021
04/06/2021	11/11/2021	37	F	6.5	57.2	R	N		
04/06/2021	11/11/2021	38	F	6.3	50.4	R	Y		
04/06/2021	11/11/2021	39	F	5.9	47.7	R	N		
04/06/2021	11/11/2021	40	F	5.5	38	R	N		

Table 2. Preliminary testing of three plastics: Polyethylene (PE), Polypropylene (PP), and Polyvinylchloride (PVC). 20 *C. maenas* individuals were presented with filter paper soaked in plastic conditioned water in pH 8.2 to assess which was the most effective to use for study.

Plastic Odour	Percentage of crabs reacted (%)
PE	75%
PP	55%
PVC	45%

Table 3. Time taken for odour cues to diffuse to the other end of the Y shaped flume. Food colouring was used to measure defusal rate of the odour cues. Food colouring was inserted at the tip of each branch of the Y shaped tank and timed until the colouring had reached the other end of the tank. This was tested over a range of flow rates to select the most appropriate for bioassays.

Flow Rate	Time to diffuse (Minutes)
0.5 L/Min	9.14
1 L/Min	5.01
1.5 L/Min	3.45
2 L/Min	2.46
2.5 L/Min	2.01
3 L/Min	1.32
3.5 L/min	0.54
4 L/Min	0.36

Table 4. Mean time of cue duration at a flow rate of 500ml per minute. As mentioned above in **Table 3.** cues were placed into the Y shaped tank and monitored. At select intervals the cues were tested for effectiveness. From this it was deduced that cues were effective for a duration of approximately 2 hours before the odour was no longer detectable by animals and needed to be replaced.

Cue	mean time (Minutes)
Pheromone	124
Food	128
Plastic	120
Control	N/A

Table 5. Timeline of when bioassays were performed, number and proportions of animals used in each test. P – Plastic odour, C – control, G – Glutathione, U – pheromone.

Date of testing	Assays tested	pH tested	Animals tested (Male, Female)	Proportion of animal types tested					
				Male			Female		
				Size	Colour	Injury	Size	Colour	Injury
08/07/21 prelim plastic testing	P v C	8.2	10 M and 10 F	7 LG, 3 SM	5 G, 5 R	5 I, 5 NI	4 LG, 6 SM	8 R, 2 G	3 I, 7 NI
	P v C	7.6	10 M and 10 F	7 LG, 3 SM	5 G, 5 R	5 I, 5 NI	4 LG, 6 SM	8 R, 2 G	3 I, 7 NI
02/08/21	P v G	7.6	24 M and 16 F	12 LG, 12 SM	19 G, 5 R	10 I, 14 NI	9 LG, 7 SM	13 R, 3 G	6 I, 10 NI
	P v G	8.2	24 M and 16 F	12 LG, 12 SM	19 G, 5 R	10 I, 14 NI	9 LG, 7 SM	13 R, 3 G	6 I, 10 NI
03/08/21	P v C	7.6	24 M and 16 F	16 LG, 8 SM	13 G, 11 R	10 I, 14 NI	10 LG, 6 SM	14 R, 2 G	5 I, 11 NI
	P v C	8.2	24 M and 16 F	16 LG, 8 SM	13 G, 11 R	10 I, 14 NI	10 LG, 6 SM	14 R, 2 G	5 I, 11 NI
05/08/21	P v U	7.6	30 M and 10 F	17 LG, 13, SM	29 G, 1 R	11 I, 19 NI	5 LG, 5 SM	8 R, 2 G	5 I, 5 NI
	P v U	8.2	30 M and 10 F	17 LG, 13, SM	29 G, 1 R	11 I, 19 NI	5 LG, 5 SM	8 R, 2 G	5 I, 5 NI
09/08/21	G v C	7.6	30 M and 10 F	18 LG, 12 SM	30 G	11 I, 19 NI	5 LG, 5 SM	9 R, 1 G	2 I, 8 NI
	G v C	8.2	30 M and 10 F	18 LG, 12 SM	30 G	11 I, 19 NI	5 LG, 5 SM	9 R, 1 G	2 I, 8 NI
	G v U	7.6	30 M and 10 F	18 LG, 12 SM	30 G	11 I, 19 NI	5 LG, 5 SM	9 R, 1 G	2 I, 8 NI
	G v U	8.2	30 M and 10 F	18 LG, 12 SM	30 G	11 I, 19 NI	5 LG, 5 SM	9 R, 1 G	2 I, 8 NI
10/08/21	U v C	7.6	30 M and 10 F	21 LG, 9 SM	30 G	13 I, 17 NI	7 LG, 3 SM	10 R	6 I, 4 NI
	U v C	8.2	30 M and 10 F	21 LG, 9 SM	30 G	13 I, 17 NI	7 LG, 3 SM	10 R	6 I, 4 NI
25/08/21	P v C	7.2	30 M and 10 F	18 LG, 12 SM	19 G, 11 R	11 I, 19 NI	6 LG, 4 SM	10 R	4 I, 6 NI
	P v G	7.2	30 M and 10 F	18 LG, 12 SM	19 G, 11 R	11 I, 19 NI	6 LG, 4 SM	10 R	4 I, 6 NI
	P v U	7.2	30 M and 10 F	18 LG, 12 SM	30 G	11 I, 19 NI	6 LG, 4 SM	10 R	4 I, 6 NI
	G v C	7.2	30 M and 10 F	18 LG, 12 SM	30 G	11 I, 19 NI	6 LG, 4 SM	10 R	4 I, 6 NI
	U v C	7.2	30 M and 10 F	18 LG, 12 SM	30 G	11 I, 19 NI	6 LG, 4 SM	10 R	4 I, 6 NI
	G v U	7.2	30 M and 10 F	18 LG, 12 SM	30 G	11 I, 19 NI	6 LG, 4 SM	10 R	4 I, 6 NI
04/11/21 winter crabs									
08/11/21 winter	P v C	8.2	30 M and 10 F	18 LG, 12 SM	19 G, 11 R	11 I, 19 NI	6 LG, 4 SM	10 R	6 I, 4 NI
	P v C	7.6	30 M and 10 F	13 LG, 17 SM	29 G, 1 R	15 I, 15 NI	7 LG, 3 SM	10 R	6 I, 4 NI
09/11/21 winter	P v G	8.2	30 M and 10 F	19 LG, 11 SM	30 G	14 I, 16 NI	7 LG, 3 SM	9 R, 1 G	6 I, 4 NI
	P v U	8.2	30 M and 10 F	19 LG, 11 SM	30 G	14 I, 16 NI	7 LG, 3 SM	9 R, 1 G	6 I, 4 NI
	P v G	7.6	30 M and 10 F	15 LG, 15 SM	20 G, 10 R	11 I, 19 NI	6 LG, 4 SM	8 R, 2 G	6 I, 4 NI
	P v U	7.6	30 M and 10 F	15 LG, 15 SM	20 G, 10 R	11 I, 19 NI	7 LG, 3 SM	9 R, 1 G	6 I, 4 NI
11/11/21 winter	P v U	7.2	30 M and 10 F	17 LG, 13 SM	27 G, 3 R	15 I, 14 NI	5 LG, 5 SM	9 R, 1 G	6 I, 4 NI
	P v G	7.2	30 M and 10 F	13 LG, 17 SM	29 G, 1 R	15 I, 14 NI	5 LG, 5 SM	9 R, 1 G	6 I, 4 NI
	P v C	7.2	30 M and 10 F	11 LG, 19 SM	26 G, 4 R	15 I, 14 NI	5 LG, 5 SM	9 R, 1 G	6 I, 4 NI

Table 6. Example test sheet of raw data.

crab ID #	size (cm)	Initial reaction (min)	Time to cue (min)	Cue chosen	reaction
28 M	6.8	00:04	02:25	GSH	Grab
29 M	6	00:02	00:26	GSH	Grab
30 M	5.4	00:05	04:41	PE	Waft
31 M	5	00:02	00:19	PE	Grab
32 M	5.6	00:02	-	-	Buried
21 M	7.2	00:04	00:53	GSH	Grab
7 M	7.5	00:04	02:25	GSH	Waft
8 M	7	00:02	00:26	GSH	Waft
9 M	7	00:05	01:41	PE	Continuous activity
10 M	7.3	00:02	00:33	GSH	Buried
11 M	6.9	00:04	02:25	GSH	Grab
12 M	7.5	00:02	00:26	PE	Grab
39 M	6.5	00:05	04:32	PE	Waft
40 M	6	00:02	00:19	GSH	Grab
41 M	6.5	00:02	02:25	GSH	Waft
42 M	5.4	00:04	00:26	GSH	Waft
43 M	6.7	00:07	03:38	GSH	Continuous activity
44 M	5.7	00:02	00:14	PE	Grab
45 M	5.5	00:05	04:41	PE	Waft
51 M	6.6	00:02	00:19	GSH	Waft
52 M	7.2	00:02	02:25	GSH	Waft
53 M	7.1	00:04	00:26	PE	Waft
54 M	6.5	00:04	00:24	PE	Buried
55 M	6.8	00:02	02:25	GSH	Continuous activity
56 M	6	00:05	00:26	PE	Waft
1 M	7.6	00:03	03:11	PE	Waft
4 M	7.5	00:02	00:18	GSH	Waft
3 M	7.1	00:04	04:58	GSH	Waft
18 M	3.5	00:04	00:19	PE	Waft
25 M	7	00:02	00:59	PE	Waft
37 F	6.5	00:05	02:25	GSH	Continuous activity
38 F	6.3	00:12	-	-	Waft
39 F	5.9	00:02	03:59	PE	Continuous activity
40 F	5.5	00:04	00:19	PE	Waft
6 F	5.6	00:03	04:41	GSH	Waft
5 F	4.5	00:02	00:19	GSH	Grab
4 F	6.3	00:05	01:45	GSH	Waft
3 F	6.2	00:02	02:23	GSH	Continuous activity
2 F	6	00:02	00:42	GSH	Waft
1 F	4.5	00:04	00:51	GSH	Waft

Table 7. All statistical outputs of the Kruskal-Wallis tests performed on initial reaction, testing for significant differences between individuals.

Kruskal wallis	**χ 2**	**df**	**p-value**
Sex x behaviour	19.566	5	0.001507
Injury x cue chosen	9.5196	4	0.04935
Condition x behaviour	14.243	5	0.01414
Initial Reaction x size	36.673	24	0.04716
Initial Reaction x colour	4.1635	1	0.0413
Cue chosen x size	49.527	24	0.001626
Cue chosen x sex	14.727	1	0.0001242
Cue chosen x weight	94.908	64	0.007296
Time to cue x injury	8.99	1	0.002715
behaviour x size	37.75	24	0.03677
behaviour x injury	4.2399	1	0.03948
pH x weight	91.095	64	0.01467
Size x injury	10.187	1	0.001414
Weight x injury	6.0947	1	0.01356

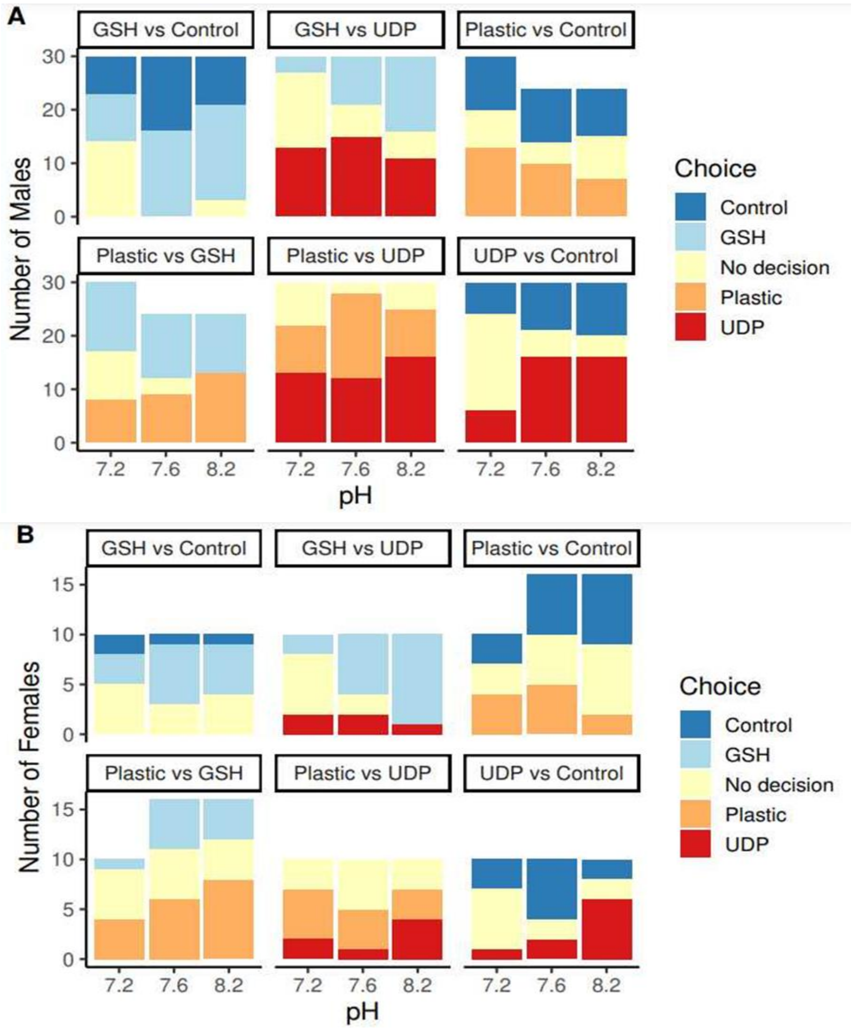


Figure 1. Figure displaying an overview of results shown from the bioassays completed in the Y shaped olfactometer flume. Whereby six experimental conditions were carried out in three pH treatments. This figure displays the resulting 'choice' individuals made under the different conditions and treatments. **A** refers to male individuals and **B** refers to female individuals.

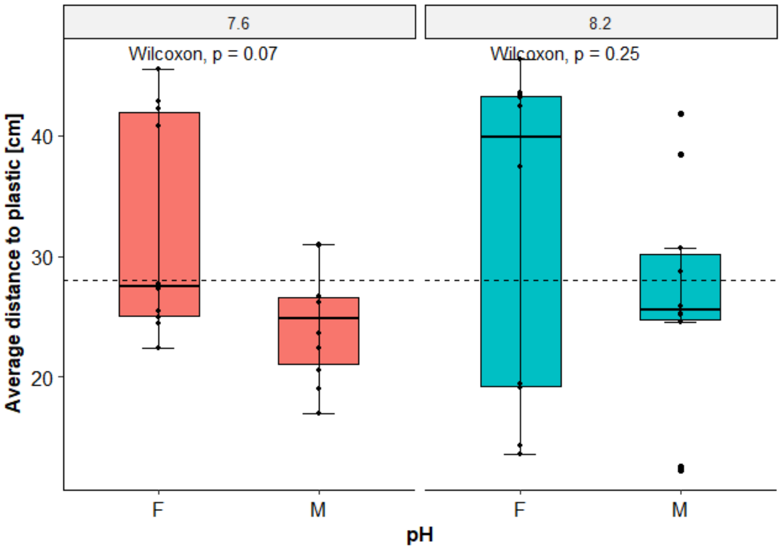


Figure 2. Boxplot showing average distance to plastic of male and female individuals in a static tank experiment. comparisons between distance in pH 7.6 and 8.2 (Shared data with Ohnstad, 2022)