Assessment of intra-individual consistency of physiology and behaviour in brachyuran crabs (*Carcinus maenas* and *Cancer irroratus*)

by

©Eugueni (Evgeni) Matveev

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Abstract

Physiology and behaviour are labile (i.e., flexible) traits with a wide intra-individual reaction scope, nonetheless individuals often exhibit consistent and predictable responses when repeatedly tested. Consistent individual-level differences in behaviour are studied as animal personality, and according to the pace-of-life syndrome hypothesis, personality differences co-vary with differences in metabolic rate. So far, much of the literature on this relationship is theoretical and based on a limited set of taxa. While experimental evidence is accumulating, the results are equivocal. Because labile traits are highly susceptible to environmental change, the lack of standardization in experimental procedures makes interpreting results from different studies complicated. My overarching thesis objective was to experimentally test the relationship between physiology and behaviour in two common species of bracyuran crab (*Cancer irroratus* and *Carcinus maenas*), and quantify the effects of common lab stressors on labile trait expression. In Chapter 2, I assess the presence of personalities and behavioural syndromes, and determine if inter-individual variation in metabolic rate can explain differences in personality. In Chapter 3, I quantify the effects of common lab stressors (prolonged lab holding, transfer into experimental chambers, and a homogenous diet) on the short- and long-term changes in crab labile traits.

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Co-authorship Statement

The work described in the present thesis was conducted by Evgeni Matveev with guidance from Dr. Iain McGaw. Evgeni Matveev was responsible for all laboratory and field data collection. Statistical modelling for chapter 3 was performed by Evgeni Matveev with assistance from Dr. Amanda Bates. All chapters were written by Evgeni Matveev with intellectual and editorial input by Dr. Iain McGaw and Dr. Amanda Bates. Any publication in the primary literature resulting from work in the present thesis and from complementary work not presented will be co-authored by Evgeni Matveev, Dr. Iain McGaw, and Dr. Amanda Bates.

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Chapter 1

General Introduction

In a heterogeneous and changing environment, a diversity of phenotypes is vital for species' adaptation and survival. Traditionally focusing on morphological traits, a phenotype is any outwardly evident and measurable characteristic of an animal, and is the product of an interaction between the genotype and the environment (Johannsen, 1911). Unlike morphological traits, which change over long periods, some physiological and behavioural traits change their expression within hours or seconds; they are thus considered "labile". Historically, inter-individual variation in labile traits was dismissed as noise surrounding the "golden mean" (Bennett, 1987), which was presumed to be the main biologically relevant measure. However, deviation from the mean may not be noise or a maladaptation, but a sign of fitness strategy diversification (Careau et al., 2008; Bergmüller and Taborsky, 2010).

Both physiology and behaviour, albeit labile, often show within-individual consistency when repeatedly measured. According to the pace-of-life syndrome hypothesis, physiological and behavioural responses co-vary along a fast-slow axis (Dammhahn et al, 2018; Mathot and Frankenhuis, 2018). The physiological parameters include everything from neuroendocrine responses to immunity, however here the focus will be on metabolic rate (MR). A fast pace-of-life is driven by a high MR, and results in early reproduction, fast growth, a short lifespan, and a proactive personality; the opposite is true for a slow pace-of-life. On an inter-species level this hypothesis underpins the idea of r- and K-selected species, with r-selected species having a fast lifestyle (short inter-generational time, short lifespan, many offspring) and K-selected species having a slow lifestyle (long lifespan, high age of maturity, few offspring: Capevilla and Salguero-Gomez, 2019).

The number of studies on behavioural and physiological individual-level consistency in invertebrates is increasing, but thus far lagging behind more conventional model organisms such as mammals and birds (Gherardi et al, 2012). Assessing the validity of the pace-of-life syndrome hypothesis in ectotherms is crucial in a time of expidited global change. In ectotherms, the MR and behaviour are highly influenced by environmental temperature, with a higher temperature driving higher MR which is predicted to be related to more active behaviours (Dillon et al., 2010; Halsey et al., 2015; Robertson and Taylor, 2002). If MR is indeed a broadscale driver of personality differences, then ecological processes such as invasive species translocation, greater seasonal temperature fluxes, or other human-induced rapid environmental change may influence animal behaviour and thus affect inter- and intra-species interactions. In this introductory chapter, I aim to provide a background on animal personality research, define terms and their broader relevance, outline some issues with measuring labile traits, and provide a rationale for using crabs as focal species.

Metabolic Rate Metrics

The metabolic rate (MR) represents the rate of energy consumption by animals (Careau et al, 2008). Most commonly, energy consumption is estimated by measuring oxygen depletion as a proxy for the oxidation of carbohydrates, fats, proteins, and other substrates by tissues during ATP production (Weir, 1948; Brown et al, 2004). The variation in MR across the animal kingdom can largely be explained by body size (Kleiber, 1932; Zeuthen, 1953); however, among individuals within a species, metabolic rates vary consistently and considerably even when body size is accounted for (Careau et al, 2008; Biro and Stamps, 2010). The specific reasons for this inter-individual variation in metabolic rate are not fully understood, but thought to be due to a mix of morphological (i.e., organ size), genetic, and environmental factors (Metcalfe et al, 2016). During periods of minimum energy consumption, when an animal is post-absorptive and resting, the energy required for maintaining homeostasis under a stable temperature is defined as the Resting Metabolic Rate (RMR). Because the RMR of an organism is the bare minimum energy expenditure, it is a non-negotiable portion of the energetic budget and drives trade-offs between life history traits such as feeding and reproduction (Biro and Stamps, 2010; Gunderley and Pörtner, 2010). In endotherms, RMR includes endogenous heat production and is termed Basal Metabolic Rate (BMR), and in ectotherms it includes only the energetic cost of homeostatic maintenance under a specific temperature and is termed Standard Metabolic Rate (SMR). On the other end of the spectrum, the highest metabolic rate that an animal can exhibit is the top boundary of substrate oxidation during aerobic metabolism and is termed Maximum Metabolic Rate (MMR). The range between the RMR and MMR is the metabolic scope, which encompasses the total energy budget available to an animal after baseline costs have been met. The metabolic scope is budgeted into activities necessary for species' survival, such as foraging and habitat defense, somatic growth and tissue maintenance, and reproduction (i.e., gonad/offspring development) (Sokolova et al, 2012; Treberg et al, 2016; Careau et al, 2008). In that sense, the MR has been called the “pacemaker” of an animal’s energy budget, and in part determines how much energy can be allocated to being active. Factors that increase an animal’s BMR or lower the MMR can therefore have significant consequences on the total energy available for processes that relate to survival (Gunderley and Pörtner, 2010). Suboptimal environmental conditions (e.g., pollutants, hypoxia, extreme temperatures, extreme salinities etc.) can require energy expenditure to support acclimatization and homeostatic maintenance (Sokolova et al., 2012). Understanding the effect of stress on the metabolic expenditure is therefore important for understanding the extent to which the metabolic budget is affected. This is especially important when considering lab associated stressors, because excessively stressed animals may not respond in ways representative of natural conditions (Biro, 2012; Biro, 2013).

Animal Personality and Ecology

The aims of animal personality research are to quantify consistent individual-level differences in behaviours, and determine their underpinning mechanisms and fitness consequences. As an emerging field, the terms and definitions vary by study and need to be explicitly laid out. For the purposes of the present thesis, I define personality as consistent individual differences in behavioural responses when repeatedly measured by standardized tests, and statistically assessed via correlation between trials (Roche et al., 2016; Careau and Garland, 2012). Careau and Garland (2012) restrict personality to five behavioural axes that are largely ubiquitous among animals and represent ecologically important traits: boldness, exploration, activity, aggression, and sociability (for definitions see Table 1 in Careau and Garland, 2012). These behaviours are predominately tested in personality research, and have well-developed standardized tests. Personalities range in behavioural type from proactive to reactive: animals exhibiting more proactive behavioural types exhibit more energy intensive behaviours (i.e., more active, bolder, more aggressive). When behaviours that are measured under different contexts (e.g., with and without predation) are correlated, they form a "behavioural syndrome" (Sih et al., 2004). Some studies use behavioural syndromes to define personality; however, this assumes the motivations of behaviours remain static under a change of context (Biro, 2013; Careau and Garland, 2012). Because of the communication barrier between humans and other animals, judgements like "bold" or "aggressive" are assumptions based on observation rather than objective fact. By defining personality as simply the consistency of a standardized behavioural trait, we allow for the possibility that behavioural expression can vary between contexts but still represent an individual's personality. Nonetheless, behavioural syndromes are important to quantify because they define the extent to which one behavioural output relates to another, and may hint towards behavioural motives or underlying physiological mechanisms of behaviour (Careau and Garland, 2012).

Multiple meta-analyses including species from drosophila to mammals, find that behaviour is approximately 25% heritable (Van Oers et al., 2005; Dingemanse et al., 2002; Ballew et al., 2017; Dochtermann et al., 2019), so behaviour is subject to natural selection and bears fitness consequences. Both reactive and proactive behavioural types have their costs and benefits. An increase in time being vigilant (for instance) has the benefit of decreased predation threat but comes at a cost of a decreased time foraging (Fortin et al, 2004). A proactive individual would be more likely to be bold and forage, whereas a reactive animal would spend more time being vigilant. Both et al. (2005) show that offspring of two fast-exploring (proactive) or two slow-exploring (reactive) great tits (*Parus major*) had the highest rate of survival success compared to mixed parents, hypothetically due to distinct advantages of both personalities. Similarly, Rangassamy et al. (2015) show that mound-building mice mating success was more likely if mating pair personalities were similar, implying there is a fitness advantage to maintaining behavioural diversity. Examples from fish aquaculture show that reactive fish are more susceptible to stress, eat less food, and are more prone to disease; however, they may also exhibit lower levels of conspecific aggression than proactive fish (Castanheira et al, 2015). Sustained consistent behavioural differences can even lead to the establishment of "keystone individuals", such as alpha males, which have a disproportionate impact compared to other conspecifics (Modlmeier et al, 2014; Pruitt and Keiser, 2014). Understanding the patterns of inter-individual behavioural variation can be important for assessing a population's resilience to environmental pressures (Sih et al., 2011) and ability to adapt to new habitats (Chapple et al, 2012; Juette et al, 2014).

Issues with labile trait studies

Behavioural and physiological treatment-response experiments can be difficult to conduct in-situ, so animals need to be either reared in lab or collected from the field. Collecting wild animals and conducting lab experiments induces acute and chronic stress responses. In wild animals, fast responses in labile traits are necessary to deal with natural acute stressors, such as predators. But stress-induced responses complicate conclusions drawn from lab experiments, and researchers should incorporate stress into the interpretation of results. The stress of capture, translocation, handling, and long-term holding can significantly affect the physiological state of animals (Thromborg and Morgan, 2007; Dickens et al., 2010). Stress notwithstanding, the stability of lab conditions compared to the fluctuating natural environment can also change the physiological response (Callaghan et al., 2016). If behaviour and physiology are intrinsically linked as hypothesized, physiological stress response is likely to change the outcomes of behavioural experiments. How to account for or mitigate lab-associated stressors (and conditions that do not represent natural environments) when assessing labile traits is a matter of some debate. Biro (2012) repeatedly measured boldness in the Ward's damselfish *Pomacentrus wardi*, and found that with repeat behavioural measures the rank order differences in traits were not maintained. The authors thus concluded that behaviours that were repeatable when initially measured were not repeatable in animals habituated to novel conditions. However, a behaviour in a novel/stressed environment is still biologically relevant but describes a different motive from that of a habituated animal (Edwards et al., 2013). Neither a high-stress nor a stress-free environment is representative of natural conditions. As Biro (2013) points out, measuring both highly stressed animals or fully habituated animals can result in a narrowing of inter-individual variation in responses, which can make the consistent inter-individual differences undetectable. Short of collecting exclusively field data, a step in the right direction would be to quantify how lab associated stressors affect animals to determine appropriate holding and settling times.

Crabs as a study species

Brachyuran decapods (true crabs) are good model organisms and have been extensitvely used to study the relationships between behaviour and physiology. They are relatively easy to maintain in a lab, display clear behavioural patterns, and are globally ubiquitous. They are also an ecologically important group that significantly affects their habitats (reviewed by Boudreau and Worm, 2012). For example, in mangroves, both sesarmid (Grapsidae) and fiddler (Ocypodidae) crabs dig burrows thereby churning up sediment layers, which alters the soil microbial communities (Kristensen, 2007). Because these two species have different burrow shapes, the effect they have on the sediment is different, underscoring the importance of understanding behaviour in ecology studies. Crab predation also has had an important role in shaping ecosystems. The European green crab *Carcinus maenas* (Fig. 1.1), is a top 100 invasive species (IUCN) that have spread globally over the last 200 years. In Newfoundland, Canada, green crabs may affect the catchability of lobster by discouraging them from approaching and entering the trap (Rayner and McGaw, 2019). On the west coast of North America *C. maenas* preyed on a native clam (*Gemma gemma*) allowing for an invasive clam (*Nutricola* spp.) to take over and cause an "invasional meltdown" (Grosholz, 2005). Conversely, the invasion of *C. maenas* in salt marshes replaced the herbivore predation niche left by overharvested blue crabs, and cordgrass quality in invaded areas improved (Bertness and Coverdale, 2013). Crabs are also a crucial commercial harvest species. In 2017, Canadian crabbing comprised 25% of the total shellfish landings (102,207 metric tonnes), and 12% of total fishery landings. In terms of monetary value, crabs amount to $1,027,199,000, or 32% of total shellfish value (28% of total fishing industry landing value) (Department of Fisheries and Oceans, Canada, 2018). Fishing is a selection pressure that not only affects population numbers and trophic cascades, but can affect behaviour (Pauli and Sih, 2016), disproportionately target more catchable individuals (Härkönen et al, 2016; Biro and Post, 2008), and target individuals with lower metabolic rates (Killen et al, 2015). In the Spanner crab (*Ranina ranina*), females responded faster to food presence than males, making them more vulnerable to trapping (Skinner and Hill, 1987). Additionally, a video analysis of Dungeness crabs around crab pots showed that some individuals guard the entrances to pots preventing others from entering (Barber and Cobb, 2009). To understand to what extent personality relates to ecological processes in crabs, we need to establish whether crabs have personalities, how personality complements compare between crab species, and whether there are physiological mechanisms that determine behavioural expression.

Thesis Objectives

The present thesis provides new insights into the presence of personalities, and the relationship between physiology and behaviour in brachyuran decapods. The main objectives are to: (Chapter 1) assess the presence of personalities and behavioural syndromes and their relationship to physiological traits in invasive (*Carcinus maenas*) and native (*Cancer irroratus*) crabs (Fig. 1.1), and (Chapter 2) establish the effect of holding time and dietary heterogeneity on crab physiology and behaviour.

I set out to test the following hypotheses and predictions:

Chapter 2:

H1. *High energy behaviours are driven by a higher metabolic rate*

I predict that crab behaviour and physiology will be consistent over time (Fig. 1.2A), and different behaviours will be correlated along the proactive-reactive axis forming behavioural syndromes (boldness will be positively correlated to activity level, Fig. 1.2B). In accordance with the pace-of-life syndrome hypothesis, I predict that proactive individuals will have higher metabolic rates than reactive individuals (Fig. 1.2C).

H2. *Crabs with a recent invasion history are selected to exhibit more proactive beahviours (and therefore have higher metabolic rates) compared to native crabs*

Invasive species may benefit from proactive behaviours compared to native counterparts (Chapple et al, 2012), and may therefore have a higher metabolic rate (Lagos et al., 2017). I therefore predict that *C. maenas* will be more active and bolder (Fig. 1.2D) and will have a higher MR than *C. irroratus* (Fig. 1.2E).

Chapter 3:

H1. *Chronic stress in a lab environment results in a gradual degradation in physiological and behavioural condition of crabs*

I predict that chronic storage under lab conditions will result in the decline of crab health over time (Fig. 1.3). This will manifest itself in deteriorated physiological state (i.e., increased resting MR (RMR), decreased MMR, decreased metabolic scope, decreased hemolymph density), and a more reactive behavioural response (decreased activity). These predictions are based similar results reported by Houlihan and Mathers (1985) after only 2-3 weeks of *C. maenas* captivity.

H2. *Providing crabs with a heterogenous rather than homogenous diet will improve crab wellbeing and result in physiological and behavioual responses related to good health*

Because crabs eat a varied diet in the wild (Griffen and Riley, 2015), I predict crabs who are given a more diverse diet will fare better than those given a single food source (Fig. 1.3). This will result in mono diet crabs having a deteriorated physiological state (i.e., higher RMR, lower MMR, lower metabolic scope, decreased hemolymph density), and a more reactive behavioural response (decreased activity, decreased strength).

Figures

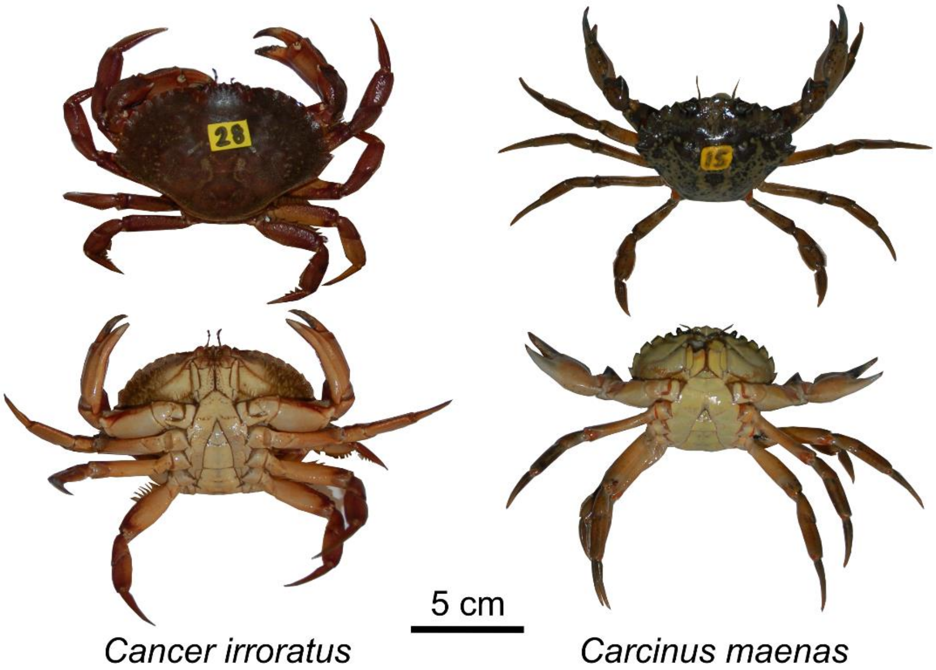

Figure 1.1. Dorsal (top) and ventral (bottom) views of male *C. irroratus* (left) and *C. maenas* (right). *C. irroratus* have a wider and dorsoventrally flatter carapace than *C. maenas*. The dorsal carapace of *C. irroratus* is consistently red, while that of *C. maenas* changes from green to red over the moult cycle (only *C. maenas* with a green carapace were used).

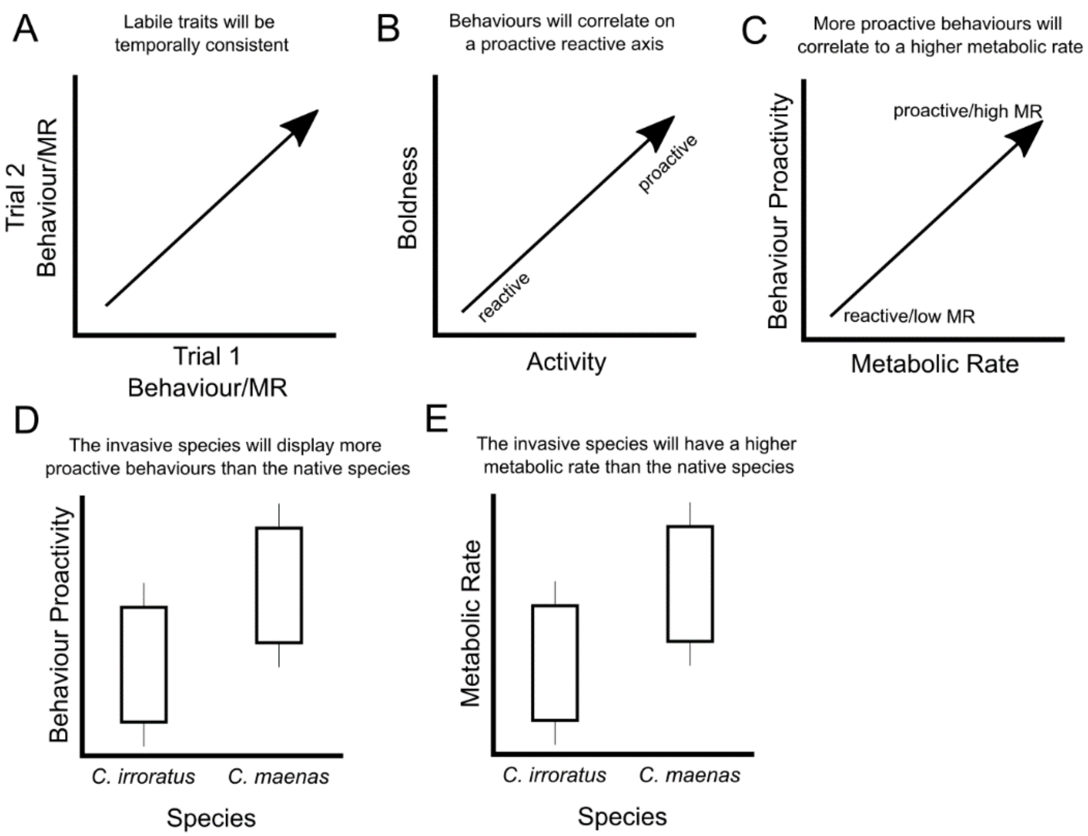

Figure 1.2. Predictions to Chapter 2 hypotheses H1 and H2. I predict that **A.** Labile traits (behaviour and metabolic rate) will be intra-individually consistent when repeatedly measured. **B.** I predict that different behaviours will be correlated on the proactive-reactive axis, as is found in other studies. **C.** I predict that a higher metabolic rate will be related to more proactive personality types. **D.** I predict that the invasive *C. maenas*, will display more proactive personality as predicted for early stage invasive species, and accordingly have a higher metabolic rate **(E)**.

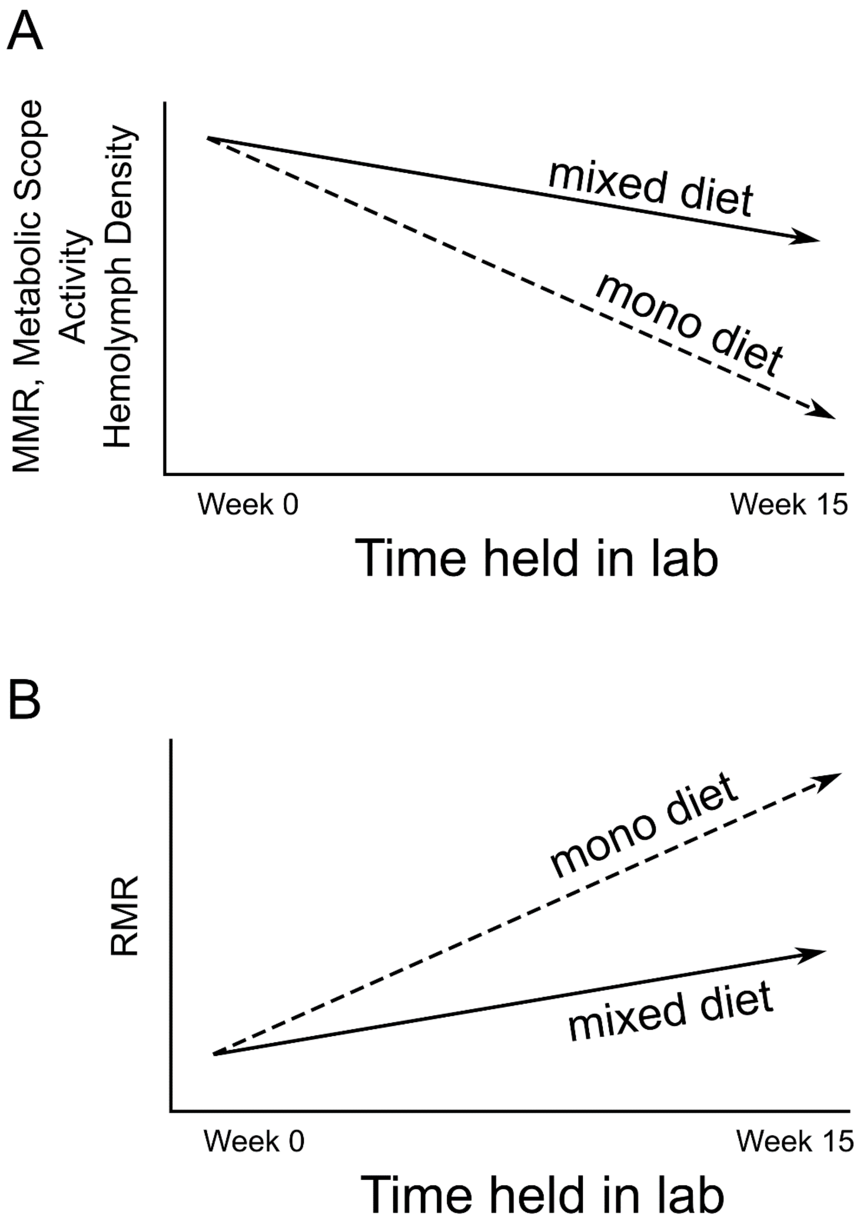

Figure 1.3. Predictions to Chapter 3 hypotheses H1 and H2. As the condition of crabs held in the lab declines, most labile traits **(A)** except for RMR **(B)** are expected to decline over time. Mono diet crabs are expected to fare worse than mixed diet crabs, because dietary heterogeneity is expected to improve crab health.

Chapter 2

Assessment of personality, behavioural syndromes, and the pace-of-life syndrome hypothesis in two crab species (*Carcinus maenas* and *Cancer irroratus*)

Abstract

Animal personality, defined as repeatable individual-level differences in behaviour, is an emerging topic with applications to research from genetics to ecology. One underpinning physiological driver of personality according to the pace-of-life syndrome hypothesis is metabolic rate, with a higher base metabolic rate relating to more ‘proactive’ (i.e., energy intensive) personalities. I tested the hypothesis that metabolic rate is a proximal driver of personality in two co-habiting and functionally similar crab species: the invasive green crab, *Carcinus maenas*, and the native rock crab, *Cancer irroratus*. I carried out repeated measures of four behavioural components (boldness, exploration, activity, and chelal compression), and measured resting and maximum metabolic rates. All behavioural metrics except chelal compression were repeatable, suggesting evidence for personality in both species. However, most behaviours were not correlated to each other (no evidence for behavioural syndromes), except for a negative correlation between boldness and chelal compression strength in *C. maenas*. Metabolic rate measures were repeatable (except RMR in *C. maenas*), however neither were related to any of the behaviours. Additionally, there were significant interspecific differences in all behaviours, with *C. irroratus* displaying more proactive behaviours. There were no interspecific differences in metabolic rate, so I found no evidence for metabolic rate underpinning behaviours at an intra- or inter-species level. This chapter provides experimental test of the pace-of-life syndrome hypothesis and underscores that inter-species differences in behaviour of functionally similar crabs can arise without differences in metabolic rate.

Introduction

Traits that are considered highly labile (i.e., flexible and variable)—such as behaviour and physiology— are increasingly found to be consistent within individuals (reviewed by Holtmann et al., 2016). The value of quantifying these consistent individual-level differences, rather than focusing solely on the population mean, is increasingly recognized as important for detecting intraspecific fitness strategy variability (Bennett, 1987; Roche et al., 2016). In the field of behavioural biology, consistent individual differences are studied as ‘animal personality’ (Koolhaas, 1999; Sih et al., 2004; Bergmüller, 2010; Dingemanse and Reale, 2012). While definitions vary, “personality” is often defined as repeatable differences between individuals when behaviours are measured by standardized tests (‘narrow-sense personality’ *sensu* Roche et al., 2016; Careau and Garland, 2012). Overall, ~35% of the variation in behaviours can be attributed to individual-level differences; however, some behaviours, such as exploration, are more repeatable than others, such as spontaneous activity (meta-analysis by Bell et al., 2009). Additionally, ~25% of the variation in behaviour can be explained by parental lineage suggesting personality is heritable (Van Oers et al., 2005; Dingemanse et al., 2002; Ballew et al., 2017; Dochtermann et al., 2019). This means natural selection can act on the diversity of personality types in a population, similarly to other phenotypes.

Different behaviours may co-vary within an individual, resulting in population level correlations between distinct behaviours. One of the first reports of co-related traits was in the stickleback *Gasterosteus aculeatus*, where aggression to conspecifics and boldness under predation were positively correlated (Huntingford, 1976). Over the past 20 years, similar correlations have been reported and conceptualized under the framework of ‘behavioural syndromes’, broadly defined as “suites of behaviours that co-vary across contexts or situations” (Roche et al., 2016; Sih et al., 2004). Within behavioural syndromes, individuals’ behavioural types range on the reactive/proactive axis; proactive animals express more energy intensive behaviours and are bolder, more exploratory, and more active than reactive animals (Koolhaas et al., 2007; Bergmüller, 2010). Evidence for behavioural syndromes has been reported in many vertebrate (fish: Wilson and Godin, 2009, amphibians: González-Bernal and Shine, 2014; reptiles: Michelangeli et al., 2016; birds: Bókony et al. 2012, and mammals: Newar and Careau, 2018), and to a lesser degree invertebrate taxa (insect: Segev et al. 2017, crustaceans: Mowles et al., 2012).

Rather than converging on one “optimal” personality, a diversity of personality types is often maintained within populations. This is thought to be advantageous by decreasing intra-specific conflict through behavioural niche segregation (Bergmüller and Taborsky, 2010), but also acts as a buffer to environmental change. Ecological processes (e.g., range expansion of invasive species) can act as a selective force on the diversity of personality types within a population to favor some personality types over others. In a classic example, western bluebirds (*Sialia mexicana*) that expanded their range into a new territory were more aggressive than both the long-established mountain bluebird (*Sialia currucoides*), and conspecifics in more established ranges (Duckworth and Badyaev, 2007); the range expansion process therefore favoured a more proactive personality type to dominate. Similar results were reported in the delicate skink (*Lampropholis delicata*), where dispersal tendency was greater in more aggressive individuals (Michelangeli et al., 2017). In the invasive mosquitofish (*Gambusia affinis*), both dispersal capacity and predation survivorship could be predicted by sociality (i.e., schooling behaviour), where the more social fish were less likely to disperse and more likely to be consumed (Brodin et al., 2019; Cote et al., 2011). The relationship between dispersal propensity and personality lead to the hypothesis that personality can play an important role in the invasion success of a species (Chapple et al., 2012).

Because personality is to some degree heritable and is documented across animal phyla, there is likely an underpinning physiological mechanism. One such mechanism could be the metabolic rate (MR). First, MR is one of the most repeatable physiological traits and therefore a likely contender for driving repeatable differences in behaviour (meta-analysis by Holtmann et al. 2016). Second, MR determines the rate of energy expenditure of an animal, with the base MR—standard MR (SMR) for ectotherms— defining the idling cost of keeping a post-absorptive resting animal alive. By determining the rate of stored energy use, MR is thought to affect behaviour: a higher MR increases the costs of idle behaviours (e.g., hiding in shelter), and necessitates proactive energy acquisition behaviours (e.g., foraging, exploring) (Biro and Stamps, 2008; Réale et al., 2010; Careau et al., 2008). The relationship between MR and personality is encompassed within the pace-of-life syndrome hypothesis, which posits that physiological and behavioural responses are intrinsically linked and co-vary with life-history traits along a fast-slow axis (Dammhahn et al., 2018; Mathot and Frankenhuis, 2018; Ricklefs and Wikelski, 2002). Animals with a fast pace-of-life have a higher MR, a proactive personality, and are hypothesized to reproduce earlier, grow faster, and have a short lifespan (Biro and

Stamps, 2008; Réale et al., 2010). In line with invasive species having more proactive personalities, some research suggests that they also have a fast pace-of-life (Lagos et al., 2016).

Experimental work on the relationship between MR and personality is limited (since Ricklefs and Wikelski, 2002), but some support for a link between MR and personality has been reported. In the ant *Temnothorax longispinosus*, more proactive behavioural types (higher foraging and exploration) were expressed during warmer years, presumably because warmer temperatures lead to higher MR in ectotherms (Segev et al., 2017). Several studies in fish also show evidence for higher MR being associated with more proactive behaviours. In the round goby (*Neogobius melanostomus*) higher resting MR is related to higher boldness (Myles-Gonzales et al., 2015; Behrens et al., 2020). Likewise, in the bluegill sunfish (*Lepomis macrochirus*) higher maximum MR is positively related to boldness (Binder et al., 2016); and dominant Ambon damselfish (*Pomacentrus amboinensis*) have higher metabolic scopes than submissive ones (Killen et al., 2014). However experimental outcomes vary. Careau et al. (2019), for instance, found that bolder fall field crickets *Gryllus pennsylvanicus*, had a lower SMR. In fact, two recent meta-analyses report that only 5-6% of variation in behaviour can be explained by physiology (Royauté et al., 2018; Niemelä and Dingemanse, 2018). This varies by organism, with considerably more support (26% of variation) in invertebrate species (Royauté et al., 2018). The pace-of-life syndrome hypothesis needs further testing, but physiology may be an important consideration when assessing the repeatability of behaviour (see also Mitchell et al., 2020).

In this chapter, I assess personalities, behavioural syndromes, and the pace-of-life syndrome hypothesis in two functionally similar invertebrates with different invasion histories: the invasive green shore crab (*Carcinus maenas,* Linnaeus, 1758), and the native Atlantic rock crab (*Cancer irroratus,* Say, 1817). *C. irroratus* are native to Atlantic coasts from Canada to South America. *C. maenas* are native to Europe and North Africa, but have invaded North American coasts over the last two centuries. The present study was carried out in Newfoundland, Canada, where *C. maenas* were first reported in 2007 (Klassen and Locke, 2007). Generally, the global invasion success of *C. maenas* is attributed to broad physiological tolerances and prey preferences (Darbyson, 2009; Young and Elliott, 2020) which allow them to survive in ship ballasts and readily establish in novel environments. *C. maenas* invasions can have many adverse effects on local ecosystems. In Atlantic Canada, foraging by *C. maenas* is responsible for uprooting eel grass (*Zostera marina*), which serves as a crucial nursery habitat for commercial species, such as Atlantic cod (*Gadus morhua*) (Matheson et al. 2016; Garbary et al., 2014). In high densities, *C. maenas* can prevent catchability of lobster (*Homarus americanus*) by detracting them from entering traps (Rayner and McGaw, 2019; Zargarpour et al., 2020). Additionally, they can feed on commercially important species (e.g., bivalves: Matheson and McKenzie, 2014; young lobster: Rossong et al., 2006), and can outcompete or displace native species (MacDonald et al., 2007).

C. maenas has invaded many of the habitats occupied by *C. irroratus*, and so the competition between the two species and their ability to co-exist has been investigated in various contexts. Generally, studies find that these two species are functionally similar.

Large *C. maenas* and *C. irroratus* capture similar prey numbers (Matheson and Gagnon, 2012a), with similar consumption rates (Breen and Metaxas, 2008; Miron et al., 2005), and diet preferences (Sungail et al., 2013). Additionally, both crab species can live in shallow coastal waters (3-6 m deep; Klassen and Locke, 2007), and their chelae exert similar crushing forces (Matheson and Gagnon, 2012a; Matheson and McKenzie, 2014). Although the maximum size of *C. irroratus* (carapace width<13 cm, Rebach and Block, 1998; Haefner, 1976) is larger than *C. maenas* (carapace width <10 cm, McGaw et al., 2011; Klassen and Locke, 2007), there is considerable overlap, and an interaction between similar sized crabs is likely. An analysis of behavioural budgets did not find that *C. maenas* presence affected the behaviour of *C. irroratus*, therefore these species may be able to coexist in the same environment (Bélair and Miron, 2009). In fact, some evidence suggests that the presence of *C. maenas* may increase the growth rate of *C. irroratus*, due to the latter consuming the former (Breen and Metaxas, 2009); although, others claim that *C. maenas* has an overall negative effect by consuming young *C. irroratus* (Griffen and Riley, 2015). Overall, it seems that despite *C. maenas* encroaching on the habitat of *C. irroratus*, these species are similar competitors sharing the same niche space.

By assessing personalities and their relationship to MR in these functionally similar species with different invasion histories, several ideas can be addressed. The first is whether these ubiquitous and widely used species have personalities that can be detected with common behavioural tests, and if different behaviours form behavioural syndromes. The second is the hypothesis that higher MR have more proactive personalities to compensate for high idling cost, as predicted by the pace-of-life syndrome hypothesis. Although using two species is not sufficient to make inter-species correlations between physiology and behaviour (as was done with multiple species of muroid rodents by Careau et al., 2009), it could help determine if consistent differences in behaviour and/or physiology can arise independent of eachother. Because *C. maenas* has life history traits generally more associated with a fast pace-of-life (Table 2.1), they are predicted to have a higher MR, and more proactive behaviours. Additionally, because invasive species are hypothesized to have more proactive personalities during the post-introduction stage (Chapple et al., 2012), I compared personalities of *C. maenas* and *C. irroratus* to determine if any potential differences might facilitate the spread and success of *C. maenas*.

My first aim was to test for intra-individual consistency of labile traits in *C. maenas* and *C. irroratus*, by repeatedly measuring four behavioural metrics (spontaneous activity, exploration, boldness, and chelal compression strength) and two metabolic rate measures (resting metabolic rate and maximum metabolic rate). Boldness, exploration, and activity were chosen as behaviours because they are repeatable and have well developed standardized tests (Roche et al., 2016; Careau and Garland, 2012). Chelal strength was initially measured to compensate for competitive ability (Block and Rebach, 1998; Sneddon et al., 2000), but was considered a behaviour because it depended primarily on the willingness of the crab to pinch down (see Discussion). Hemolymph protein density was also measured because it is a common measure of crustacean physiological condition and predictive of food intake, moult stage, and stored energy (Chapter 2; Wang and McGaw, 2014). Finally, the amount that crabs ate was measured to determine if energy input is higher for animals with higher metabolic rates, and/or those with more proactive personality types (as predicted by pace-of-life syndrome hypothesis).

Materials and Methods

Animal Collection and Storage

Intermoult male Atlantic rock crabs (*Cancer irroratus*) were collected using baited traps during October 2018 in Holyrood, Newfoundland, Canada (47°23'18.6"N, 53°07'36.7"W). Male European green crabs (*Carcinus maenas*) were collected in October 2018 in Fox Harbour, Newfoundland, Canada (47°19'06.9"N, 53°54'40.4"W) where they have been detected since 2007 (Klassen and Locke, 2007). Only *C. maenas* with a green ventral carapace colour were used, to reduce any potential influence of moult stage (McGaw et al., 1992; Reid et al., 1997). For both species only male crabs between 60-110 g were used in experiments to reduce any potential influence of sex and size, and because official regulations by the Department of Fisheries and Oceans (Canada) prevent use of female *C. maenas*. Although the carapace width of *C. maenas* was smaller (67-79 mm) than that of *C. irroratus* (72-97 mm), the crab mass frequency distributions were equal because *C. irroratus* is more dorsoventrally flattened than *C. maenas*. Crabs were transported in a cooler to the Department of Ocean Sciences, Memorial University, where they were stored individually in 4 x 15 x 22 cm perforated plastic containers (1 crab per container, to reduce effect of inter/intra-specific agonistic interactions). The containers were kept in a flow-through seawater tank that was maintained at 11±1 ℃ at a salinity of 31-32 ppt with constant air-stone oxygenation, and under constant dim light. The crabs were acclimated to these conditions for three weeks prior to the start of experiments. Crabs were fed a mixed diet of fish and mussel twice weekly (see details below), but were fasted for 3 days prior to being used in experiments to ensure they were in a post-absorptive state (McGaw, 2007). Behavioural and physiological parameters were first measured after a 3-week acclimation period, and repeat measures were carried out on the same individuals 10 days later (which was an estimated trade-off between temporal autocorrelation and maintaining the crabs in good health while in captivity). Hemolymph density, chelal strength, and weight were measured once immediately after collection, and again after the second behavioural and metabolic rate trials finished. These latter measures were separated from others so that the crabs had time to recover from the stress of excessive handling and withdrawal of a hemolymph sample.

Feeding

Food intake was measured so it could be included as a factor in the statistical models to determine any potential influence of energy intake on behavioural or physiological measures. Crabs were fed a mixed diet of chopped herring (*Clupea harengus*) and shucked mussels (*Mytilus edulis*). Feeding took place once every 5 days, and crabs were fed in individual mesh-lined containers placed inside the storage tank. Prior to feeding, the food was soaked in seawater overnight so that osmotic absorption during feeding had a minimized effect on food weight change. Food was patted dry with a paper towel, weighed before feeding, introduced into the chamber, and the crabs were given 2 h to eat. After the feeding period, any uneaten food was picked out with forceps, patted dry and re-weighed to the nearest 0.01 g. As a control, food was weighed before and after a 2 h period in feeding chambers without crabs. A linear regression was run on the pre- and post-soak control weights, and the line equation was used to correct for erroneous changes in mass not due to feeding. During the feeding period water from the tank was pumped into feeding chambers so there was sufficient oxygenation and water replacement.

Crab Mass

Crab mass was measured and was included in the models to determine any potential influence of crab size on measured traits. Crabs were removed from the tanks and allowed 5-7 min to empty the branchial chambers, they were then dabbed dry with a cloth towel and weighed to the nearest 0.1 g using a digital scale (Sartorius LP 5200P). Crabs were weighed before they had consumed food, and any limb loss was accounted for. Mass was measured once during the acclimation period, and again after the experiments had been completed.

Hemolymph Protein Density

Hemolymph protein density was used as an indicator of general physiological condition and prior food intake (Wang and McGaw, 2014). Hemolymph was collected by inserting an 18-gauge needle through the arthrodial membrane of a walking leg.

Approximately 0.3 mL of hemolymph was collected and placed into the sample well of the Brix/RI-Check Digital Pocket Refractometer (Reichert Analytical Instruments, Depew, NY). The refractometer was calibrated with deionized water prior to each hemolymph measurement. The Refraction Index (RI) was recorded for each crab and converted to hemolymph protein density (in dg/L) using the equation $HD=510(RI_{water}-RI_{hemolymph})-1.81$ (Sunderman, 1944; Wang and McGaw, 2014).

Chelal Strength Index

Chelal strength was measured because it is a good predictor of physical competitive ability (Sneddon et al., 2000). A digital hanging scale (Brecknell Electrosampson) was mounted to a clamp stand and fastened with zip ties. A block of wood with an immovable latch was clamped to the base of the stand, such that the latch was 0.5 cm away from the hook on the digital scale (Fig. 2.1). A crab claw was placed with the bottom (fixed finger) on the immovable latch, and the top (dactyl) into the hook on the digital scale (Fig. 2.1 C). The crab was agitated by handling to make it pinch down, thereby pulling on the scale. The three strongest pinches for each claw were recorded to the nearest 0.1 kg, and the maximum pinch strength was used as the measure of strength of each crab. Because a small number (8%) of crabs exceeded the maximum capacity of the scale (10 kg), their maximum strength were recorded as 10.1 kg and thus represents a measurement threshold, but still indicates relatively strong individuals within the population. Chelal compression strength is a composite of the claw size (Block and Rebach, 1998; Sneddon et al., 2000), and the willingness of the crab to pinch down. To compensate for the claw size, compression strength was divided by claw height to calculate a chelal strength index (kg/cm). By compensating for claw size, the chelal strength index was more of a behavioural measure representing the willingness of the crab to pinch, and thus might be considered as a score of aggressive propensity.

Boldness/Exploration Trial

Boldness and exploration were chosen as they are frequently measured traits for behavioural syndrome assessment, especially for invasive species (Monceau et al., 2015; Cote et al., 2011; Damas-Moreira et al., 2019; Michelangeli et al., 2020), and were two behaviours previously measured in *C. maenas* (Fürtbauer, 2015). Two plastic coolers (55 x 25 cm (length x width), 30 cm (deep)) were placed side by side in a flow-through seawater tank with constant air-stone oxygenation. Water from the tank was circulated into the coolers at a rate of 10 L/min using a water pump. A shelter was made by attaching two pieces of corrugated plastic at a 30° angle to each other, with a 10x25 cm opening at the side opposite to water inflow (Fig. 2.2A). The shelter was large enough to allow a crab to fully retreat underneath it. Each shelter took up approximately 1/3 of the cooler. The shelter was positioned in opposite directions in each cooler to ensure that lighting was not a factor in shelter use. An IDS uEye camera was secured directly above the coolers and connected to a computer running IDS uEye Cockpit software. The entire apparatus was illuminated with dim red light because decapods appear to be unable to detect this wavelength (Bruno et al, 1973; Cronin and Forward, 1988).

After metabolic rate measurement (see below) the crabs were taken out of the respiration chambers, and placed into the side of the cooler opposite to the shelter where their behaviour was filmed for 1 h. There was no settling period in the apparatus for these behavioural measures because I was testing the responses of the crabs to a novel/stressful environment. Additionally, an immediate transfer from the metabolic chambers negated any considerable changes in animal physiological state, enabling a more direct comparison between the physiological and behavioural measures (Mitchell et al., 2020). The crab position in relation to the shelter (completely out, partway out, and completely in) was logged using the BORIS event-logging software V7.5.2 (Friard and Gamba, 2016) (Fig. 2.2B). A boldness score was assigned to each crab based on the percent of time the crab spent outside or partly outside the shelter, similar to the methodology used in Su et al. (2019). A bolder crab was one that spent more time outside the shelter. Additionally, the number of times a crab went in and out of shelter was used as a measure of "exploration". This was deemed a reasonable measure of exploration as preliminary observations showed that there was a difference between crabs using shelter for hiding and exploring the shelter as a novel environment for brief moments (~5-30 seconds). For a visual distinction between exploration and boldness behaviours see Fig. A.1.

Spontaneous Activity

Spontaneous activity is a trait commonly measured for behavioural syndrome assessment, often in the context of dispersal ability (Michalangeli et al., 2017; Geffroy et al., 2015). Additionally, in crabs, activity can be related to boldness (Brodin and Drotz, 2014; Su et al., 2019), which was also measured. Within 5 min of completion of the boldness/exploration trials, crabs were transferred into the activity chambers. Because the goal was to relate different behavioural traits, this minimal transfer time allowed for a change in experiment, without a considerable change in physiological condition, which could affect behaviour (Mitchell, 2020).

Four 30-cm diameter perforated buckets were placed into a flow through water table with 15-cm of oxygenated sea water (11±1℃). The water table was surrounded by dark curtains to shield crabs from visual disturbance, and recording was carried out under constant dim lighting to decrease potential diurnal rhythms (Rebach, 1985) while allowing a camera to see the crabs. A digital video camera (Panasonic WV-BP334) was placed above the water table, centered between all four buckets ensuring they were fully within the field of view. The camera was connected to a computer running Noldus Ethovision software (Noldus et al, 2001). The buckets were outlined as circular arenas and a pixel-to-centimeter calibration was done each day using an object of known length. Four crabs were recorded at one time, with one individual crab in each bucket (i.e., chamber). The buckets were opaque, so crabs could not see eachother. Recording began immediately after the crab was placed in the chamber, so I could record maximal (stressed) activity and the time to settle in the novel environment. The crabs were tracked by thresholding the dark crab body against the background of the white bucket and tracking the center point at 1-second intervals. The velocity and distance moved per second was recorded as a time series and the total distance moved in 20 h was used as the measure of crab activity. Total distance was log transformed for statistical analyses to account for non-normally distributed data, as some crabs moved considerably further than others.

Metabolic Rate

Mass-specific rates of oxygen uptake (mg O_2 kg^{-1} h^{-1}) were measured using an L-DAQ intermittent flow respirometry system (Loligo systems, Viborg, Denmark). This fully automated system is equipped with two pumps. The first pump continually flushes seawater through a cylindrical chamber (20 cm diameter x 12 cm depth) to ensure adequate water saturation during the non-measurement phase. For measurements, the flush pump is automatically turned off, the chamber is sealed and a second pump re-circulates the water through the chamber at a rate of 10 L/min ensuring that oxygen gradients do not build up within the chamber. The experiments were carried out in constant dim light, which helps reduce possible diurnal rhythms (Rebach, 1985) and the apparatus was surrounded by black plastic sheeting to avoid visual disturbance to the animal. Oxygen concentration was recorded on a Loligo data acquisition system which calculated MO_2 as mg O_2 kg^{-1} h^{-1}. Oxygen uptake (MO_2) was calculated during a 20 min decline in oxygen levels while the chamber was sealed and the flush pumps were off. The system had four chambers, so four crabs could be tested at one time. Oxygen uptake was recorded for 20 h, and erroneous oxygen uptake values (i.e., very low values caused by crabs sitting against outflow valve during recirculation) were manually removed from the record and an average was taken between the adjacent points. The highest recording (obtained consistently during the first hour) was used as the MMR. The resting metabolic rate (RMR) was calculated as the lowest 10% of records after 10 h post-transfer to respirometry chambers (Chabot et al., 2016).

Statistical Analysis

To determine trait consistency between trials, a linear regression was run between trial 1 and trial 2 for each repeatedly measured trait (boldness, exploration, activity, chelal strength index, RMR, MMR) for each species separately (*C. maenas* and *C. irroratus*). Intraclass Correlation (ICC) was not used because rank-order consistency in traits was deemed a more relevant measure (Biro and Stamps, 2015).

To determine whether the traits varied significantly between the trials or species, a linear mixed effects (LME) model was run using each trait metric as the dependent variable and trial (β_{Trial}, 1 or 2) and crab species (β_{Species}, *C. irroratus* or *C. maenas*) as the independent variable. Crab mass ($\beta_{\text{Crab Mass}}$, grams) and amount crabs ate ($\beta_{\text{Amount Eaten}}$, grams) were included as covariates in each model to ensure that behavioural and physiological measures were not due to size differences or hunger levels. Crab ID (numbered name) was included as a random factor to account for intra-individual variation of repeat measures. The LME model variance was partitioned according to this equation:

$$\text{Trait}=\beta_{\text{Species}}+\beta_{\text{Trial}}+\beta_{\text{Crab Mass}}+\beta_{\text{Amount Eaten}}+(1|\text{ Crab ID})+\varepsilon$$

To assess whether the models had different variance structures between the two species, Akaike Index Criterion (AIC) values were compared between a model with a heterogenous species-level among-individual variance weighting component and one without. In cases where including variance structure considerably improved the model fit (ΔAIC>4; Burnham et al. 2011) it was said that variance differed between species.

To assess behavioural syndromes, pairs of linear regression models were run between boldness, exploration, spontaneous activity, and strength index measures. The relationship between boldness and exploration was not assessed because these traits were measured in the same trial (however, a visual inspection of an exploration vs. boldness plot showed no linear relationship). To assess the evidence for the pace of life syndrome hypothesis (i.e., physiological underpinning of behavioural differences), linear models were run between mean metabolic rate parameters (RMR and MMR) and mean behavioural parameters (spontaneous activity, boldness, exploration, and strength index). All models were visually inspected for assumptions of residual normality and homogeneity with qq-norm plots and residual-fit plots, respectively. Minor deviations from normality where qq-norm plots did not follow a perfectly linear relationship were considered acceptable. All statistical analysis was carried out in R-Studio (RStudio Team, 2016), and packages used are outlined in Table 2.2. Final plots were edited in Inkscape v1.0 (Inkscape Project, 2020).

Results

Intra-individual labile trait consistency and inter-species trait comparison

Boldness and exploration experiment

Boldness, measured as percent of time crabs spent outside of shelter, was an intra-individually consistent trait for both *C. irroratus* (Regression, *r*=0.81, $F_{1,16}$=30.18, p<0.001) and *C. maenas* (Regression, *r*=0.65, $F_{1,17}$=12.26, p=0.002) (Fig. 2.3A). Boldness means did not vary between trials (LME, $beta_{Trial2}$= 0.04±0.03 SE, t=-1.44, p=0.16). *C. maenas* were considerably less bold than *C. irroratus* (LME, $beta_{C.maenas}$=-0.19 ±0.09 SE, t=-2.23, p=0.03) (Fig. 2.3B, C). Neither crab mass (LME, beta=-9e-10 ±0.002 SE, t=-0.38, p=0.70) nor amount eaten (LME, beta= -0.007 ±0.03 SE, t=-0.24, p=0.81) had a significant effect on boldness. Including species-specific variance resulted in a model with a considerably better fit than one that did not include variance (ΔAIC = 8.23), therefore variance was said to differ between species. Based on variance parameter estimates, in *C. irroratus* boldness was about 0.45 times as variable as that of *C. maenas*.

Exploration, measured as the number of times crabs entered shelter during the boldness trials, was an intra-individually consistent trait for both *C. irroratus* (Regression, *r*=0.75, $F_{1,16}$=25.14, p<0.001) and *C. maenas* (Regresion, *r*=0.74, $F_{1,17}$=20.27, p<0.001) (Fig. 2.3D). Exploration means did not vary between trials (LME, beta= -0.84±2.01 SE, t=-0.42, p=0.67). *C. maenas* were less exploratory than *C. irroratus* (LME, beta= -9.13±2.73 SE, t=-3.33, p=0.002) (Fig. 2.3E, F). Neither amount of food eaten (LME, beta= -1.13±1.14 SE, t=-0.98, p=0.33) nor crab mass (LME, beta= -0.12±0.07 SE, t=-1.76, p=0.08) had a significant effect on exploration. Including species-specific variance resulted in a model with a marginally better fit than one that did not include variance (ΔAIC = 4.35), therefore variance was said to differ slightly between species: according to the variance weighting parameter, the exploration score in *C. irroratus* was about 1.54 times as variable as that of *C. maenas*.

Spontaneous Activity

Spontaneous activity measured as the total distance crabs travelled in 20 h was not an intra-individually consistent trait in either *C. irroratus* (Regression, r=-0.28, $F_{1,14}$=2.31, p=0.15) or *C. maenas* (Regression, r=-0.06, $F_{1,16}$=0, p=0.95). The mean activity did not vary between trials (LME, $\text{beta}_{\text{Trial2}}$= 0.15±0.14 SE, t=1.05, p=0.29). *C. maenas* crabs were less active than *C. irroratus* (LME, $\text{beta}_{C.maenas}$= ± SE, t=-5.25, p<0.001). Neither the amount eaten (LME, beta= -0.01±0.09 SE, t=-0.13, p=0.89) nor crab mass (LME, beta= 0.009±0.007 SE, t=1.35, p=0.18) had a significant effect on activity. Including species-specific variance did not result in a model with a better fit than one that did not include variance (ΔAIC = -1.64), therefore variance was said not to differ between species.

When the record was subset to include only the first hour post-transfer into activity chambers (termed "maximal activity", and encompassing a post-transfer acute stress response) the distance moved was repeatable for both *C. irroratus* (Regression, r=0.45, $F_{1,15}$=5.75, p=0.03) and *C. maenas* (Regression, r=0.48, $F_{1,15}$=6.93, p=0.01) (Fig. 2.4A). Maximal activity was therefore more consistent than overall activity. During the first hour *C. maenas* were less active than *C. irroratus* (LME, $\text{beta}_{C.maenas}$ =-1.14±0.24 SE, t=-5.7, p<0.001), and neither trial (LME, $\text{beta}_{\text{Trial2}}$=0.1±0.11 SE, t=0.87, p=0.38), amount eaten (LME, beta=0.02±0.09SE, t=0.25, p=0.8) or crab mass (LME, beta=0.003±0.006 SE, t=0.51, p=0.61), had a significant effect on activity (Fig 4B, C). Because activity within the first hour was more comparable to the 1 h boldness/exploration trials, I used this measure for behavioural syndrome analysis.

I also subset activity rates of settled crabs (10 h post transfer into chambers). The distance moved was not repeatable for either *C. irroratus* (Regression, *r*=0.38, $F_{1,14}$=2.33, p=0.15) or *C. maenas* (Regression, *r*=0.3, $F_{1,15}$=1.58, p=0.22), so settled activity was not a repeatable trait. Settled activity in *C. maenas* was significantly lower than that of *C. irroratus*, and trial (LME, beta= -0.19±0.04 SE, t=-4.63, p<0.001), and amount eaten (LME, beta= -0.003±0.017 SE, t=-0.22, p=0.82) did not have significant effects. Crab mass had a very marginal effect on settled activity (LME, beta=0.002±0.001 SE, t=2.12, p=0.04), with larger crabs being slightly more active. The settled activity was more comparable to the RMR measures, therefore it was used to determine if there was a relationship between RMR and activity.

Chelal Strength Index

Chelal strength index, measured as the maximum compression strength of a claw divided by the claw height (kg/cm), was not an intra-individually consistent trait for either *C. irroratus* (Regression, $F_{1,16}$=3.55, *r*=0.20, p=0.07) or *C. maenas* (Regression, *r*=0.25, $F_{1,17}$=1.15, p=0.29) (Fig. 2.5A). The mean chelal strength did not vary between trials (LME, $\text{beta}_{\text{Trial2}}$= -0.003±18 SE, t=-0.01, p=0.98). Species had a significant effect of strength index, with *C. maenas* being stronger (LME, $\text{beta}_{C.maenas}$= 0.91±0.3 SE, t=2.99, p=0.005) (Fig. 2.5B, C). Neither the amount eaten (LME, beta= 0.07±0.11 SE, t=0.66, p=0.50) nor crab mass (LME, beta= 0.01±0.008 SE, t=1.87, p=0.06) had a significant effect on the strength index. When crab strength not compensated for claw size (kg) was used as the response variable, larger crabs were on average stronger (LME, beta= 0.05±0.01 SE, t=2.99, p=0.005). Including species-specific variance structure resulted in a model with a better fit than one that did not include variance structure (ΔAIC = 8.47), therefore variance was said to differ between species. According to the variance weighting parameter, *C. irroratus* strength index measures were about 0.51 times as variable as that of *C. maenas*.

Metabolic Rate

The resting metabolic rate (RMR) (mg O_2/kg/hr) was an intra-individually consistent trait for *C. irroratus* (Regression, $F_{1,17}$=16.27, *r*=0.72, p=0.001) but not for *C. maenas* (Regression, $F_{1,15}$=0.38, *r*=0.15, p=0.54) (Fig 6A). The mean RMR did not vary between trials (LME, $beta_{trial2}$= 1.21± 1.46SE, t=0.84, p=0.40), or crab species (LME, $beta_{C.maenas}$=-3.24 ± 1.46 SE, t=-1.07, p=0.29) (Fig 2.6B, C). Neither the amount of food eaten (LME, beta=-0.21±1.14 SE, t=-0.13, p=0.89) nor crab mass (LME, beta= 0.02±0.09 SE, t=0.17, p=0.85) had a significant effect on RMR; it should be noted that mass-specific metabolic rate was used to calculate RMR. Including species-specific variance structure did not result in a model with a better fit than one that did not include variance (ΔAIC = -1.97): therefore, both species had similar inter-individual variation in RMR.

The maximum metabolic rate (MMR) (mg O_2/kg/hr) was an intra-individually consistent trait for both *C. irroratus* (Regression, $F_{1,15}$=20.27, *r*=0.76, p<0.001) and *C. maenas* (Regression, $F_{1,17}$=9.36, *r*=0.6, p=0.007) (Fig. 2.6D). MMR means did not vary significantly between trials (LME, $beta_{Trial2}$= -3.85±3.87 SE, t=-0.99, p=0.32), or crab species (LME, $beta_{C.maenas}$=6.79 ±9.57 SE, t=0.71, p=0.48) (Fig. 2.6E, F). The amount eaten (LME, beta= -1.64±3.34 SE, t=-0.49, p=0.62) did not have a significant effect on

MMR, however crab mass (LME, beta=-0.61 ± 0.27 SE, t=-2.18, p=0.03) did, with larger crabs having a lower MMR proportional to their body mass. Including species-specific variance structure did not result in a model with a better fit than one that did not include variance (ΔAIC = -1.96), therefore both species had similar intra-individual variation in MMR.

Behavioural Syndromes

Behavioural syndromes form when suites of behaviours are correlated (Roche et al., 2016, Sih et al., 2004). None of the behavioural traits (spontaneous activity, boldness, exploration, strength index) were significantly correlated to one another (Fig. 2.7), except for a negative correlation between chelal strength and boldness in *C. maenas* (Regression: r=-0.65, $F_{1,17}$=12.36, p=0.002) (Fig. 2.7E). This suggests that none of these behaviours were part of the same behavioural syndrome within either species.

Relationships between physiology and behaviour

According to the pace of life syndrome hypothesis, individuals with higher metabolic rates should have more proactive behaviours due to increased costs of idling. However, none of the pairwise correlations between metabolic rate measures (RMR and MMR) and behavioural traits (boldness, exploration, spontaneous activity, or strength index) were significant (Table 2.3).

Discussion

Over the past two decades an interest has emerged in understanding consistent individual-level differences in behaviour, widely referred to as animal "personality" (Roche et al., 2016; Careau and Garland, 2012). The proximal mechanisms underpinning personality differences are still largely unknown, but theoretical work with some experimental backing suggests that a higher metabolic rate (MR) drives more energy intensive "proactive" behaviours (Biro and Stamps, 2008; Réale et al., 2010; Careau et al., 2008). Here, I report evidence for personality, defined as repeatable differences between individuals when behaviours are measured by standardized tests, in *Cancer irroratus* and *Carcinus maenas* crabs. However, I report no evidence that the behaviours measured were part of the same behavioural syndrome, i.e., there were no correlations between the different behaviours. There were interspecific differences in all behaviours, and *C. irroratus* were consistently more proactive than *C. maenas*. I found that despite maximum metabolic rate (MMR) and resting metabolic rate (RMR) being repeatable traits, there was no relationship between MR and any behaviour either intra- or interspecifically.

Personality and Behavioural Syndromes

Boldness and Exploration

Boldness was an intra-individually consistent trait, which supports previous studies on crabs (*C. maenas*, Fürtbauer, 2015; *Uca mjoebergi*, Reaney and Backwell, 2007; hermit crabs, Mowles et al., 2012; Garcia et al., 2020). There was a significant interspecies difference in boldness measures, with *C. irroratus* spending on average 15% more time outside shelter than *C. maenas*. Similarly, McDonald et al. (2001) report that the Dungeness crab *Cancer magister* used shelter about half as much as *C. maenas* but in that study both species spent more time out of shelter. In the present study, although *C. maenas* boldness was intra-individually consistent, inter-individually it was highly variable, with some individuals spending almost 100% of the time out of shelter, and others spending almost 100% of the time inside shelter. In contrast, Rossong et al. (2006), did not find that *C. maenas* entered shelter at all. Shelter use therefore seems to be a consistent trait, but the extent to which shelter is used varies by study and may depend on experimental conditions: e.g., shelter size or season (Beck, 1997; Beck, 1995), competitor presence (Jensen et al., 2002; McDonald et al., 2001), light conditions (Cobb, 1971), and potentially settling time (as discussed below).

Although shelter was initially presented as a refuge, many crabs repeatedly entered and exited the shelter, which may relate to the exploration of a new habitat. Because exploration represents the interest an individual has in a novel object/environment (Roche et al., 2016), here I measured exploration as the number of shelter entrances. Exploration propensity was a highly intra-individually consistent trait in both species. Every crab entered the shelter at least once, and some entered and exited upwards of 50 times in 1 h. In both trials, *C. irroratus* were significantly more exploratory than *C. maenas,* entering shelter on average twice as frequently. Based on the settling time observed during the 20 h activity recording (see also Chapter 3), the boldness and exploration experiments may have been conducted on stressed animals, and may not represent settled behaviour. In that sense, these experiments are more likely to represent an “anxiety”-like response, rather than a “curiosity”-like response (Roche et al., 2016). The results of this experiment therefore do not represent naturalized behaviours, and should not be used to draw conclusions about the extent of shelter use in the wild. Nonetheless, both of these behaviours were intra-individually consistent, so both species had personality types ranging on the bold/shy and exploratory/non-exploratory axes.

Spontaneous Activity

While few studies compare the relative distance travelled by different crab species, it might be an important metric for assessing species-level energy budgets and dispersal propensity. Here, *C. irroratus* were significantly more active than *C. maenas* in both trials: on average, *C. irroratus* travelled over three times further in 20 hours than *C. maenas*. The highest activity levels were consistently recorded immediately post transfer into the chambers in both species, suggesting that high activity may be a stress response to handling and transfer into chambers. Activity was not an intra-individually consistent trait during the whole 20 h record, nor after 10 hours of settling time in the chambers. However, when using only the first hour of the record, during the period of maximal activity (i.e., maximal handling/transfer stress), activity was repeatable in both species.

I therefore found that post-transfer stress increased behavioural consistency, and it is likely that stressed and habituated responses represent different behaviours (Edwards et al., 2012). The increase in behavioural repeatability under stressed conditions (usually in the context of predation) has been noted in fish (Ehlman et al., 2019), birds (Mathot et al., 2011), mammals (Dammhahn and Almeling, 2012), and crabs (Toscano et al., 2013). The specific reasons for this increase is unknown. However, Ehlman et al. (2019) suggest that a risky environment limits intra-individual behavioural plasticity by driving behaviours that are consistently effective at avoiding predation. A future study should establish to which extent stress hormone release (i.e., crustacean hyperglycemic hormone) relates to increased repeatability of behaviours and how personality relates to more direct measures of fitness consequences (i.e., survival and progeny).

Strength Index

Strength index, calculated as the maximum force exerted by the claw divided by claw height, was initially measured as a potential covariate representing competitive ability (Sneddon, 2000). Interestingly, the strength index was not a consistent trait in either species, which contrasts with findings from other crustaceans: e.g., crayfish *Cherax dispar* (Bywater et al., 2008), *C. maenas* (Taylor et al., 2009), and the fiddler crab *Uca pugnax* (Levinton and Judge, 1993). Most of these studies, attribute strength to claw size. However, in my study, morphology would not have changed considerably over 10 days, and claw size was included in the strength index calculation. Instead, the lack of repeatability was likely because claw compression was driven by aggressive behaviour (i.e., willingness of the crab to pinch down). Although not intra-individually consistent, my strength measures were comparable to other studies on both species (Block and Rebach, 1998; Warner et al., 1982; Taylor et al., 2009). That *C. maenas* were stronger even when size differences were accounted for is an important consideration for designing competitive contest experiments. Often, crab sizes for experiments are designated based on carapace width (e.g., Matheson and Gagnon, 2012a); however, *C.*

maenas size matched by carapace width with *C. irroratus* would not only be significantly heavier due to different body dimensions, but even mass-matched *C. maenas* would be stronger. Additionally, the effect of crab mass was very weak, and the strength index measures of *C. maenas* were significantly more variable than those of *C. irroratus*. Matching competitors by size or mass may therefore not be the best way to achieve similar contestants, and even using claw compression measurement techniques could yield variable results.

Behavioural Syndromes

To analyse the presence of behavioural syndromes, pairwise cross-correlations of behavioural metrics were performed to see if any behaviours co-varied on a proactive/reactive axis. None of the behaviours showed any relationship, except boldness and chelal strength index in *C. maenas* which were significantly negatively correlated. Shelter use is a risk-averse behaviour because it protects from predation, but it also increases the chance of intraspecific agonistic contests for the shelter (e.g., Savvides et al., 2015). McDonald et al. (2001), for instance, concluded that the reason *C. maenas* spent more time in shelter than *C. magister* was because they won interspecific contests. Therefore, the difference in species' shelter use may be less of an indication of boldness, but rather of a crab's resource holding ability (Briffa et al., 2015). This could explain why stronger *C. maenas* spent more time in shelter. While this relationship was not significant in *C. irroratus*, they were weaker and spent significantly more time out of shelter. Alternatively (or additionally) boldness and claw strength could be negatively correltated because they are co-specialized for reducing predation risk (i.e., shyer, stronger crabs are better at avoiding predatation); similar results are reported for eastern chipmunks (*Tamias striatus*) (Newar and Careau, 2018) and Karoo bush rats (*Myotomys unisulcatus*) (Agnani et al., 2020). Of the other correlations, it was surprising that exploration and activity were not correlated. Both metrics involve locomotor activity, and since exploration was measured as the number of times the crab entered shelter, it is similar to the "threshold-crossing" method some studies use to assess activity in decapods (e.g., Aguzzi et al., 2004; Snyer and Peeke, 2001). Activity and exploration were measured within a 2-hour timespan, so it is unlikely that any change in the 'physiological state' of the crab would have caused behavioural differences. I suspect the presence of a novel object (shelter) changes the context of active behaviour from intrinsic locomotor output to object exploration. This may also explain why activity measures were considerably less repeatable than exploration measures.

Interspecies Personality Differences

Although there were no substantial correlations among behaviours, *C. irroratus* were consistently more proactive than *C. maenas* in boldness, exploration, and activity measures, which contrasts with previous studies. Matheson and Gagnon (2012b), for example, found no difference in time spent moving between these same species, and several others report that *C. maenas* is generally more active (Saxton et al., 2020; Bélair and Miron, 2009; Breen and Metaxas, 2009). It seems that *C. maenas* in my study were less active than previously reported. Fürtbauer and Fry (2018) found that *C. maenas* travelled up to 80 m in 10 min (median 8.8 m), which is roughly the distance *C. maenas* travelled in the first hour (max= 83 m, median=9.1 m). Ameyaw-Akumfi and Naylor

(1987) report estuarine *C. maenas* travelling up to 2 km in 6 h, which is considerably further than either crab species here. Both crab species in this chapter were collected from and stored in similar temperatures (10-12 °C); however, one possibility is that the seasonal change in water temperature from fall to winter affects these species differently. *C. irroratus* abundance and activity rates are higher at low temperatures (6-7 °C), while *C. maenas* abundance are higher at temperatures >10 °C (Bélair and Miron, 2009). In fact, in Chapter 3, *C. irroratus* collected from the same location in late June were considerably less active (approximately 150-200 m in 20 h) then the ones in the present study, suggesting some seasonal influence. This can bear ecological consequences because, as shown by Mathewson and Gagnon (2012b), *C. maenas* in warmer water (12°C) initiate more contests with *C. irroratus* than those in cold water (4°C). If this discrepancy is corroborated by in-situ studies, it might mean that there are prolonged offset periods during colder months when the two species are not in direct competition (as suggested by Bélair and Miron, 2009). It is, however, also possible that the interspecies difference is not driven by temperature, but rather represents an intrinsic interspecies personality type difference in Newfoundland. Theoretical work suggests that post-establishment, successful invasive populations are selected to have individuals with proactive personalities because they increase dispersal propensity and competitive ability (Chapple et al., 2012). It was therefore surprising that the invasive *C. maenas* was more reactive than the native *C. irroratus*. However, if dispersal is too quick post invasive species establishment, it may lead to a considerable population density decline, and therefore insufficient reproduction to maintain population growth (i.e. Allee effect, Chapple et al., 2012). Additionally, reactive behaviours may be important to some invasive species when the risk of predation is high, and the benefit of exploration and active foraging is low. Both crab species primarily prey on sessile organisms (Sungail et al., 2013), therefore the benefit of proactive hunting maybe low. Both species are preyed on by gulls (*Larus* spp.), lobster, and fish, so reactive behaviours in *C. maenas* may serve to decrease predation risk. Alternately, *C. irroratus* could make a better invader once it establishes in a new location. Since 2007, invasive *C. irroratus* has slowly outcompeted Icelandic *C. maenas* and the spider crab *Hyas araneus*, becoming the dominant brachyuran crab species in coastal regions (Gíslason et al., 2020). Gíslason et al. (2014) propose that the invasion of *C. irroratus* to Iceland has been facilitated by ballast transport from North America, and that there are reports of *C. irroratus* larvae surviving in ballasts through to Europe. Although the longer larval stage of *C. maenas* (50-80 days vs. 37-58 in *C. irroratus*) may allow this species to survive longer in ballasts, *C. irroratus* could be a viable competitor to *C. maenas* if sufficient propagule pressure overcomes high ballast mortality.

Physiology and Behaviour

Like behaviour, metabolic rate (MR) is often an intra-individually consistent trait, leading to the hypothesis that MR is a proximal physiological driver of behaviour (meta-analyses: Nesplo and Franco, 2007; Holtmann et al. 2016). Previous studies show that higher metabolic rate measures are related to more proactive behaviours (resting MR: Myles-Gonzales et al., 2015; Behrens et al., 2020; maximum MR: Binder et al., 2016; metabolic scope: Killen et al., 2014). However, there were no significant correlations between any

MR and behavioural measures. Moreover, while *C. irroratus* and *C. maenas* differed significantly in all behavioural measures, none on the physiological measures varied significantly by species. In other words, *C. irroratus* expressed more proactive behaviours without significantly increasing its metabolic expenditure. It was especially surprising that MMR and maximal activity were not correlated because both were measured within the first hour post transfer into chambers (therefore measured after handling/transfer stress), and both were repeatable traits. This finding contrasts the expectation that active individuals would have higher MMR measures (Brill et al., 2015; Full, 1987), and previously reported relationships between locomotor activity and heart rate in crabs (Styrishave et al., 1999; Aagaard et al., 1995), both of which relate to metabolic rate (Hamiltan and Houlihan, 1992; Gribble and Broom, 1996). In fact, Aagaard et al. (1995) suggest that heart rate increase precedes locomotor activity, "preparing" the crab in advance to deal with the metabolic demand of activity. I found no relationship between MMR and maximal activity level, and the correlation coefficient was close to 0 in both species. It is possible that measuring both traits simultaneously (rather than 24 h apart) would yield a clearer relationship, but in Toscano and Monaco (2015), the relationship between MR and activity was negative or positive depending on context (i.e., how activity was measured). Therefore, it is more likely that handling stress increases both metabolic rate and activity, but one is not a proportional or causal consequence of the other. As shown by Aagaard et al. (1995), stressed immobilized crab can have a higher heart rate than free running crabs, which would suggest that stress induced increase in respiration is separate from active movement. Measuring other stress-related metabolic costs (i.e., enzyme production, gluconeogenesis; Burton et al., 2011) may explain a higher proportion of the variation in MMR. My results suggest that the consistent individual differences in physiology do not drive consistent individual differences in behaviour either within or between these crab species. This does not support the pace-of-life syndrome hypothesis.

Conclusions

I found evidence for consistent individual differences in behaviour (i.e., personality) in two species of crab, but no evidence that any of the measured behaviours were part of the same behavioural syndrome. There was considerable interspecific difference in behaviours: the native *C. irroratus* was more proactive (bold, exploratory, active) than the invasive *C. maenas*. One possibility for future research is to test whether this difference is because the two species respond differently to seasonal temperature change; *C. maenas* may be more averse to decreasing temperatures than *C. irroratus*. It should also be tested whether this creates an offset period where *C. irroratus* has an advantage over *C. maenas*, and suggests this as an interesting hypothesis to explore in-situ. Alternately, the invasion process has selected for more reactive *C. maenas* in Newfoundland as a predator avoidance tactic/to decrease Allee effect, or that post-establishment *C. irroratus* could outcompete *C. maenas*. Although most behaviours and metabolic rate measures were repeatable, no metabolic rate measure was significantly correlated to any behavioural measure. Furthermore, there were no significant differences in maximum metabolic rate (MMR) or resting metabolic rate (RMR) between the two species, despite every behaviour being significantly different between the two species. Considering that *C. irroratus* consistently displayed more energy intensive behaviours, my findings do not support the hypothesis that a higher metabolic rate drives more proactive behaviours. Instead, other physiological metrics such as endocrine release, should be considered as a proximal driver of personality.

Tables

Table 2.1. Comparison of life history traits of *C. irroratus* and *C. maenas*.

Life History Trait	***C. irroratus***	***C. maenas***
Maximum Lifespan	7-8 years[1]	5-7 years[3]
Age at Maturity	2 to 3 years[2]	<1 to 2 years[4]
Egg Clutch Size	107,045±103,590[1]	195,833±83,673 SD[5]

1. Reilly and Saila, 1978
2. Griffen and Riley, 2015
3. Young and Elliott, 2020
4. Klassen and Locke, 2007
5. Audet et al., 2008

Table 2.2. R packages used for data management, data visualization, and statistical analysis.

Package Name	**Citation**
ggplot2	Wickham, 2016
ggpubr	Kassambra, 2020
tidyverse	Wickham et al., 2019
patchwork	Pedersen, 2020
Hmisc	Harrell, 2020
nlme	Pinheiro et al., 2020
reshape2	Wickham, 2007

Table 2.3. Results of linear regressions between physiological and behavioural trait pairs. None of the physiological traits measured correlated significantly with any behavioural trait in either species.

Physiological Trait	Behavioural Trait	Species	*r*	p-value
RMR	Boldness	*C. irroratus*	-0.28	0.273
RMR	Boldness	*C. maenas*	-0.11	0.58
RMR	Exploration	*C. irroratus*	-0.18	0.764
RMR	Exploration	*C. maenas*	0.08	0.091
RMR	Settled Activity	*C. irroratus*	0.09	0.724
RMR	Settled Activity	*C. maenas*	0.3	0.266
RMR	Strength Index	*C. irroratus*	-0.009	0.94
RMR	Strength Index	*C. maenas*	0.4	0.092
MMR	Boldness	*C. irroratus*	-0.30	0.249
MMR	Boldness	*C. maenas*	-0.08	0.758
MMR	Exploration	*C. irroratus*	0.038	0.639
MMR	Exploration	*C. maenas*	0.001	0.879
MMR	Maximal Activity	*C. irroratus*	-0.07	0.89
MMR	Maximal Activity	*C. maenas*	-0.012	0.79
MMR	Strength Index	*C. irroratus*	-0.15	0.419
MMR	Strength Index	*C. maenas*	0.41	0.078

Figures

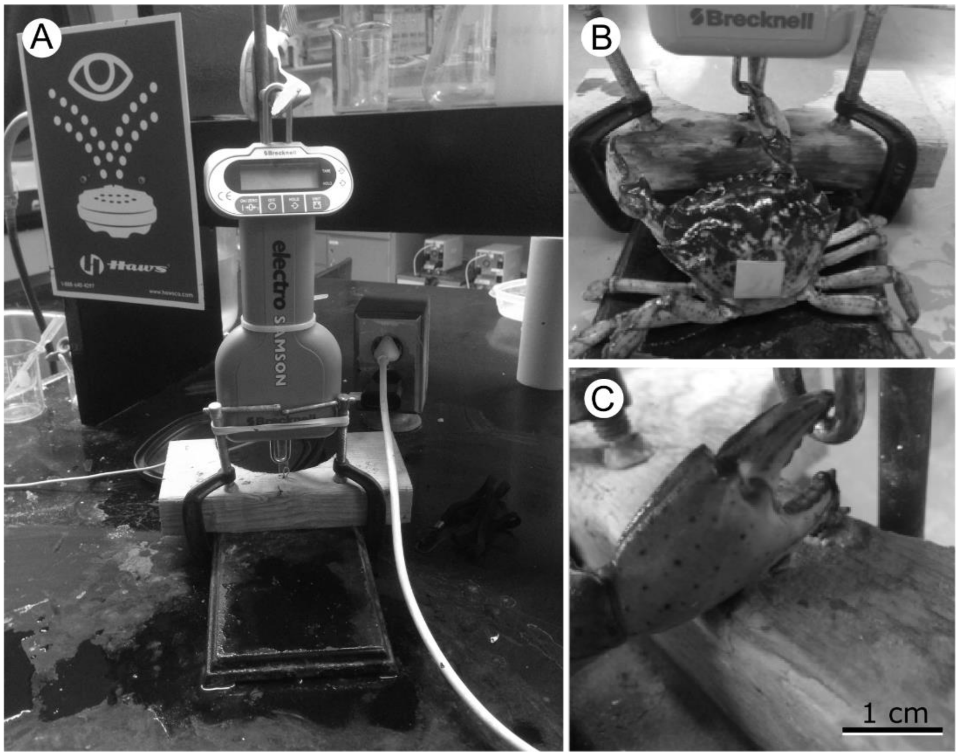

Figure 2.1. Chelal strength measuring apparatus. **A.** the scale setup on a clamp stand. **B.** Placement of *C. maenas* on strength measuring apparatus. **C.** Fixed finger of claw in immovable latch and dactyl on scale latch.

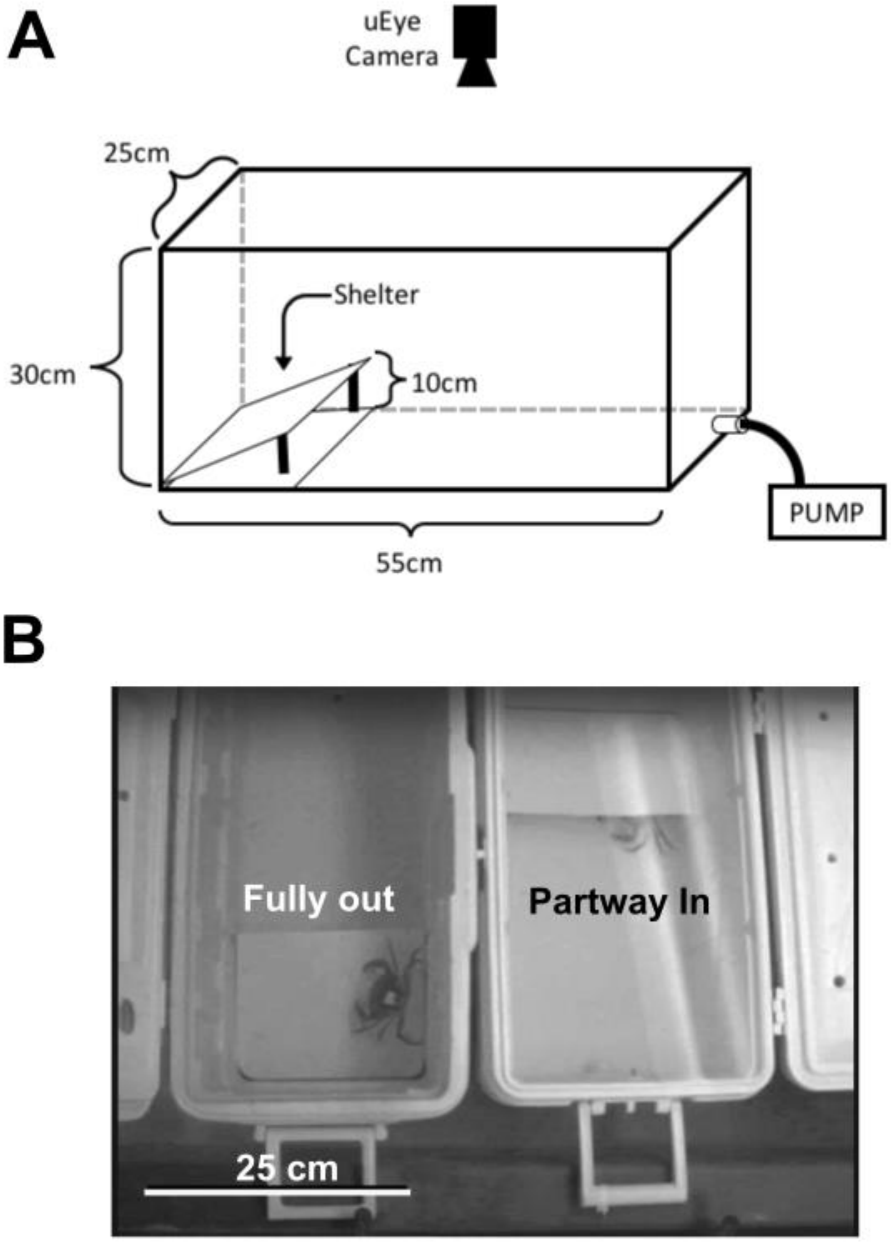

Figure 2.2. Boldness and exploration chamber. **A.** Diagram of cooler showing all dimensions and shelter placement. **B.** Top view of both coolers with shelters; one crab is fully out of shelter and another partway in shelter. When a crab was fully in the shelter it was invisible from the top.

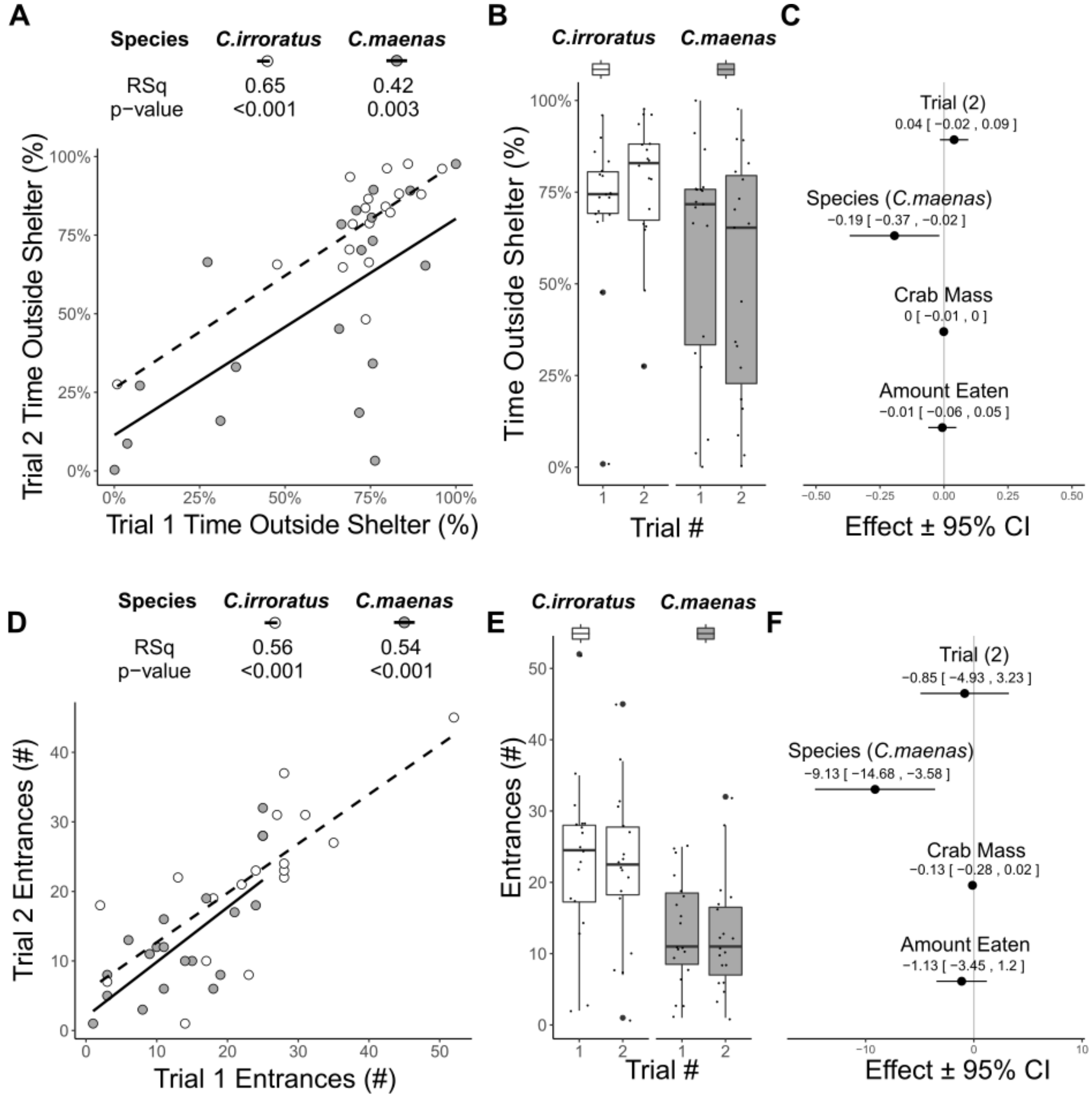

Figure 2.3. Boldness (measured as percent time spent out of shelter) and exploration (measured as the number of entrances into shelter) for *C. maenas* (grey, solid) and *C. irroratus* (white, dashed) measured between 2 trials. On boxplots (B, E), the horizontal line is the median, box hinges are 25th and 75th percentiles, whiskers represent 1.5*IQR, and outliers are large points. **A.** Linear regression between trial 1 and 2 shows that boldness was a consistent trait for both species. **B.** Boxplot of boldness measures during both trials for both species. **C.** Fixed effect estimates (±$CI_{95\%}$) from the linear mixed effect models, using boldness as the response variable and crab ID as the random factor show that only species type had a significant effect on boldness, with *C. maenas* being less bold. **D.** Linear regression between trial 1 and 2 shows that exploration was a consistent trait for both species. **E.** Boxplot comparing exploration measures between trials for

both species. **F.** Fixed effect estimates (±$CI_{95\%}$) from the linear mixed effect models, using exploration as the response variable and crab ID as the random factor show that only species type had a significant effect on exploration, with *C. maenas* being less exploratory.

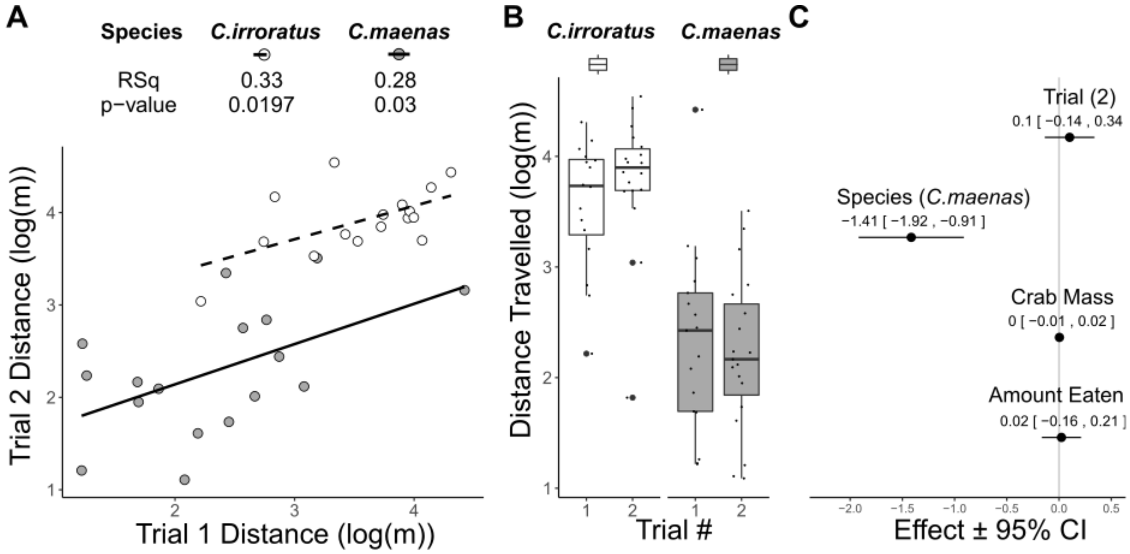

Figure 2.4. Spontaneous activity (total distance moved in 1 h) for *C. maenas* (grey, solid) and *C. irroratus* (white, dashed). Activity measures were log-transformed to improve data normality. **A.** Linear regression between trial 1 and trial 2 shows that activity was an intra-individually consistent trait in both species. **B.** Comparison of activity between trials and between species. Small points represent raw data. Boxplot stats: horizontal line represents the median, box hinges represent 25th and 75th percentiles, whiskers represent 1.5*IQR, and outliers are large points. **C.** Fixed effect estimates (±$CI_{95\%}$) from the linear mixed effect models, using activity as the response variable and crab ID as the random factor show that only species type had a significant effect on activity, with *C. maenas* being less active.

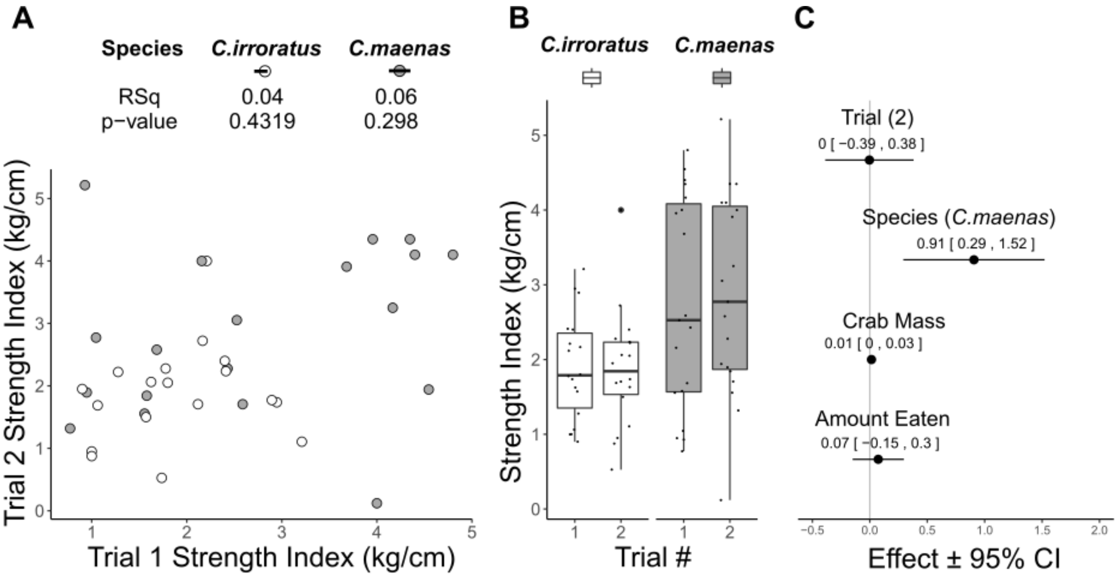

Figure 2.5. Strength index measures (compression strength/claw height, kg/cm) for *C. maenas* (grey, solid) and *C. irroratus* (white, dashed) crabs. **A.** Linear regression between trial 1 and trial 2 shows that strength was not an intra-individually consistent trait in either species. **B.** Comparison of strength of both species between tri als. Small points represent raw data, horizontal line represents the median, the box hinges represent 25th and 75th percentiles, whiskers represent 1.5*IQR, and outliers are large points. **C.** Fixed effect estimates (±$CI_{95\%}$) from the linear mixed effect models, using strength as the response variable and crab ID as the random factor show that only species type had a significant effect on strength index, with *C. maenas* being stronger than *C. irroratus*. Crab mass had a very minimal but positive effect on crab strength.

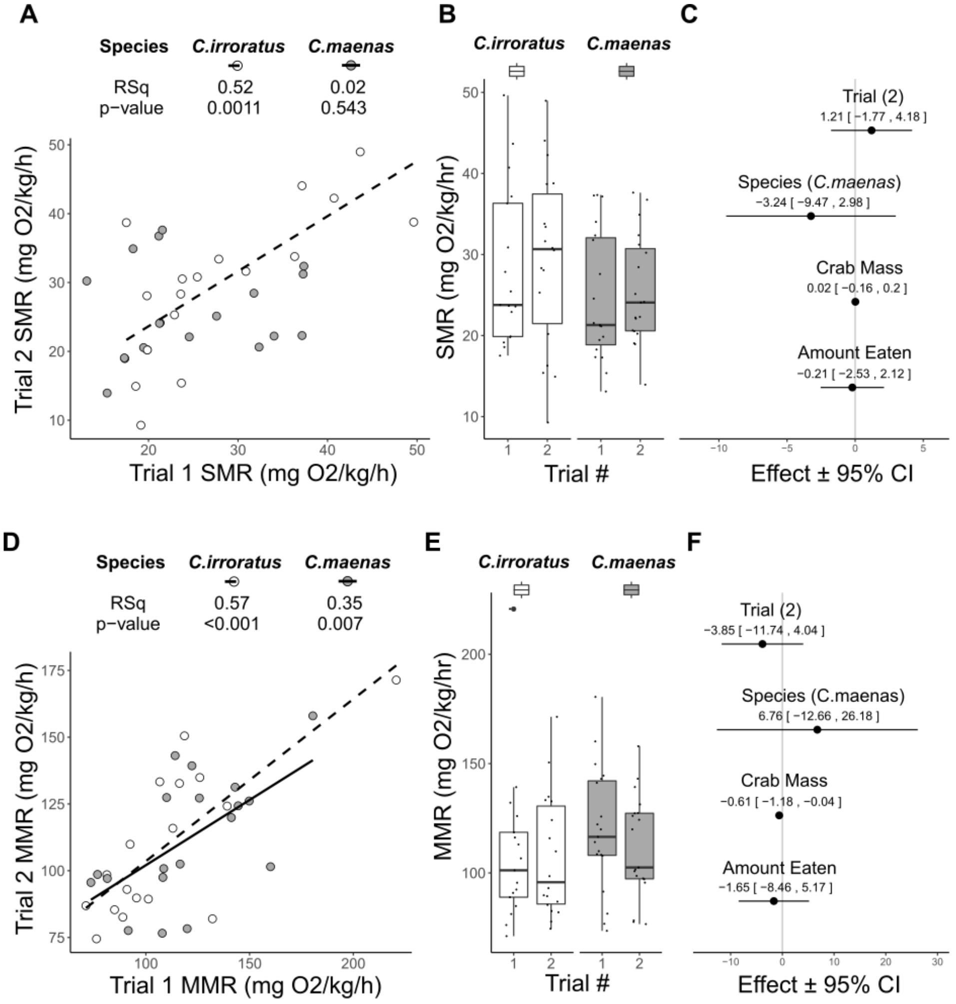

Figure 2.6. Resting and maximum metabolic rate (oxygen uptake in mg O_2/mg/kg), for *C. maenas* (grey, solid) and *C. irroratus* (white, dashed) measured in two separate trials 10 d apart. On boxplots (B, E), the horizontal line represents the median, box hinges represent 25th and 75th percentiles, whiskers represent 1.5*IQR, and outliers are large points. **A.** Linear regression between trial 1 and trial 2 shows that RMR was an intra-individually consistent trait in *C. irroratus* but not *C. maenas*. **B.** Boxplot of RMR during both trials for each species. **C.** Fixed effect estimates (±$CI_{95\%}$) from the linear mixed effect models, using RMR as the response variable and crab ID as the random factor show that none of the factors explained a significant proportion of variation in RMR. **D.** Linear regression between trial 1 and trial 2 shows that MMR was an intra-individually consistent trait in both species. **E.** Boxplots of RMR during both trials for each species. **F**. Fixed effect estimates (±$CI_{95\%}$) from the linear mixed effect models, using MMR as the response variable and crab ID as the random factor show that none of the factors explained a significant proportion of variation in MMR.

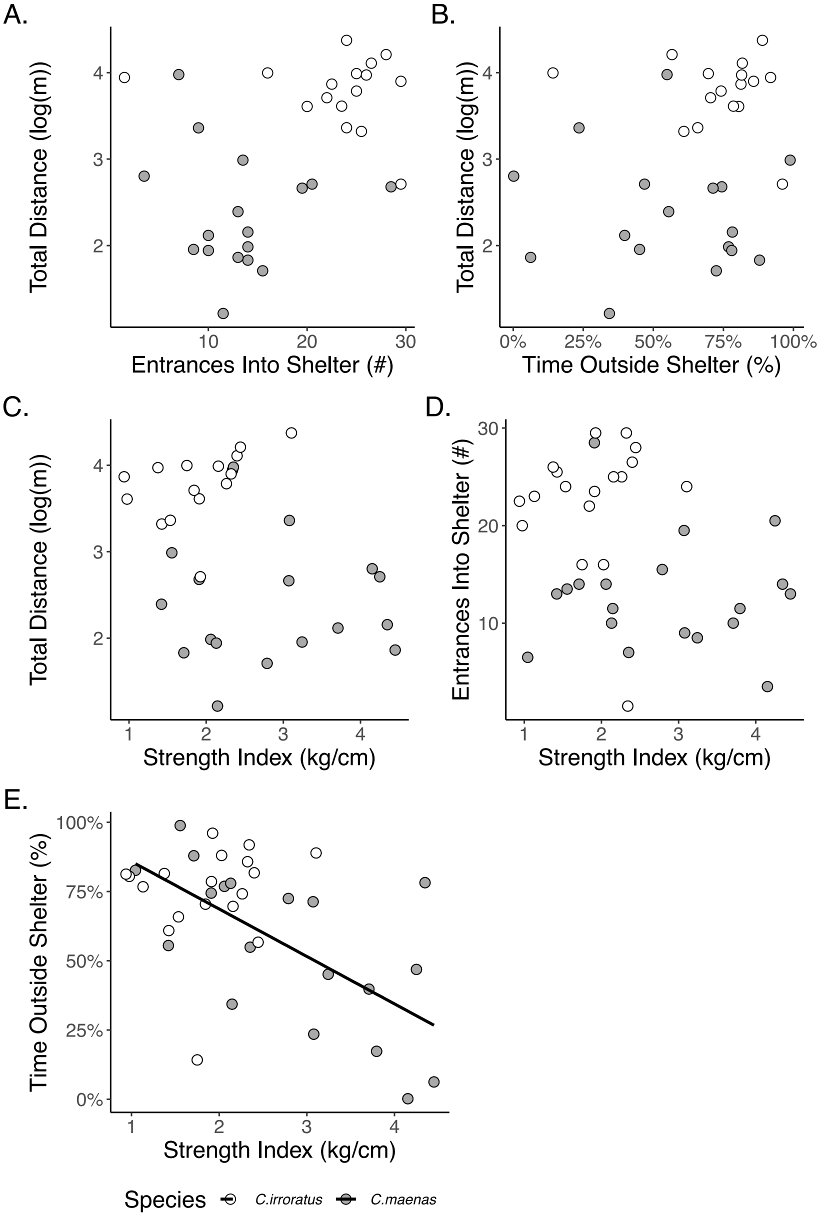

Figure 2.7. Analysis of behavioural syndromes (pairwise linear regressions of different behaviours) for *C. maenas* (grey, solid) and *C. irroratus* (white, open) crabs. In *C. maenas*, chelal strength index was negatively correlated to boldness (time outside shelter). None of the other behaviours were significantly correlated in either species.

Chapter 3

Quantifying the effects of dietary homogeneity, lab holding time, and handling stress on labile traits in the brachyuran crab *Cancer irroratus*

Abstract

Collecting animals from the field and bringing them into the laboratory elicits acute and chronic stress responses which may affect the interpretation of experimental outcomes. I quantified the effects of prolonged lab holding and transfer to experimental chambers on the labile traits (metabolic rate and spontaneous activity) of the Atlantic rock crab *Cancer irroratus*. Additionally, I assessed whether providing a heterogenous vs. homogenous diet affected indicators of crab health condition (hemolymph protein density, crab mass, and chelal compression strength). *C. irroratus* displayed a clear preference for mussel, and an aversion to herring (*Clupea harengus*) and algae. The amount crabs ate in the lab was negatively correlated to the hemolymph protein density at the time of collection. This affirms the strong link between nutritional status and hemolymph protein in crustaceans, and suggests that crabs in good nutritional condition may forgo eating even a high quality meal if they are stressed. Overall, providing a heterogenous did not significantly improve survival rates or affect crab labile trait response. I also showed that prolonged holding in the lab had considerable effects on labile traits by elevating RMR for 1 week post-translocation and depressing variation in locomotor activity for roughly 4 weeks. Transfer to experimental chambers resulted in elevated RMR for 15-16.5 h in the first two weeks in the lab, and 9-12.5 h thereafter. The crabs also exhibited increased locomotor activity for between 3.5 and 10 h post-transfer, likely a stress response to handling. Recommendations for holding and settling times are made.

Introduction

Collecting *in situ* field data on animals is invaluable, but fieldwork is constrained by the complexity of the natural environment. Multiple biotic and physiochemical factors affect animals in combination, which makes causal relationships difficult or impossible to interpret (Hilário et al., 2015; Auer et al., 2016; Sloman et al, 2008). To circumvent complexities associated with fieldwork, animals are collected and maintained in constant and optimal species-specific laboratory conditions. This controlled reduction in environmental variability decreases intra-individual trait variation, and simplifies the relationships between experimental treatment and response variables (Lee and Berejikian, 2008; Morash et al., 2018; Höjesjö et al., 2002). However, lab experiments come with their own sets of challenges. Acute stress responses (e.g., stress hormone release and metabolic rate increase) are commonly recorded in animals during capture, transport, handling, and introduction to novel surroundings (Crear and Forteath, 2001; Haukenes et al., 2009; Belanger, 2001; Davis and Schreck, 1997; Dickens et al., 2010). To account for collection and transport to aquaria conditions, common practices dictate that animals collected from the field are given time to adjust to novel conditions prior to the start of experiments. For the purposes of the present chapter, I will distinguish between "holding time" as the period animals spend in a research facility after translocation from the field and before use in experiments, and "settling time" as the period after transfer to experimental conditions but prior to the start of measurement (often termed "acclimation"). Not giving animals enough time to settle after transfer to novel conditions can yield results affected by acute stress response, but holding animals for prolonged periods under stable lab conditions can also alter labile trait responses. For instance, Callaghan et al. (2016) show that rainbow trout (*Oncorhynchus mykiss*) exposed to a fluctuating thermal regime (as compared to a stable regime) adjusted their metabolite output to better deal with heat shock. The authors suggest that while a fluctuating environment may result in symptoms of stress (i.e., heat shock protein release), environmental variability better prepares the animal for subsequent exposure to acute stress. The stability of the lab environment can also amplify the significance of experimental outcomes. A study replicating lab experiments on the benefits of a dominant behavioural type to salmon fitness, found that natural environmental heterogeneity resulted in a less clear advantage of a dominant behaviour (Höjesjö et al., 2002). Moreover, if the conditions under which animals are held are suboptimal, animals can experience chronic stress effects (review by Dickens et al, 2010). For example, ambient noise from water pumps has long-term physiological effects on seahorses (*Hippocampus erectus*), resulting in measurable weight loss and increased levels of cortisol (Anderson et al., 2011). In *Carcinus maenas* just two weeks of captivity results in a significant decrease in activity levels and aerobic scope (Houlihan and Mathers, 1985), suggesting a decline in condition during lab holding conditions. Prolonged periods of captivity-related chronic stress can also increase mortality, or cause permanent changes to animals, making them unsuitable as experimental subjects (Morgan and Tromborg, 2007).

While rigorous studies examining captivity stressors have been carried out for common experimental models or commercial species, such as farm animals (Morgan and Tromborg, 2007; Dickens et al., 2010), less is known about the effects of lab holding and settling times in invertebrate model systems. In studies on decapod crustaceans, holding/settling times vary greatly, and few studies cite a rationale for appropriate adjustment period duration. A brief survey of 54 papers on the physiology and behaviour of adult crabs from the last decade (2009-2019) (Fig. 3.1, Table A.1) shows that holding times last between 1 day to 9 months (median = 13 d ± 46.11 SD). The settling time in experimental apparatus also varies from no settling (0 h) to 48 hours (median = 4 h ± 11.46 SD). Of the papers surveyed, only 7 cite a justification for holding and/or settling time (Table A.1). How, and if, the duration of adjustment time affects fast-changing "labile" traits that are often measured during experiments (i.e., physiology and behaviour) should be understood to draw accurate conclusions from experiments.

One potential chronic stressor in a lab environment is a change in diet. Not only are natural foraging instincts curtailed due to regulated feeding (Morgan and Tromborg, 2007), dietary composition is often limited to one food type. While this is practical and standardizes the nutritional background of a study organism, prolonged feeding on a suboptimal diet can result in restricted eating, which can in turn change labile trait expression (e.g., Careau et al., 2014). For generalist omnivorous feeders, such as many scavenging decapods, the homogenization of dietary intake may affect welfare (i.e., ability to maintain homeostasis; Broom, 1991). Wild crabs feed on bivalves, algae, and detritus (Griffen and Riley, 2015; Hudon and Lamarche, 1989), but a brief survey of 52 papers from 2000-2020 shows that 73% (38/52) of lab studies give crabs a diet composed of a single food type (Table 3.1 & Table A.2). Previous research suggests that diet has a significant effect on crab labile traits. Blue crabs (*Callinectes sapidus)* fed a small amount of food, or a diet exclusively of seaweed (*Ulva lactuca*) have higher aggression levels, decreased lipid content, and smaller egg volume (Belgrad and Griffen, 2016). Shrimp larvae (*Litopenaeus setiferus*) fed with a heterogenous diet (consisting of commercial diet supplemented with *Artemia*, flagellates, and diatoms) had higher growth rates, soluble protein content, and enzymatic activity than those fed with just the commercial diet, suggesting dietary variety is important for animal welfare (Brito et al., 2000). Because animals have preferences for certain food types, even if a nutritious diet is provided it might not be preferred (Meakin et al., 2008; Sclafani, 1995). Dietary preference, in turn, could determine how much animals eat (Belgrad and Griffen, 2016), and if a non-preferred diet is given animals could experience symptoms of starvation (Meyer and Oettl, 1995; Wang and McGaw, 2014).

The aim of my study was to use the Atlantic rock crab *Cancer irroratus* (Say, 1817) to quantify any changes in labile traits during prolonged holding in the lab and after handling and transfer into experimental chambers. Additionally, I investigated the effects of long-term feeding on a heterogenous “mixed” diet (fish, mussel, and kelp) versus a homogenous “mono” diet (only fish). As a physiological response metric, I assessed metabolic rate (via oxygen uptake), because it is consistently shown to be affected by handling stress in crustaceans (Jouve-Duhamel and Truchot, 1985; Stoner 2012; Crear and Forteath, 2001; McMahon et al., 1979). Additionally, I measured spontaneous locomotor activity (i.e., un-stimulated movement in an open arena) as a behavioural metric, because it was found to decline in *C. maenas* after 2-3 weeks of captivity (Houlihan and Mathers, 1985). These labile traits were paired with more stable indicators of health/condition: claw closure strength (Sneddon et al, 2000), hemolymph protein density (Wang and McGaw, 2014; Lorenzon et al., 2008), and crab mass. Additionally, I measured the amount crabs ate of each food type provided to determine if dietary preferences could be detected, and if so, what effect they had on crab labile traits and survival rates.

C. irroratus is ubiquitous along eastern Atlantic coast, inhabiting rocky and sandy substrates (Grosholz & Ruiz 1996) between Iceland (Gíslason et al., 2017) and North Carolina (Haefner Jr., 1976; Stehlik, 1993). Male *C. irroratus* have an average carapace width of about 11.6 cm, while females tend to be slightly smaller (Gíslason et al., 2014). *C. irroratus* is a good model species because it is easy to collect and maintain, a fact that probably accounts for its widespread use in laboratory experiments (Penney et al., 2016; Hobbs et al., 2017; Matheson and Gagnon, 2012a; Matheson and Gagnon, 2012b; Bélair and Miron, 2009; Wells et al., 2010; Mayrand and Dutil, 2008). Because *C. irroratus* has a wide habitat distribution (Haefner, 1976), it also has a wide tolerance range to temperature (4-25 °C, Breen and Metaxas, 2008) and salinity (75% sea water, Cantelmo et al., 1975), which makes it useful for prolonged holding in controlled stable conditions. *C. irroratus* is considered an opportunistic scavenger with a broad and varied diet, feeding on bivalves, polychaetes, other crustaceans, seaweed and small fish (MacKenzie Jr., 1977; Miron et al., 2005; Elner and Jamieson, 1979; Stehlik, 1993; Griffen and Riley, 2015). While this makes it easy to feed *C. irroratus* in a lab setting, the effect of reduction in dietary heterogeneity should be investigated.

The overall goals of this study are threefold:

1. Test whether long-term holding in a lab environment resulted in the change of labile trait expression, specifically spontaneous activity and metabolic rate (as concluded by Houlihan and Mathers, 1985).
2. Quantify duration of acute stress response after handling and introduction to experimental chambers and determine appropriate acclimation and settling times.
3. Test whether a "mixed" diet vs. a homogenous "mono" diet has an effect on crab labile traits and overall condition.

I predicted that crabs would have the highest spontaneous activity immediately post collection, and that it would decrease over time held in the lab as crabs deteriorated in condition. In contrast, aerobic scope would increase over time due to chronic stress (as found in Houlihan and Mathers, 1985). I also predicted that that crabs given a heterogeneous diet would fare better at the end of the 15-week lab holding period than crabs maintained on a homogenous diet.

Materials and Methods

Study system

Intermoult male Atlantic rock crabs (*Cancer irroratus*) were collected between June 23-28th in 2018 using herring-baited traps deployed for 1-3 days from a recreational boat dock in Holyrood, Newfoundland (47°23'18.6"N 53°07'36.7"W). Crabs were selected to a carapace width of 8.4-10.6 cm to diminish any potential effects associated with animal size, and only crabs that had both claws and no more than two missing legs were used. Crabs were transported in a cooler with seaweed (to prevent desiccation) during a 40-minute drive to the Department of Ocean Sciences, Memorial University. They were labeled with ID numbers using plastic foam tags glued on their dorsal carapace, and stored in individual-specific 4 cm x 15 cm x 22 cm heavily perforated plastic containers at 11±1°C in a flow-through seawater (31-32 ppt) tank with constant air-stone oxygenation.

Experimental Design

The first aim of this experimental series was to determine if and how labile traits (oxygen uptake, spontaneous activity, hemolymph protein density, and claw compression strength) of *C. irroratus* changes during 15 weeks (three months) of holding in the lab, and what role dietary heterogeneity plays in crab condition (see below). The crabs were separated into 6 groups (n=10 each) based on diet type and trait measured (2 diets= mixed and mono; 2 traits measured= oxygen uptake, spontaneous activity). Different sets of crabs were used for oxygen uptake (n=20; 10 mixed, 10 mono) and activity trials (n=20; 10 mixed, 10 mono). An extra 10 crabs in each diet treatment were used for measurement of chelal strength and hemolymph density, but not activity or metabolic rates. Claw strength, hemolymph protein density (HD), and weight were measured for all 60 experimental crabs (a visual representation of the data collection schedule can be seen in Fig. A2). Because some crabs died over the course of the experiment and some corrupted records had to be discarded, the sample sizes used for analysis varied by experiment and by sample time (Table A.3). Because of the logistics associated with experimental period and number of experimental chambers, each set of oxygen uptake and activity measurements was collected over several days. Oxygen uptake and spontaneous activity were measured 7 times throughout 15 weeks; HD, claw strength and crab mass were measured 6 times (Table A.3).

Feeding and diet treatment

A diet treatment was introduced to determine if diversifying food choice influenced crab traits. Half of the crabs (30) were fed a homogenous "mono" diet of herring (*Clupea harengus*), while the other half (30) were given a heterogeneous "mixed" diet of herring, mussels (*Mytilus edulis*), and algae (mostly kelp or dulce collected from the field site). Mussels and algae were used as diversifying food items because they represent a measurable proportion of gut contents in wild rock crabs (Griffen and Riley, 2015). Crabs were presented food for 2 h once every four days, and were fasted for two days before experiments to standardize hunger levels (Wallace, 1973). Twelve crabs were fed per day in individual mesh lined containers kept inside the storage tank. Each feeding chamber had an inflow tube ensuring oxygenated water was pumped in and recirculated. Each time the crabs were fed, the amount they consumed was calculated as follows. Prior to feeding the food was soaked in seawater overnight so that osmotic absorption or loss had a minimal effect on food weight change. Food was patted dry with a paper towel, weighed before feeding, and introduced into the feed chambers. After 2 h of feeding, any uneaten food was picked out with forceps, patted dry, and re-weighed to the nearest 0.01 g. As a control, food was weighed before and after a 2 h period in feeding chambers without crabs. A linear regression was run on the control pre- and post-soak weights; the line equation was used to correct for potential changes in mass of uneaten food in crab chambers.

Hemolymph Protein Density

Hemolymph protein density (HD) is related to a higher mass and lower moisture of the heart, hepatopancreas, and muscles and was used as an indicator of physiological condition (Wang and McGaw, 2014; Lorenzon et al., 2008; Moore et al., 2000; Hagerman, 1983). Hemolymph was collected by inserting an 18-gauge needle through the arthrodial membrane of a walking leg. Approximately 0.5 mL of hemolymph was collected and placed into the sample well of the Brix/RI-Check Digital Pocket Refractometer (Reichert Analytical Instruments, Depew, NY). The refractometer was calibrated with deionized water prior to each hemolymph measurement. The Refraction Index (RI) was recorded for each crab and converted to hemolymph protein density (in dg/100 mL) using the equation $HD=510(RI_{water}-RI_{hemolymph})-1.81$ (Sunderman, 1944; Wang and McGaw, 2014).

Weight

Crabs were weighed at the same time intervals that the HD was measured (Table A.3). Crabs were removed from the tanks and allowed 5-7 min to empty the branchial chambers. They were then dabbed dry with a cloth towel and weighed to the nearest 0.1 g using a digital scale (Sartorius LP 5200P). In each case crabs were weighed before they had consumed food, and any limb loss was accounted for.

Chelal Strength

A digital hanging scale (Brecknell Electrosampson) was mounted onto a clamp stand and fastened with zip ties. A block of wood with an immovable latch was clamped to the base of the stand, such that the latch was 0.5 cm away from the hook on the digital scale (Fig. 3.2). Crab claw strength was estimated based on the compression power of the right claw (predominately the larger of the two). A crab claw was placed with the bottom (fixed finger) on the immovable latch, and the top (dactyl) in the hook on the digital scale. The crab was agitated (via handling) until it pinched down, thereby pulling on the scale. The three strongest pinches for each claw were recorded to the nearest 0.1 kg, and the maximum pinch strength was used as the measure of strength of each crab. In some tests (34 out of 384) crab claw compression exceeded the maximum capacity of the scale (10 kg), in such cases their maximum strength was recorded as 10.1 kg, which was an underestimation but nonetheless represented a strength maxima. To compensate for claw size, claw strength was measured as kg compression per claw cm width (kg/cm), as claw width is the best predictor of claw strength (Block and Rebach, 1998). In this experiment crab strength was both a function of the physical ability of the crab to pinch down, and its willingness to do so in an experimental setting, therefore it is not an objective measure of strength alone but a composite of strength and aggressive propensity (see also Chapter 2).

Oxygen Uptake

Rates of oxygen uptake (mg O_2 kg^{-1} h^{-1}) were measured using an L-DAQ intermittent flow respirometry system (Loligo systems, Viborg, Denmark). This fully automated system is equipped with two pumps. The first pump continually flushes seawater through a cylindrical chamber (20 cm diameter x 12 cm depth) to ensure adequate water saturation during the non-measurement phase. For measurements, the flush pump is automatically turned off, the chamber is sealed and a second pump re-circulates the water through the chamber at a rate of 10 L/min ensuring that oxygen gradients do not build up within the chamber. Oxygen uptake (MO_2) was calculated during a 20 min decline in oxygen levels while the chamber was sealed, then the chamber was continuously flushed for 10 min between readings. Data were recorded on a Loligo data acquisition system which calculated MO_2 as mg O_2 kg^{-1} h^{-1}. Erroneous Oxygen uptake values (e.g., very low values caused by crabs sitting against outflow valve during recirculation) were manually removed from the record and an average was taken between the adjacent points to fill in the data. The experiments were carried out in constant dim light, which helped reduce possible diurnal rhythms (Rebach, 1985) and the apparatus was surrounded by black plastic sheeting to avoid visual disturbance to the animal. To measure maximum metabolic rate (MMR), the crabs were fastened to a weighted plastic platform using elastic bands. The crabs were placed inside the respirometry chambers in an inverted position which caused them to struggle vigorously trying to right themselves. The constraints were removed after 1-2 h and the crabs were allowed to settle while oxygen use was recorded for a further 18 h. The highest recording (obtained consistently during the first hour) was used as the MMR. The resting metabolic rate (RMR) was calculated as the lowest 10% of records post 10 hours of recording (Chabot et al., 2016). The metabolic scope was calculated as the difference between MMR and RMR.

Spontaneous Activity

The spontaneous activity of each crab was measured in a 30-cm diameter perforated bucket; 4 individual buckets were placed into a flow through water table with 15-cm depth of oxygenated sea water (11±1°C). A Panasonic WV-BP334 video camera was placed above the table, centered between all four buckets ensuring they were fully within the field of view. The camera was connected to a computer running Noldus Ethovision software (Noldus et al., 2001). The buckets were outlined as circular arenas and a pixel:centimeter calibration was carried out each day. Crabs were placed individually into buckets and recording began immediately: the software tracked each individual animal by thresholding the dark crab body against the white bucket and tracking the center point at 1 Hz. The distance moved per second was recorded as a time series, and the total distance moved in 20 h was used as the measure of crab activity. The distance moved per 30 min period was used to assess activity patterns over the 20 h record. The water table was surrounded by dark curtains to shield crabs from visual disturbance and recording was carried out under constant dim lighting to decrease potential diurnal rhythms (Rebach, 1985).

Statistical Analysis

Statistical analyses and graphs were performed in R-Studio v1.1.456 (RStudio Team, 2015). Graphs were made using the 'ggplot2' package (Wickham, 2016).

Diet and Hemolymph Protein Density: The amount crabs ate of each food type (kelp, mussel, and/or fish) and the total amount eaten over 15 weeks in each diet (mixed or mono), was assessed with Generalized Linear Mixed Effects models (GLMER) using

Crab ID as a random factor. Because the feeding data had a large number of 0 data cells (crabs that did not eat anything), a Poisson error distribution was used to account for non-normally distributed residuals. Models were tested against null models (no treatment effect) and Akaike Information Criterion (AIC) values were compared to determine if treatment components improved model fit; a ΔAIC of > 4 was used as a cut-off for the judgement for strong model improvement over the null (Burnham et al. 2011). The models were also assessed for residual homogeneity using residual-fit plots and residual normality was assessed using qq-norm plots. Predicted model fits with 95% confidence intervals were calculated using the 'AICcmodavg' package (Mazerolle, 2019). For models with normally distributed residuals, General Linear Models (GLMs) were used to test relationships between traits measured, and models were assessed for homogeneity and normality of residuals using residual-fit plots and quantile-quantile plots. Chi-squared tests were used to assess differences in surviving crabs between diet treatments and weight loss/gain between diet treatments.

The effect of long-term holding on RMR, MMR, Metabolic Scope, and Spontaneous Activity: To assess the effect of long term holding on RMR (mg O_2/kg/hr), MMR (mg O_2/kg/hr), and spontaneous activity (distance, in m), I used the 'nlme' package (Pinheiro et al, 2020) to run Generalized Linear Mixed Effects models (GLMER) using time (weeks since collection) as the independent variable and Crab ID as the random factor. For spontaneous activity, distance measures (m) were log-transformed to improve model normality. Time (weeks since collection) was used as a continuous variable to test for directional effects of holding time, and a discrete variable to assess whether traits varied between weeks. A Tukey HSD post-hoc test was run to test pair-wise comparisons between weeks. Based on preliminary plots, variance was hypothesized to change with time in RMR, MMR, and spontaneous activity. AIC values of Generalized Least Squares models with and without a variance structure component were compared. In models where the AIC was lower when the variance structure was included (ΔAIC > 4), the variance was considered to have changed over time (Burnham et al, 2011).

Effect of handling stress on metabolic rate and spontaneous activity: To test the acute effect of translocating crabs from holding tanks to test chambers, I ran a Generalized Additive Model (GAM) using activity or oxygen uptake as dependent variables and time in the test chamber (measured at 30 min intervals) as the independent variable. A fitted line was produced, and the difference between values predicted by the GAM fit at $t+1$ and t were calculated. Where the difference between two sequential time-points reached 0 the activity/oxygen uptake were determined to have stabilized, suggesting settling post translocation to the testing chamber.

Results

The amount of food consumed by the crabs was influenced by the diet and food type. Within the mixed diet, the model including food type (fish, kelp, or mussel) was a stronger predictor of amount eaten than the null model (ΔAIC= 122491.4). Crabs ate more fish (GLMER, model predicted mean = 0.21 g, 95% CI [0.18, 0.24]) than kelp (GLMER, model predicted mean = 0.031 g, 95% CI [0.026, 0.036]), and more mussel (GLMER, model predicted mean = 1.96 g, 95%CI [1.68, 2.29]) than either kelp or fish

(Fig. 3.3A). When comparing the amount of fish eaten between the mixed and mono diet, including the diet type improved the model fit over the null (ΔAIC = 22.44). Crabs in the mono diet ate more fish (GLMER, model predicted mean = 0.76 g, 95% CI [0.43, 1.33]) than crabs in the mixed diet (GLMER, model predicted mean = 0.08 g, 95% CI [0.046, 0.142]). When comparing total amount eaten in the mixed and mono diets (Fig. 3.3A, right panel), including the diet improved model fit over the null (ΔAIC = 16.91); crabs overall ate 3.14 times more food in the mixed diet (GLMER, model predicted mean = 2.20 g, 95% CI [1.64, 2.97]) than in the mono diet (GLMER, model predicted mean = 0.79 g, 95% CI [0.59, 1.07]). The proportion of crabs eating more than 0.29 g per sitting was also calculated: this value was used because it was the average change in food mass between pre- and post-2 h soak of control "feeding" (no crabs). Crabs fed the mixed diet ate >0.29 g 90 % of the time, whereas crabs maintained on the mono diet ate >0.29 g 65 % of the time (Table 3.2).

Holding time, measured as number of weeks since collection, did not have a strong effect on the amount eaten for either diet (Fig. 3.3B). Including week since collection did not substantially improve the model fit relative to the null model for either the mixed (ΔAIC= 0.742) or mono diets (ΔAIC= 0.274), and weeks since collection did not have a significant effect on the amount eaten (GLMER, mixed diet: *p*=0.125, model estimate ±SE = -0.021± 0.014; mono diet: *p*=0.083, model estimate ± SE= 0.05±0.029). However, the proportion of crabs that ate >0.29 g gradually increased in the mono diet (from 43% during week 0 to 88% in week 15); in contrast there was little change in crabs that ate > 0.29 g in the mixed diet (93% in week 0, 83% in week 15) (Table 3.2). Over the course of the 15-week experimental period, 8 crabs fed the mono diet and 5 crabs fed the mixed diet died (Fig. 3.3C). All crabs in the mixed diet survived 6 weeks of holding, while 5 crabs died in the mono diet during this time. Despite this, there was no significant difference in number of crabs that died as a function of diet type (chi-square=1.25, p=0.26).

Hemolymph Density

There was a negative correlation between the initial HD (pre-experimental, week 0) and the average amount eaten by individual crabs (of both diets) over the course of 15 weeks (Regression, R^2=0.48, $F_{1,45}$=37.01, p<0.001) (Fig. 3.4A). The initial HD did not vary significantly between diets (ANOVA, $F_{1,43}$ =2.58, p=0.11). In addition, while there was an overall trend towards a decrease in mean HD over time, there was no significant difference in HD between week 0 and week 15 (t-test, t-value=-1.6, p=0.11). There was no statistically significant effect of diet (ANOVA, $F_{1,43}$= 0.3269, p=0.57) or an interaction between diet and amount eaten (ANOVA, $F_{1,43}$= 0.0482, p=0.82) on ΔHD ($HD_{week15} - HD_{week0}$). However, the ΔHD was positively correlated with the average amount eaten by each crab (Regression, R^2=0.53, $F_{1,43}$=51.32, p<0.001); crabs that consumed high amounts of food exhibited an increase in HD, while HD decreased in crabs that consumed less than about 2 g of food (Fig. 3.4B). Overall, crabs that had a lower HD at the start of trials (week 0) ate more and thus exhibited an increase in HD and crabs with a higher initial HD tended to show a decrease in HD over time. This resulted in a decrease in the variation of HD over time (Week 0 mean HD±SD=4.22±1.59 dg/L, Week 15 mean HD±SD =3.96±1.11 dg/L). The HD of the crabs measured at Week 0 was also significantly related to the ΔWeight (loss or gain) between Week 0 and Week 15 (ANOVA, $F_{1,44}$=72.58, p<0.001); crabs with a higher initial HD on average lost weight, whereas crabs with a lower initial HD on average gained weight (Fig. 3.4C). Average amount eaten over 15 weeks also had a significant effect on weight loss/gain (Regression, $F_{1,45}$=57.15, p<0.001), with crabs that ate more on average gaining weight and crabs that ate less on average losing weight (Fig. 3.4D). Mean HD was also positively correlated to mean crab claw strength (Regression, R^2=0.25, $F_{1,54}$=18.05, p<0.001) (Fig. 3.5): crabs with a higher HD were able to generate greater closure forces with the chelae.

Metabolic Rate

The type of diet that the crabs were offered did not have a significant effect on any of the metabolic parameters during the 15-week period (ANOVAs; RMR: $F_{1,18}$=0.2177, p=0.64; MMR: $F_{1,18}$=1.021, p=0.32; Scope: $F_{1,18}$=1.16, p=0.29), and so data for the different diet types was combined. There was a directional effect of holding time (weeks since collection as a continuous variable) on the RMR (ANOVA, $F_{1,99}$ = 4.67, p=0.03) which was evident as an overall decline during the 15-week period (Fig. 3.6A). To assess differences between individual weeks, the weeks since collection was also considered as a categorical variable. The RMR measured at week 0 (49.18±0.81 SE mg O_2/kg/hr) was significantly higher than all subsequent weeks (Table 3.3); thereafter RMR did not vary significantly by week (Tukey HSD post hoc test, Table A.4). Including the variance structure in the RMR model improved model fit (ΔAIC = 5.15); in all weeks RMR variance was lower than Week 0, except Week 2 and Week 15 (Table 3.4).

No significant directional effects of week since collection were found for the MMR (ANOVA, $F_{1,120}$= 0.4218, p=0.51, Fig. 3.6B), or the metabolic scope (ANOVA, $F_{1,120}$= 0.99, p=0.3201, Fig. 3.6C). However, when weeks since collection was considered as a categorical variable there was a significant effect on both MMR (ANOVA, $F_{7,114}$= 5.59, p<0.001) and metabolic scope (ANOVA, $F_{7,114}$= 4.15, p<0.001) but the week-to-week differences in these metrics were somewhat random (Fig. 3.6B-C, Table 3.3). Unlike for RMR, including variance did not improve the model fit for MMR (ΔAIC = -2.63) and scope (ΔAIC = -10.58), therefore variance was stable over time.

In all experiments transferring the crabs from the holding tanks into the respirometry chamber had a visible effect on the pattern of oxygen uptake (Fig. 3.7). This was observed as elevated oxygen uptake at the start of each experiment that declined gradually over the 20 h experimental period to reach stable levels (Fig. 3.7). A Generalized Additive Model (GAM) fit (Fig. 3.7, solid black line) was used to determine when the change in predicted oxygen uptake between adjacent timepoints reached 0 (i.e., had stabilised) each week (Fig. 3.7, dashed line). At week 0, which was also the time when the highest RMR was recorded (Fig. 3.7, Table 53.), a slow decrease in oxygen uptake over time was measured (Fig. 3.7, week 0), and the model predicted line stabilized 15.5 h after transfer into the test chamber (Table 3.5). In contrast the lowest mean RMR was measured during week 6, and here the oxygen had uptake largely reached stable values after 10 h (Fig. 3.7). The mean time until oxygen uptake stabilised (all weeks) was 12.75 ± 0.33 SE h post translocation (Table 3.5).

Spontaneous Activity

There was no significant effect of diet type on spontaneous activity (ANOVA, $F_{1,18}$=0.0031, p=0.95), and diet was thus removed as a factor in the analysis. Spontaneous activity, measured as distance travelled (m) in 20 h (log transformed for model normality), varied significantly by week since collection as a continuous variable, indicating directional change (ANOVA, $F_{1,113}$=35.59, p<0.001). The lowest distance travelled (90.18 ± 2.98 SE m, Table 3.6) was measured at week 0, and crabs were most active during week 10 (241.10 ± 25.94 SE m, Table 3.6). The median activity rates measured during week 0 were significantly lower than all activity rates during all other weeks except those recorded during week 1, and activity recorded during week 1 was significantly lower than all weeks except during week 2 (Table 3.5, Fig. 3.8A).

In addition to an increase in the median distance travelled after week 0, there was also a visible increase in variation of distances travelled (Fig. 3.8A), and including the variance structure considerably improved the model fit (ΔAIC = 130.80). Compared with week 0, the variance was considerably higher in weeks 6, 8, and 15 (Table 3.4). Further, visualization of activity in individual crabs showed a biphasic pattern (Fig. 3.8B) which accounted for the increase in variation. For 14 out of the 19 crabs, activity rates were low during week 0 and had only a slight increase during the following weeks. In contrast, for 5 out of the 19 crabs (CrabID: 23, 28, 33, 34, 36) activity was consistently and considerably higher in later measurements (Fig. 3.8B).

In line with changes in oxygen uptake, crab spontaneous activity was highest immediately after the crabs were introduced to the chambers and decreased thereafter during the 20 h experimental period (Fig. 3.9). A Generalized Additive Model fit (Fig.

3.9, black line) was used to determine the time when the activity stabilised (Table 3.5). The stabilisation time was variable, and ranged from 12 hours post translocation during week 10, to 3.5 hours post translocation during week 15. The median time until activity stabilized was 7.31 ± 0.37 h post translocation.

Discussion

Effects of dietary homogeneity

When crabs are brought into the lab for experiments, most studies (73%) report that the crabs were fed only one food type, with bivalves (46%) or fish (33%) being the most common food (Table 3.1, Table A.2). It is possible that limiting dietary heterogeneity may impact captive crab performance (i.e., ability to maintain homeostasis) by decreasing nutrient diversity and/or offering suboptimal diets that result in lowered food consumption rates (Brito et al. 2000; Belgrad and Griffen, 2016).

The main effect of providing a "mixed" diet (mussel, fish, and kelp) increased feeding rates by a factor of 3, compared to a homogenous "mono" diet of just fish. While direct nutrient absorption tests were not conducted, it is unlikely that nutrient/caloric content played a role, and more likely that crabs displayed a strong preference for a familiar food type (mussel). Crabs given the mixed diet ate about 9 times more mussel tissues than fish, and did not eat measurable amounts of kelp. Although some algae is found in the gut contents of *C. irroratus* (Griffen and Riley, 2015; Hudon and Lamarche, 1989), their disinterest for kelp in the lab is also reported by Sungail et al. (2013) who suggest that wild *C. irroratus* eat soft decomposing algae, or consume algae unintentionally while eating algal fouling epiphytes growing on desired food items (e.g., barnacles). Therefore, providing fresh kelp does not improve crab energy intake rates, and does not serve to diversify nutrient intake. Previous lab and field studies also report that mussel is a preferred food type of crabs (Griffen and Riley, 2015; Sungail et al., 2013; McGaw and Penney, 2014). It is unlikely that mussel is preferred to maximise energy intake because the caloric content of herring *Clupea harengus* (wet = 2.3 kcal/g, dry = 6.4-7.15 kcal/g; Härkönen and Jørgensen, 1991; Perez, 1994; Fisher et al., 1992) is about 35% higher than that of the blue mussel *Mytilus edulis* (wet = 1.52 kcal/g, dry= 4-5.5 kcal/g; Petersen, 1981; Gardner and Thomas, 1987; Sungail et al., 2013). Mussel is more likely to be a familiar food source to *C. irroratus* (Griffen and Riley, 2015), which may influence preference by olfactory attraction (Ristvey and Rebach, 1999). Overall, one clear advantage of dietary heterogeneity is that dietary preference can be elucidated and a preferred diet can be proided. Importantly, mussel provided in this experiment was removed from its shell, considerably decreasing the resource cost (Sungail et al., 2013; Matheson and Gagnon, 2012; Hughes and Seed, 1981). In a more natural environment, a fish carcass might be a valuable source of food due to lower handling cost.

Fish is frequently provided to captive crabs as the only food source, but I found that crabs fed only fish (i.e., mono diet) ate considerably less than those provided a mixed diet, especially at the start of the experiment. Even though no other food was available to mono diet crabs, immediately post-collection only approximately 40% ate considerable amounts (>0.29 g) of food (compared to 90% in the mixed diet). By the end of three months, the number of mono-diet crabs consuming a considerable amount of fish gradually increased to 88%. This suggests that fish was not a preferred food, and that crabs avoided eating it until food deprivation made eating a necessity. Omnivorous crabs fed exclusively on a fish diet (as detailed in other articles, Table 3.1 & Table A.2), may therefore experience symptoms of food deprivation when they are maintained in the lab. This might not influence homeostasis *per se*, as wild crabs experience frequent periods of natural food deprivation (Sacristán et al., 2019; Roberts, 1957; Schultz and Shirley, 1997). For example, Griffen and Riley (2015), found that only 15% wild caught *C. irroratus* had food in their guts. Nonetheless, studies should consider that crabs given a suboptimal diet may be underfed.

Hemolymph protein density as a proxy for energy stores?

Unexpectedly, I found that hemolymph protein density (HD) could accurately predict food intake rates, which might bear relevance to a crab's competitive status. The initial HD (measured when crabs were first collected - week 0) was negatively correlated to the average amount crabs would eat (per meal) during three subsequent months in captivity: i.e., crabs that had higher HD at week 0 ate significantly less food during their time in captivity and *vice versa*. It is unclear why HD might determine feeding propensity. Crustacean hemolymph contains a diverse array of proteins, including, but not limited to, hemocyanin, coagulogen, apohemocyanin, hormones, enzymes and lipoproteins (Depledge and Bjerregaard, 1989). Previous work showed that HD can be influenced by factors such as nutritional condition (Moore et al., 2000; Wang and McGaw, 2014), salinity changes (Perazzolo et al., 2002), stress (Lorenzon et al., 2008), immune response (Fredrick and Ravichandran, 2012; Hall, 1999), and moult stage (Wang and McGaw, 2014; Hagerman, 1983). Additionally, HD has been hypothesized to represent stored protein energy (Sánches-Paz et al., 2006; Depledge and Bjerregaard, 1989; Busselen, 1970). Because crabs with a higher HD ate less, it is possible that crabs with sufficient energy stores may choose to forgo eating if they are stressed post capture. Such an effect as was found in brown trout *Salmo trutta* where translocation/handling stress caused a decrease in food intake (Pickering et al., 1982). Food intake is therefore not only determined by the quality of food provided, but the extent to which a crab can rely on stored energy to sustain homeostasis. As found by previous studies (Moore et al., 2000; Wang and McGaw, 2014), the amount crabs ate was positively correlated with the change in HD between week 0 and week 15 (ΔHD); crabs that ate more than about 2 g (~1.5% of body mass) per meal exhibited an increase in HD. This corroborates the supposition that HD is representative of feeding history, and therefore energy storage. One of the assumptions of maintaining animals in captivity is that trait expression diversity is reduced due to a reduction in environmental heterogeneity. Indeed, I found a significant reduction in variation in HD among individual animals by the end of the 15-week period. This suggests that in the wild, natural processes such as intraspecific competition and foraging efficacy diversify the access of individuals to high quality food and thereby increase the variation in HD. Therefore, at least with regards to HD, a period of acclimation has the effect of removing previous environmental influences. What specifically drives foraging efficacy differences is unknown, but because mean HD was significantly correlated to chelal compression strength, it is possible that stronger crabs are more competitive (Sneddon, 2000) and therefore outcompete weaker crabs for food.

Effect of diet on labile traits and survival

Contrary to expectations, diet did not have a significant effect on any metabolic rate measures (RMR, MMR, metabolic scope), or spontaneous activity. Therefore, even crabs fed a suboptimal diet ate sufficient amounts of food to maintain their basic metabolic machinery and were not undergoing any form of "starvation" as this is known to depress metabolic rate in crustaceans (Wallace, 1973; Roberts, 1957; Meyer and Oettl, 1995). Other physiological changes not measured here are, however, possible. While the final mortality rates were not significantly different between diets (mixed diet: 17%, mono diet: 27%), the mortality rate was more rapid in the mono diet. Five crabs (17%) died in the first 6 weeks in the mono diet, while all crabs in the mixed diet survived during this time. It could be that lab holding coupled with the stress of a suboptimal diet affects health and thus survival. Providing a diversified diet may improve long-term survival rates (>3 months), but *C. irroratus* deal well with low food intake and maintaining crabs on a suboptimal diet without adverse effects, at least with respect to the measured traits here, appears possible.

Effects of prolonged holding and translocation on metabolic rate

Capturing animals from the wild and maintaining them in a stable and regimented lab environment is a common procedure but can be a stress-inducing procedure, or at least, does not represent wild conditions (reviews by Dickens et al., 2010 & Morgan and Tromborg, 2007). Conducting experiments immediately post collection risks measuring physiological or behavioural responses related to prior environmental history and/or the transport and handling stress (Stoner, 2012). Subsequently holding animals for prolonged periods in stable conditions can also affect animal welfare (i.e., the ability of maintaining homeostasis; Broom, 2011) and labile trait expression (e.g., Callaghan et al., 2016; Anderson et al., 2011). Here both holding time in the lab and transfer into experimental chambers influenced the metabolic rate and spontaneous activity suggesting the length of holding/setting periods is an important consideration for labile trait studies.

When crabs were translocated from the field and brought into the lab, there was a detectable effect on the metabolic rate. RMR, a measure of the basal metabolic requirements of a post-absorptive, resting animal, was on average about 30% higher within the first four days post-collection compared to 1 week of holding, and decreased by almost 50% after 6 weeks of holding (lowest RMR measured). Because higher oxygen uptake is indicative of stress in crustaceans (Jouve-Duhamel and Truchot, 1985; Stoner 2012), the capture, handling, and translocation to a novel environment likely increased the initial basal metabolic demand of crabs. MMR and metabolic scope varied significantly week by week, but there was no directional decline or increase during prolonged holding. Nonetheless, the metabolic scope was significantly lower immediately post-collection as compared to most remaining weeks, likely due to the high RMR and relatively lower MMR.

Overall, although I found some differences in metabolic parameters over time, I did not find a noticeable decline in metabolic scope as reported by Houlihan and Mathers (1985) in *C. maenas* after just 2-3 weeks of holding. While there could be some discrepancy among species, it is more likely that the different holding conditions (Table 3.7), are responsible. In Houlihan and Mathers (1985) *C. maenas* were maintained in a recirculating seawater system distant from the ocean whereby water was “cleaned” through filters and sumps (Aberdeen University, pers. comm.). In contrast, our current flow-through seawater system pumped seawater directly from the ocean for a one-time use. Recirculating systems can rapidly accumulate waste products and bacterial load, especially if stocked to high density (Noble and Summerfelt, 1996; Hoang et al., 2018). This likely accounts for the discrepancy between my study and this earlier study and underscores the importance of specific details of holding systems on animal health.

Metabolic rate was highest immediately after animals were placed into respirometry chambers, and gradually declined over 9 to 16 h before stabilizing. This increase in metabolic rate is well known and part of an overall stress response associated with acute handling and transfer between water and air (McMahon et al., 1979; Crear and Forteath, 2001; Haukenes et al., 2009). As such, most studies do allow a settling time in experimental apparatus after handling, although as mentioned earlier there appears to be very little justification for specific times (Table A.1). The mean settling time of 12.75 ± 0.3 h obtained here was slightly higher than those reported for the crayfish *Panulirus cygnus*, where oxygen uptake measures returned to pre-stressed conditions after 5-8 hours (Crear and Forteath, 2001), but within the range of 8-20 h reported in vigorously exercised *Cancer magister* (McMahon et al., 1979). Nonetheless these studies have roughly similar settling times, which suggests that settling time is fairly consistent across decapods. During the first two weeks of holding, settling times (15-16.5 h) were roughly 5 h longer than during subsequent weeks (9-12.5 h). Therefore, there was a synergistic effect, whereby a recent transfer from the field to the lab and the transfer into respirometry chambers cumulatively increased the metabolic rate. It should be noted that because I conducted repeat measurements on the same individuals, the decrease in settling time length could be due to habituation to experimental procedures (Biro, 2012). Repeating the experiment on naïve crabs held for several weeks would resolve this issue, and is suggested as a future direction. Additionally, determining the repeatability of individuals' settling time would be important to design experimental procedures based on individual with the longest settling times; this would ensure that all, rather than most, inividuuls are settled.

Effects of prolonged holding and translocation on spontaneous activity

Spontaneous activity also varied significantly by week since collection, with the mean distance travelled in 20 h gradually increasing until the 4th week. This result was opposite from Houlihan and Mathers (1985), who found that activity declined in *C. maenas* after 2-3 weeks of holding; as discussed above this was likely due to deterioration of the green crabs in poor holding conditions. Although activity was lower in week 0 than week 15 in all but one surviving individual, the degree of increase was not equal. Seven individuals barely increased in their distance travelled, while others increased it by 14-fold. This resulted in the variance of activity rates being 22-27% higher after 8-15 weeks of holding compared to week 0 variance. There was a biphasic response and the overall increase in activity rates was primarily due to 5 individuals that showed consistently high activity after 4 weeks of holding. The fact that activity rates were consistently high from week to week in the same crabs suggests that animal personality differences emerge after prolonged holding. While lab holding may reduce the effects of prior environmental history (as was seen in HD) it may not abolish, but rather accentuate, personality differences. Alternately, it was not holding time that influenced activity rates, but because *C. irroratus* migrate in the fall (Comeau et al., 2012; Haefner and van Engel, 1975), it is possible that the increase in activity represented an endogenous seasonal rhythm that some individuals responded to more rapidly than others.

Similar to the metabolic rate measures, the activity levels were highest immediately post transfer into the experimental chambers, declined steadily over 3.5-12 h, and stabilized thereafter. This increased activity was likely a general stress response caused by handling and transfer of crabs (Paterson, 1993; Jensen et al., 2013). Interestingly, while locomotor activity was lowest after bringing animals in to the lab, it was highest immediately post transfer into activity chambers; therefore two different stressors had opposite effects on locomotor activity. This may be because locomotor activity represents different behaviours depending on the context. Gherardi et al. (1988) for example, distinguish between short foraging movements and long "wandering" movements in the crab *Potamon fluviatile*. Changing the context from introducing the crabs to a completely novel habitat to later trials when they became habituated to the testing chamber may therefore change the behavioural context (Edwards, 2013). It is also possible that the different (non-directional) settling times varied during the 15 weeks as a result of endogenous rhythms that were not completely removed during holding in the lab. Due to logistics, I did not start activity experiments at the exact same time each day (broadly, during early afternoon), and it is known that the time of day that an experiment is started can influence both the physiology and behaviour by unmasking circadian rhythms (Scott et al., 2018).

Conclusions

My goal were to: 1. test whether long-term holding in a lab environment resulted in the decline of animal condition as measured by activity and metabolic rate measures, 2. quantify duration of acute stress response after handling and introduction to experimental chambers, and determine appropriate holding/settlining times and 3. Test whether a "mixed" diet vs. a homogenous "mono" diet has an effect on crab labile traits and overall condition.

I found that prolonged lab holding did not result in a considerable decline in crab survival or condition, as measured by activity and metabolic rate measures. However, time stored in the lab does affect labile physiological and behavioural traits, but the effect depends on the trait and the time held in the lab. Using the current data I recommend that studies hold crabs for approximately 4 weeks post-collection and allow 16 h for settling post transfer into experimental chambers.

I showed that a heterogenous diet can improve feeding rates in *C. irroratus*, but does not appear to elicit noticeable effects on crab physiology or behaviour. Additionally, I suggest that HD may be used to predict food intake, weight change, and chelal compression strength, and that HD might be related to competitive ability in the field. The mean HD was correlated to the average chelal compression strength of crabs, likely because higher HD relates to higher muscle mass (Wang and McGaw, 2014). As competitive ability is related to chelal strength (Sneddon et al., 2000), the relative HD within a population could potentially be used to determine competitive ability (assuming other sources of HD variation are accounted for).

Finally, I showcase that labile traits are affected in diverse and complex ways by laboratory-associated procedures. Because this experiment was carried out during one season and on one species from one population, extrapolations should be done with caution. I therefore recommend that future studies consider quantifying the effects of lab procedures prior to extrapolating lab findings to natural systems.

Tables

Table 3.1. Summary of meta-analysis on food types offered to a variety of crab species that were held and fed in the laboratory. Data was gathered from articles published during the past 2 decades (2000-2020). Mixed diet refers to more than one food type provided, while mono diet refers to a single food type provided. References, species types, and specific foods provided can be see in Table A.2.

Food Type	Proportion of Studies
Bivalves	46%
Fish	33%
Commercial Feed	12%
Squid	10%
Shrimp	8%
Algae	6%
Other	6%
Mono diet	**73%**
Mixed diet	**27%**

Table 3.2. Percent of crabs (both diets and all weeks), that ate > 0.29 g (average error in measurement of control feeds without crabs). The percentage of crabs that consumed >0.29 g of the mixed diet remained stable, but increased over time for crabs fed the mono diet. On average crabs in the mixed diet were more likely to eat some food than those in the mono diet.

	Percent that ate >0.29 g	
Week	Mixed	Mono
0	93%	43%
1	93%	39%
1.5	90%	39%
2	97%	52%
3	97%	56%
4	87%	58%
5	90%	62%
6	87%	64%
7	86%	84%
9	93%	95%
10	81%	71%
14	92%	100%
15	83%	88%
Average	90%	65%

Table 3.3. Resting Metabolic Rates (RMR), Maximal Metabolic Rates (MMR) and Metabolic Scope. The data are the mean ±SE values for each week. Number of crabs used for each trial are shown as n.

Week	**Mean RMR ± SE (mg O2/kg/hr)**	**Mean MMR ± SE (mg O2/kg/hr)**	**Mean Scope ± SE (mg O2/kg/hr)**
0	49.18±0.81, n=16	130.49±1.68, n=16	83.21±1.69, n=16
1	34.16±0.84, n=15	160.52±1.72, n=20	123.54±1.76, n=20
2	29.08±1.15, n=13	161.23±1.89, n=19	123.42±2.61, n=19
4	27.18±0.54, n=19	147.08±1.34, n=19	120.46±1.46, n=19
6	25.66±0.39, n=17	142.11±1.10, n=19	116.76±1.3, n=19
8	27.10±0.58, n=13	137.70±1.43, n=17	107.61±1.82, n=17
10	29.57±0.65, n=15	131.93±1.66, n=15	103.28±1.84, n=15
15	34.99±1.44, n=12	156.79±1.22, n=16	122.81±1.82, n=16
Average	32.11± 0.95, n=8	145.98±1.55, n=8	112.63±1.78 n=8

Table 3.4. Relative variance for GLMMs where variance structure considerably improved model fit. Variance is compared proportional to week 0, where a value <1 means a variance decrease, and >1 means a variance increase.

Week	**Variance relative to Week 0**	
	RMR	Activity
0	1	1
1	0.899	0.994
2	1.070	1.003
4	0.711	0.911
6	0.447	1.044
8	0.544	1.274
10	0.689	1.265
15	1.226	1.221

Table 3.5. Time for oxygen uptake and activity rates to stabilize after being introduced into the apparatus (e.g., reaching stable values without further change). Note that separate crabs were used for oxygen consumption and activity. Data was based on running General Additive Mixed Model predictions and estimating where the difference between *t* and *t+1* was 0. The Mean time ± Standard Error of all weeks is given at the bottom.

Week	**Time until stabilization (h)**	
	Oxygen Uptake	Activity
0	15.5	10.5
1	16.5	4.5
2	15	5
4	12.5	7.5
6	10	8.5
8	11.5	7
10	12	12
15	9	3.5
Mean ± SE	**12.75 ± 0.33**	**7.31 ± 0.37**

Table 3.6. Spontaneous activity rates (distance travelled over 20h, m) for each measurement week. Data are the median ± SE. Number of crabs used for each mean are shown as n.

Week	**Median Distance ± SE (m)**
0	90.18±2.98, n=16
1	133.21±2.44, n=19
2	165.73±3.48, n=19
4	212.74±7.99, n=19
6	233.96±11.16, n=19
8	135.36±15.71, n=18
10	241.10±25.94, n=17
15	187.47±21.80, n=15
Average	174.96±6.68, n=8

Table 3.7. Comparison of holding conditions used for *Carcinus maenas* Houlihan and Mathers (1985) and the present study for *Cancer irroratus*.

	Houlian and Mathers (1985)	**Present Study**
Crab Species	*Carcinus maenas*	*Cancer irroratus*
Collection Location	Scotland	Newfoundland, Canada
Sample Size	310	60
Holding Time	0-3 weeks	0-3 months
Experimental design	Three distinct groups (no repeated measures)	Repeated measures
Tank Size	1x1 m tank	1.5 x 1 m (crabs stored in 15x10 cm individual perforated containers)
Aeration & Flow Through	Recirculated aerated water	Direct flow through of ocean seawater
Photoperiod	12L:12D	Constant dim
Holding Temperature	14-18℃	11±1℃
Crab Sex	Male and Female	Male Only
Crab Mass Limits	80-105 g	79-179 g
Feeding	Squid or mussel (random)	Mixed diet= kelp, mussel, herring Mono diet= herring only

Figures

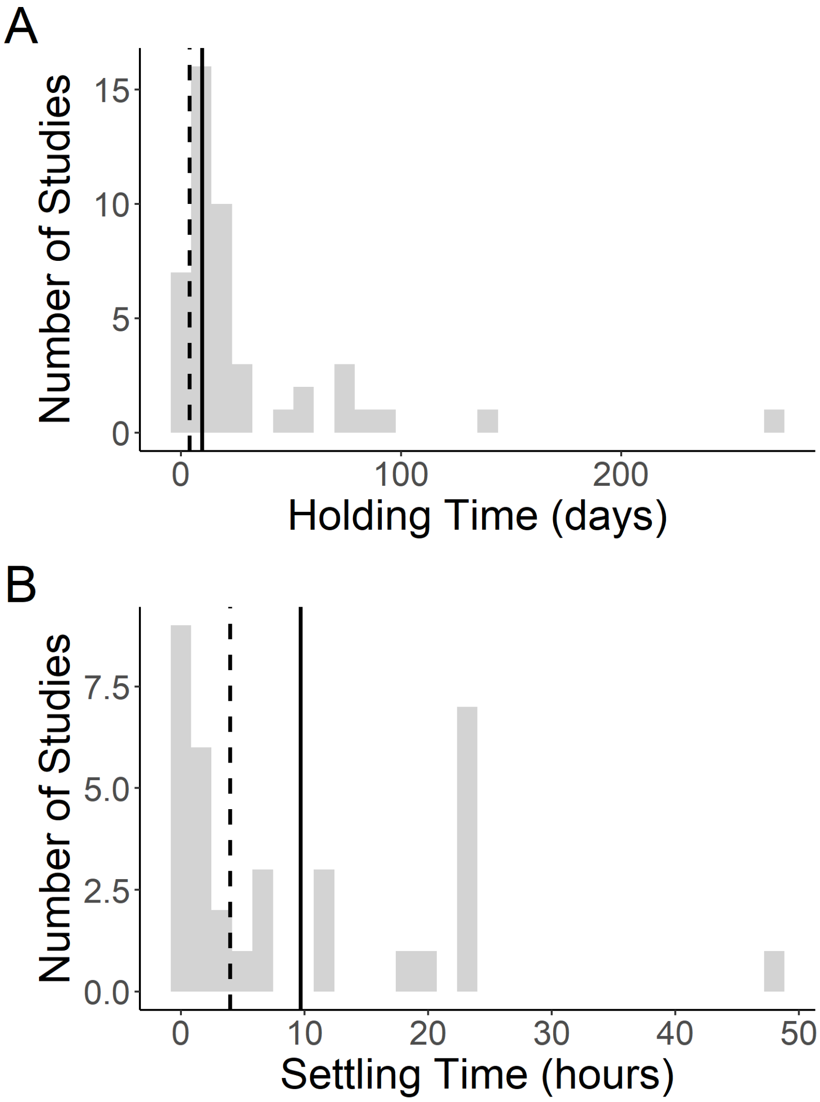

Figure 3.1. Histograms representing the holding (**A**) and settling (**B**) times reported in studies on labile traits in crabs from 54 papers published between 2000-2020. Dashed line represents the median, solid line represents the mean. There was a holding range of 1-270 days, and 2 of the holding times were cited. There was a settling/acclimation period range of 0 to 48 hours, and 5 studies included citations or justification. For meta-analysis details and citations, consult Table A.1.

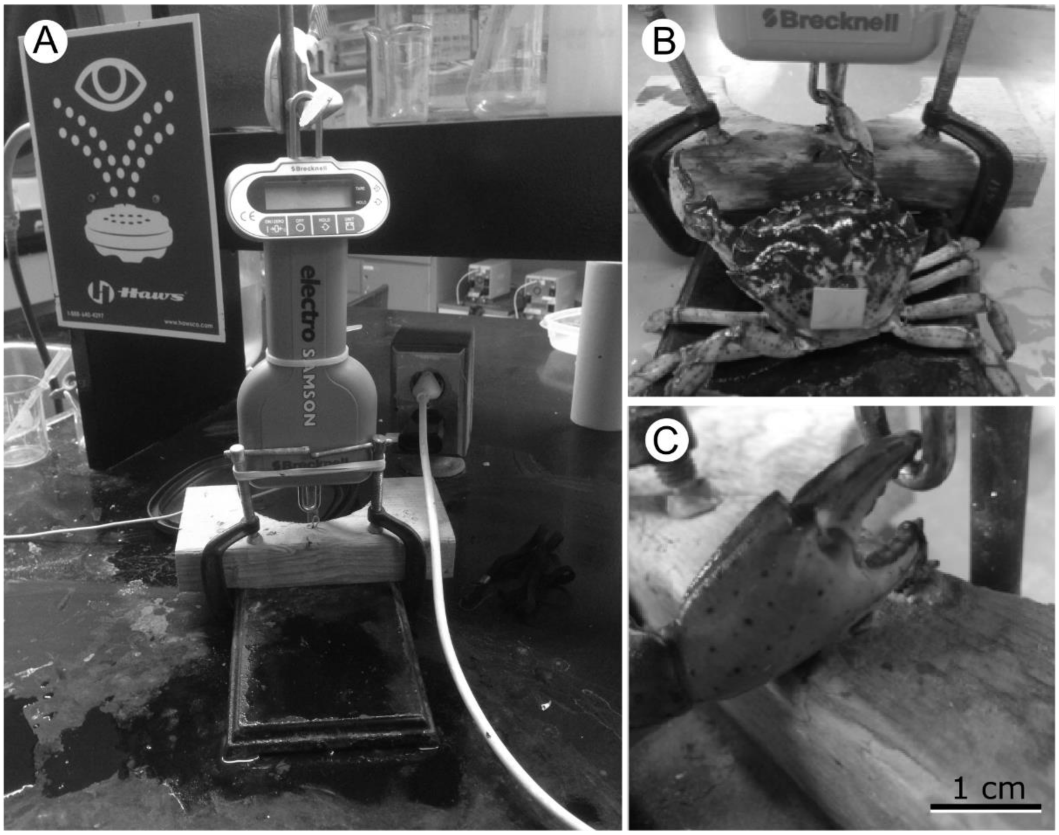

Figure 3.2. Chelal strength measuring apparatus. **A.** the scale setup on a clamp stand. **B.** Placement of *C. maenas* on strength measuring apparatus. **C.** Fixed finger of claw in immovable latch and dactyl on scale latch.

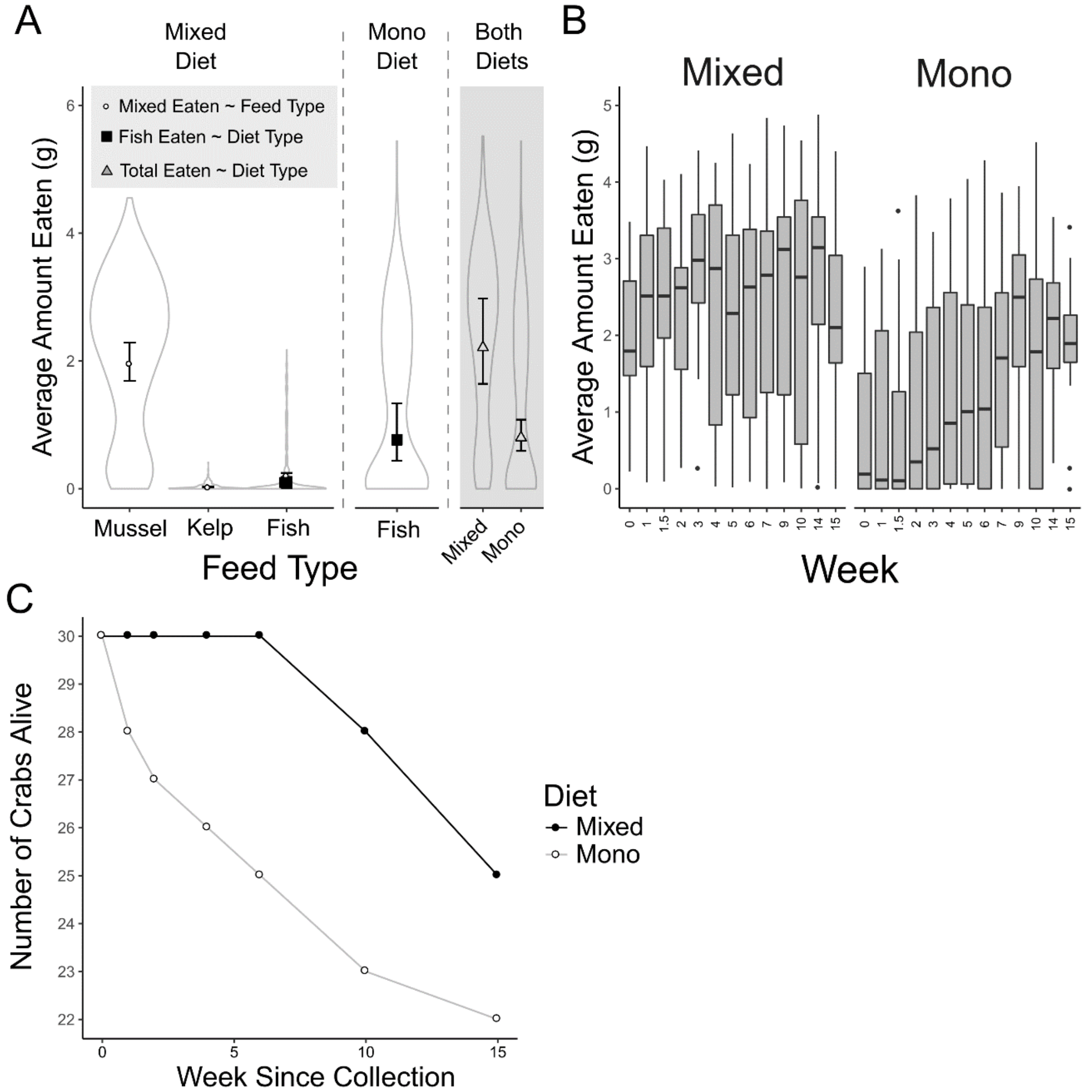

Figure 3.3. Effect of diet type. **A)** Violin plots representing the distribution of the amount of each food type eaten (fish, kelp, mussel) in each diet (mixed or mono). Symbols and error bars represent model predicted means with 95% CI. **B)** Amount of food eaten (g) by crabs over the 15-week experimental period represented as boxplots for each diet. Boxplots show medians (horizontal line), 25th-75th percentile quartiles (grey), 1.5*IQR (whiskers), and outliers (black points) **C)** Number of crabs alive in each diet treatment during the 15-week experimental period.

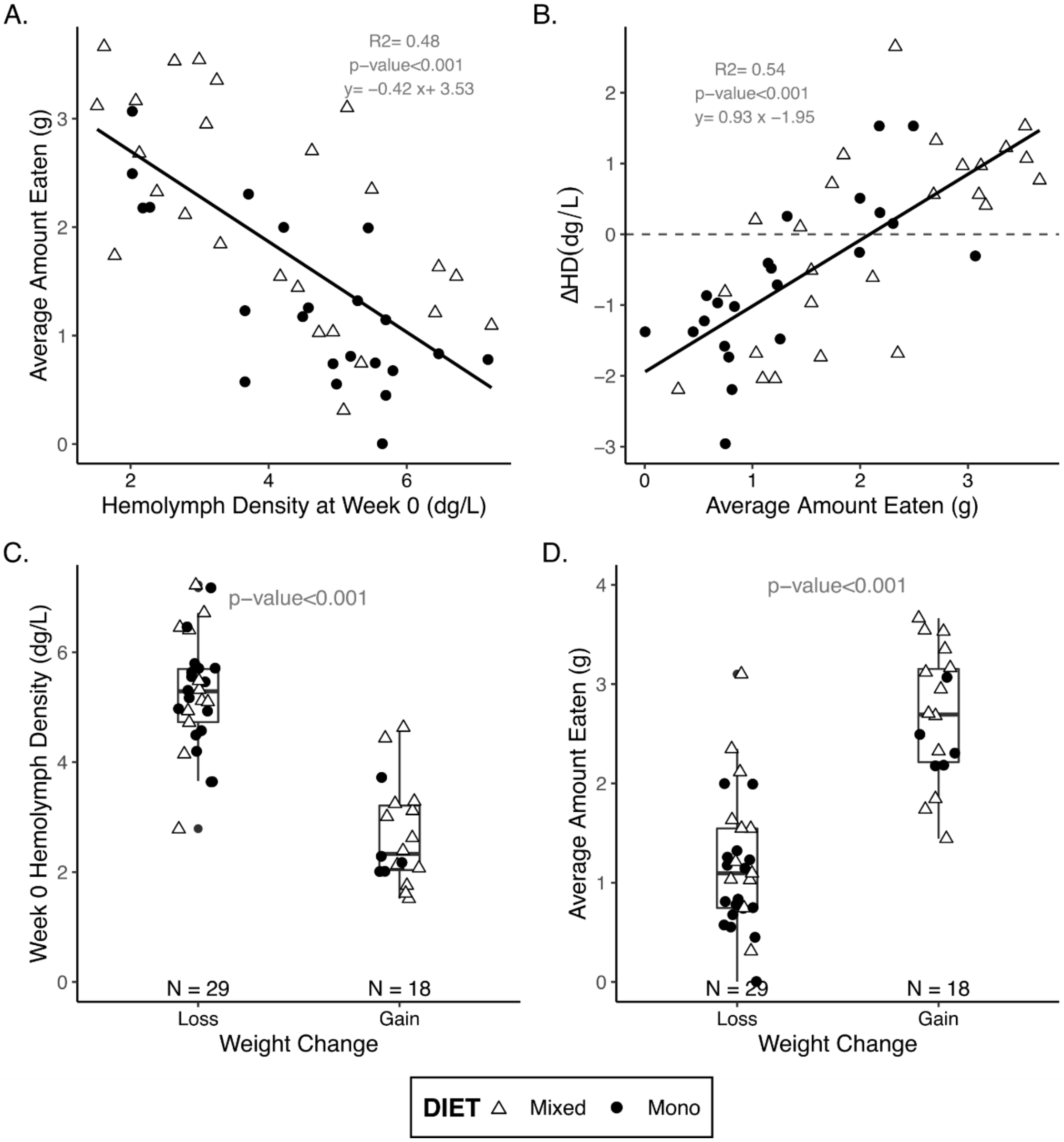

Figure 3.4. Relationship between hemolymph protein density (HD) and amount eaten by the crabs. **A)** Relationship between HD at week 0 and average amount eaten over three months for mixed (white triangle) and mono (black points) diets **B)** Relationship between amount crabs consumed and the difference in hemolymph protein density between the start and end of 15-week experimental period (ΔHD) for mixed (white triangle) and mono (black circle) diets. Positive values indicate gain in HD, negative values indicate loss. **C)** Crabs with a higher initial HD at week 0 tended to lose mass over the 15-week experimental period, whereas crabs with a lower HD at week 0 exhibited an overall gain in mass. Boxplots show medians (horizontal line), 25th-75th percentile quartiles (grey), 1.5*IQR (whiskers), and outliers (black points). **D)** Crabs that ate more overall gained mass, whereas crabs that ate less tended to lose mass.

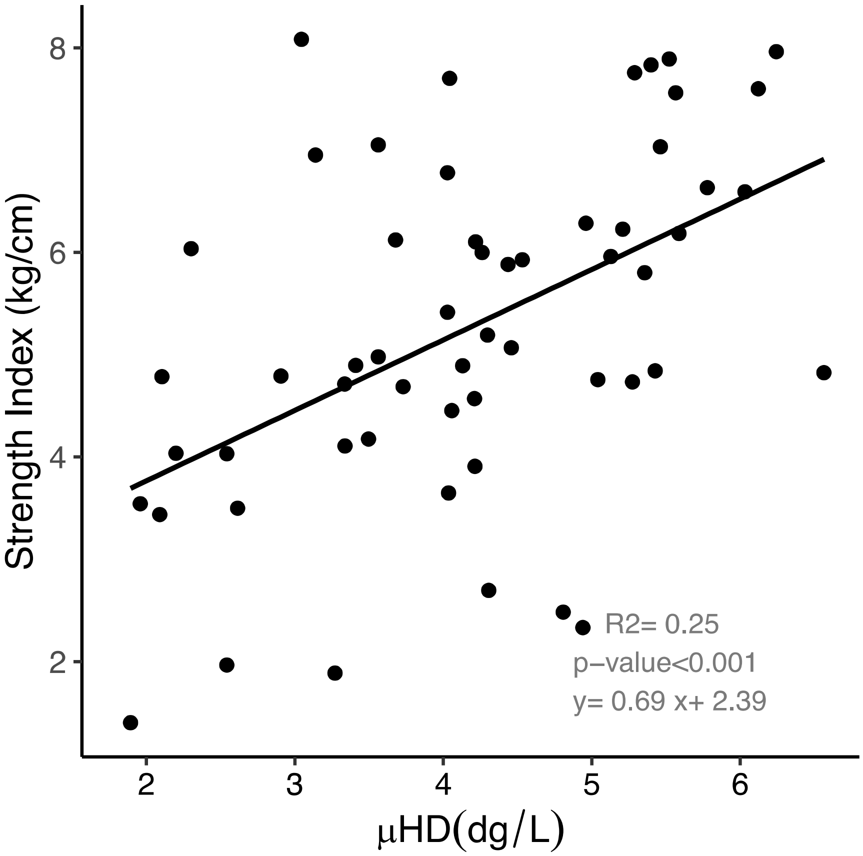

Figure 3.5. Relationship between mean crab chelae strength index (kg/cm) and the mean crab hemolymph protein density (HD, dg/L) during the 3-month period. Data was not separated by diet type.

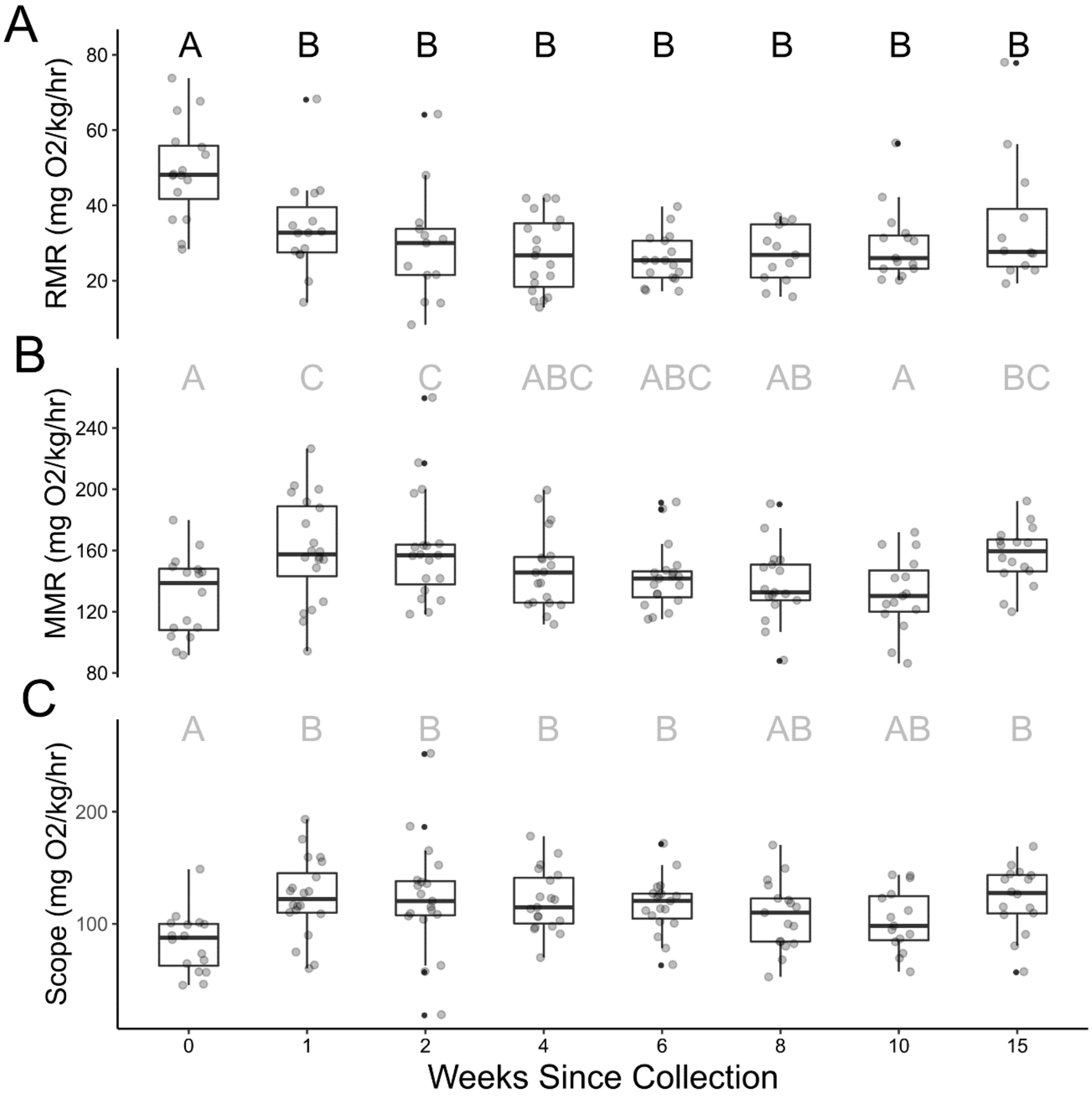

Figure 3.6. Oxygen uptake (mg O_2/kg/hr) measured in *C. irroratus* at regular intervals during the 15-week experimental period **A.** Resting Metabolic Rate (RMR), **B.** Maximal Metabolic Rate (MMR) and **C.** Metabolic Scope (MMR minus RMR). Different letters at each time period denote oxygen uptake levels that were significantly different from each other (Tukey HSD pairwise comparison). Boxplots show medians (horizontal line), 25th-75th percentile quartiles (grey), 1.5*IQR (whiskers), and outliers (black points), grey points represent data points.

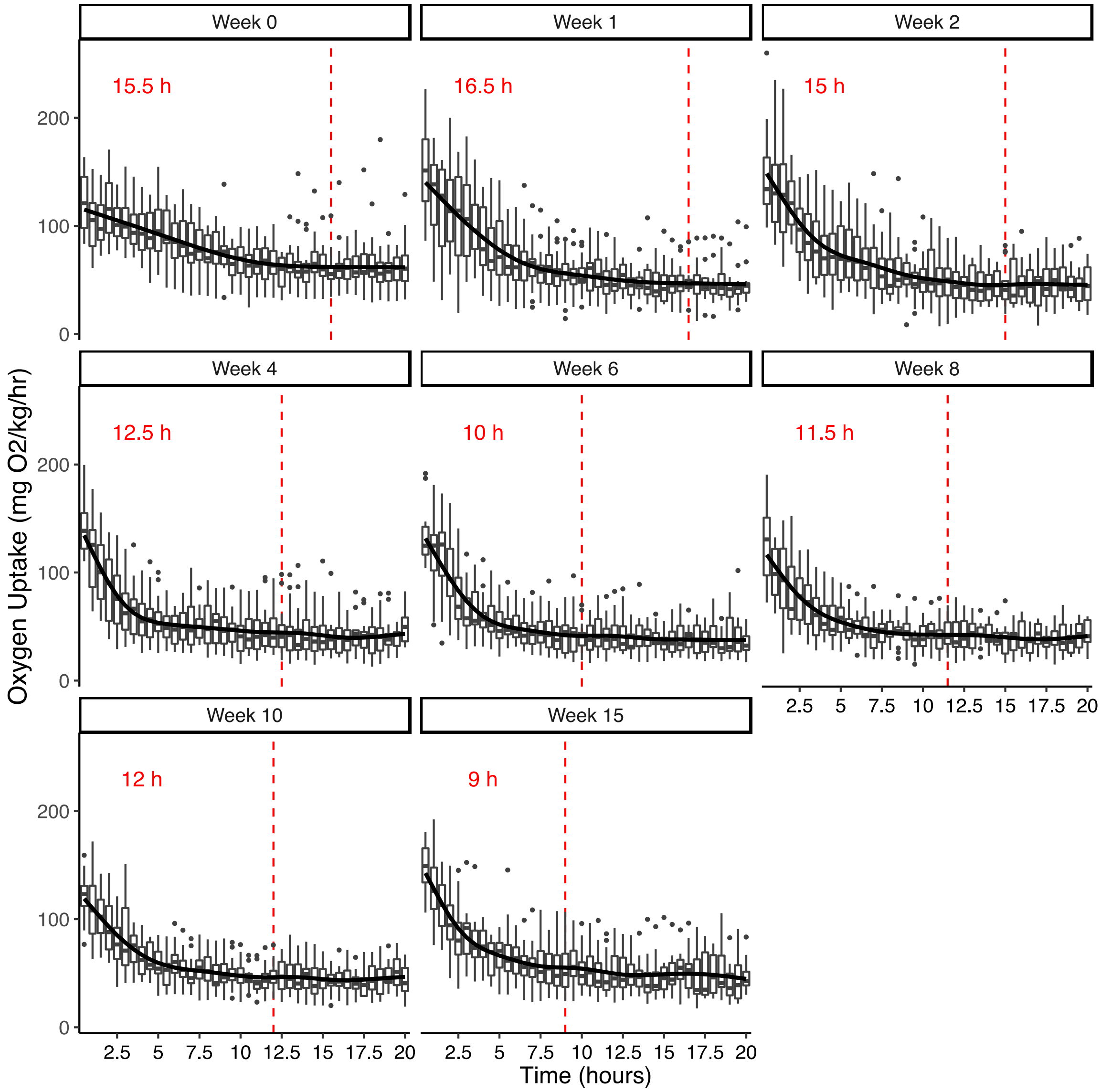

Figure 3.7. Oxygen uptake (mg O2/kg/h) levels of *C. irroratus* (n=15-20) measured over 20-hour trials during experimental weeks (0-15). A Generalized Additive Model (GAM) was fit through the data to visualize the general declining trend (solid black line). Where the difference in predicted values of the GAM fit between *t* and *t+1* reach 0, was counted as the time it took for oxygen uptake to stabilize post translocation (vertical dotted line, h=hours post translocation). Boxplots placed at 30-min intervals show medians (horizontal line), 25th-75th percentile quartiles (grey), 1.5*IQR (whiskers), and outliers (black points).

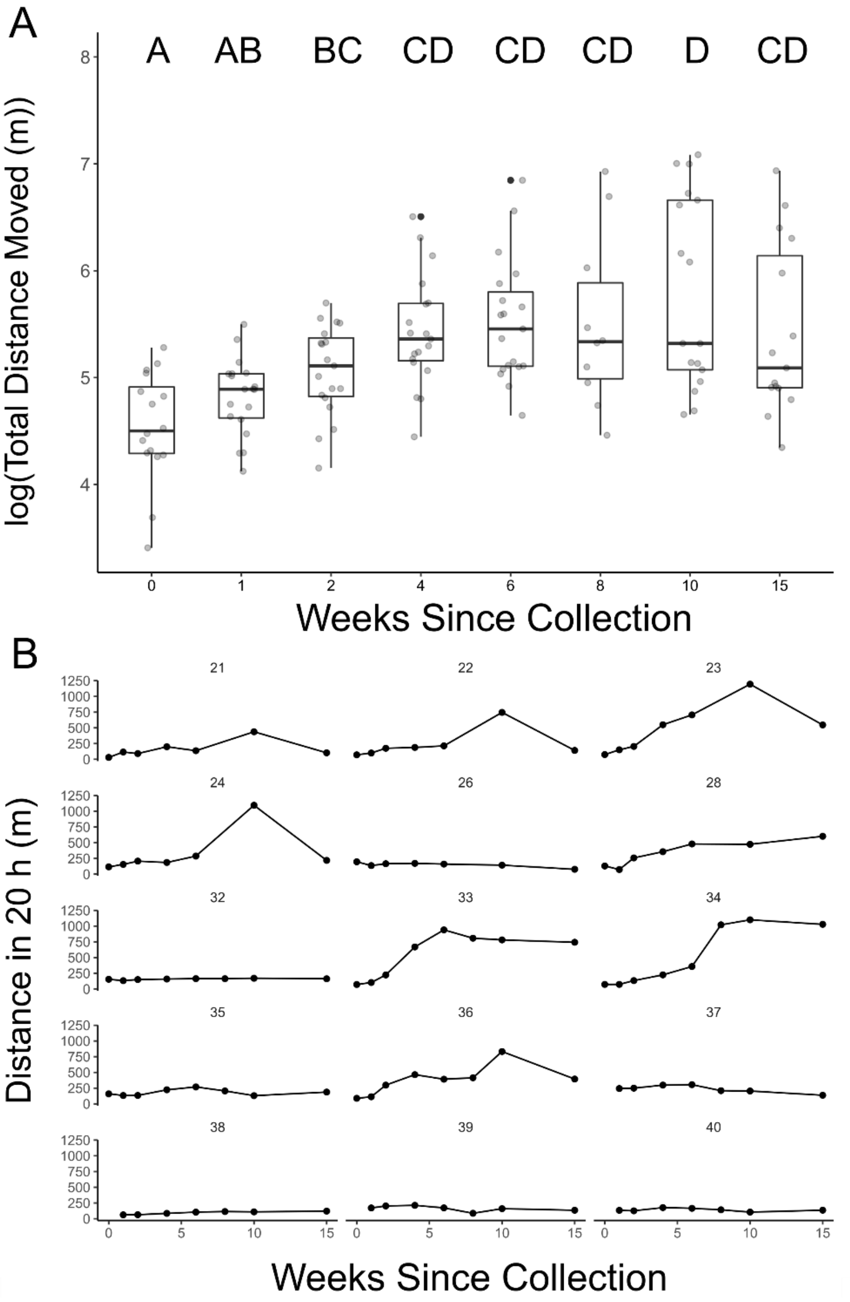

Figure 3.8. Spontaneous activity measured repeatedly in *C. irroratus* (n=15-19) over 15 weeks. **A.** Total distance (m, log transformed) travelled by crabs during a 20 h trial. Different letters denote where distances travelled were significantly different between measurement weeks (Tukey HSD pairwise comparison). Boxplots show medians (horizontal line), 25th-75th percentile quartiles (grey), 1.5*IQR (whiskers), and outliers (black points) **B.** 20 h spontaneous activity records for each individual crab measured repeatedly over 15 weeks. Data shows that some crabs (ID=23, 28, 33, 34, 36) were consistently active during later weeks. The remaining crabs had consistently low activity levels throughout the experimental monitoring period.

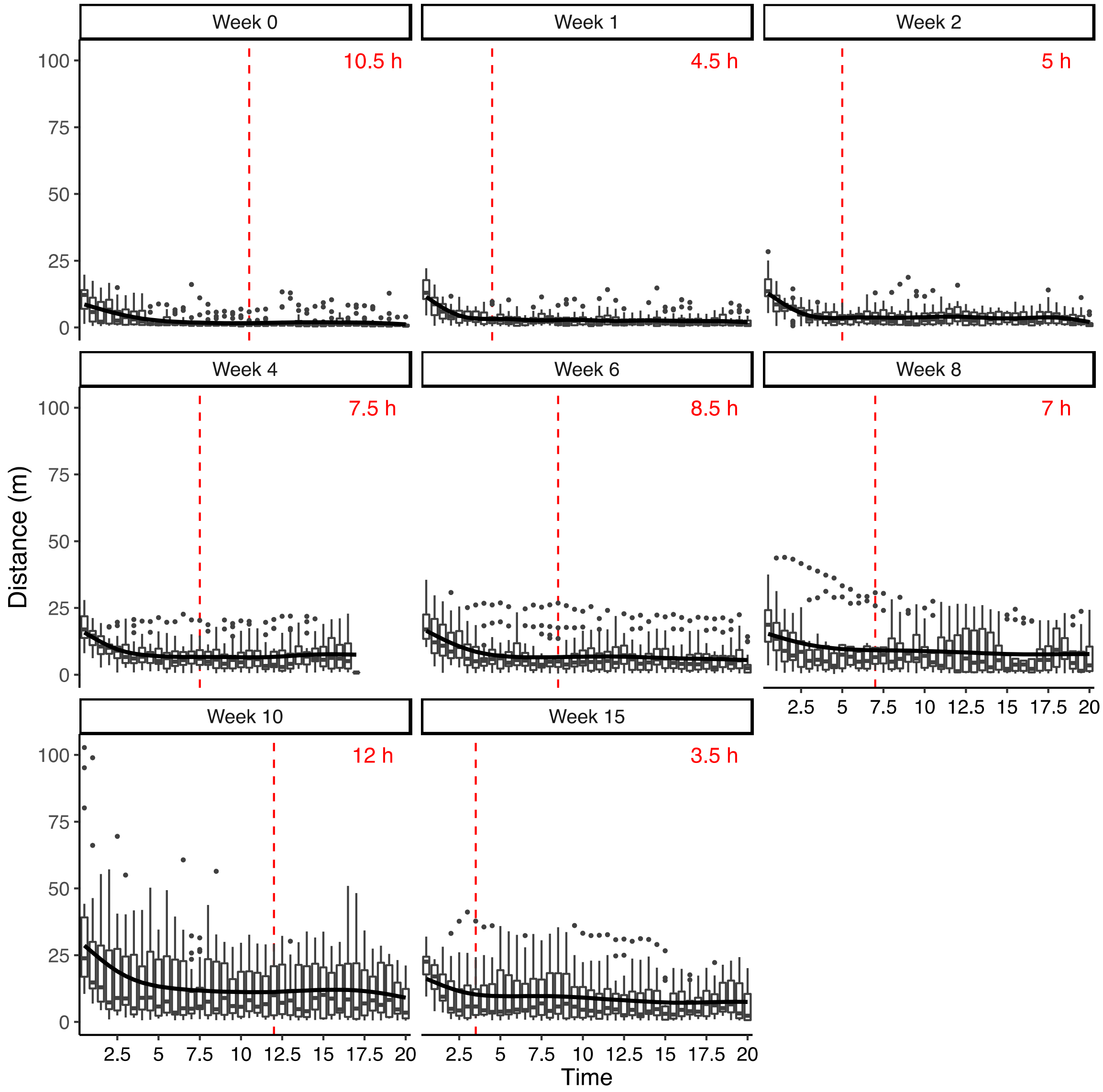

Figure 3.9. Spontaneous activity of crabs over a 20h recording period (n=15-19). Over the course of a 20 h trial, crab spontaneous activity decreased in most crabs during all weeks. A Generalized Additive Model (GAM) was fit through the data to visualize the general trend (black solid line). Boxplots placed at 30-min intervals show medians (horizontal line), 25th-75th percentile quartiles (grey), 1.5*IQR (whiskers), and outliers (black points) Where the difference in predicted values of the GAM fit between *t* and *t+1* reach 0 was said to be the time crabs settled post translocation (vertical dotted line, h=hours post translocation).

Chapter 4

General Discussion

Summary

The present thesis aimed to assess individual-level consistency of labile traits, while quantifying the effect of lab holding and dietary homogeneity on labile traits in brachyuran crabs. First, in Chapter 2, I tested the pace-of-life syndrome hypothesis prediction that physiology and behaviours co-vary on a fast-slow axis, and compared labile trait expression in two cohabiting crab species in Newfoundland (native Atlantic rock crab *Cancer irroratus*, and invasive European green shore crab *Carcinus maenas*). Although I found evidence for consistent individual differences in behaviour (i.e., personality), I did not find evidence for cross-correlations between different behaviours (i.e., no behavioural syndromes), and did not find that behaviour correlated to any metabolic rate metric. Additionally, I found that while behaviours were more proactive in *C. irroratus* than in *C.maenas*, there were no significant difference in resting and maximum metabolic rates. My experiment showed that labile traits were repeatable, but I report no evidence for metabolic rate being related to behaviour either intra- or inter-specifically.

In chapter 3, I used *C. irroratus* to test how prolonged lab holding affects labile traits, and whether diversifying the dietary composition for a generalist feeder affects survivorship or labile trait expression. Mussel was a preferred food type compared to fish, and kelp was not consumed in measurable amounts. I found that prolonged holding significantly affected labile trait expression; the resting metabolic rate (RMR) declined after 1 week of holding, and spontaneous activity rates increased (especially in some individuals) after 4 weeks of holding. I did not find that diversifying the diet resulted in better crab survivorship, or that any labile traits were significantly affected by diet. I did, however, find that crabs fed a heterogenous diet ate significantly more than those fed a homogenous diet (because mussel was a preferred food type). I found that hemolymph protein density at the time of collection was negatively related to the amount eaten per meal. In turn, the average amount eaten was positively related to the change in hemolymph density over 15 weeks. I therefore corroborate previous suggestions that hemolymph protein represents energy stores, and show that crabs with higher energy stores are more likely to forego eating in a lab environment. Additionally, I quantified appropriate settling times that allow for metabolic rate and spontaneous activity to stabilize after transfer-induced stress. These results can be used by future studies on crustaceans to minimize the effect of experimental procedures; my specific recommendations are discussed in the following section.

Recommendations for holding/settling times

My study on the effects of dietary diversity and holding time (Chapter 3) was conducted on just one species of crab (*C. irroratus*) during one season, therefore the following recommendations should be applied with caution. Nonetheless, they are based on empirical data, which is generally lacking in current literature. Additionally, *C. irroratus* has robust physiological tolerances owing to it's wide use in experiments; therefore a more sensitive species may experience greater lab stressor-associated effects. The following recommendations assume that researchers will use the results in order to significantly reduce transport, holding, and transfer stress on captive-held wild animals.

1. Crabs collected from the field should be held in lab conditions for approximately 4 weeks to acclimatize them to a novel environment. This allows for RMR to decline to stable levels, and for variation in activity rates to emerge.
2. Experiments aiming to decrease transfer stress should allow 12-16 h of settling time after transfer into experimental chambers. This allows for both activity and metabolic rate to decline to stable levels after acute transfer stress.
3. Diversifying the diet of generalist omnivorous crabs did not result in significant survivorship improvement, and I did not detect effects on labile traits. However, dietary preference should be accounted for to maintain high food intake rates.
4. I found that prolonged feeding on a lab diet resulted in a significant decrease in the interindividual variation of hemolymph protein density. Studies should consider that animals stored for prolonged periods, or those reared in-lab, may have lower inter-individual trait diversity due to environmental homogeneity.

Future Directions and Considerations

Drawing conclusions from single studies is especially difficult when labile traits are measured because they change quickly in response to environmental conditions, and lab studies incur stressful stimuli. While my research adds to the growing body of work on personalities and their relationship to physiology in invertebrates, there are many factors that need to be considered and follow up experiments to be conducted.

1. *Effects of stress on trait consistency*

In Chapter 2 I found that activity rates were repeatable when measured during the first hour post-transfer to experimental chambers but not when measured after 10 h of settling time. Similarly, while MMR measures were repeatable in both crab species, RMR was only repeatable in *C. irroratus*. This suggests that transfer stress might increase intra-individual trait consistency. My boldness and exploration trials consisted of hour-long recordings with no settling time, and it cannot be excluded that repeatability would decrease if crabs were given time to settle. Biro (2012) found that boldness in Ward's damselfish (*Pomacentrus wardi*) was repeatable during the first tests in a new environment, but not thereafter. While acute stress is a natural response, the extent to which behaviours are repeatable outside of stressful conditions may influence ecological implications. For instance, in Höjesjö et al. (2002) the fitness advantage of a non-aggressive personality type of brown trout (*Salmo trutta*) was found to be more difficult to detect in a heterogenous natural environment than in the lab. Future studies should consider measuring behaviour alongside more direct stress response metrics (i.e., hemolymph lactate concentrations, crustacean hyperglycemic hormone) to determine if stress affects labile trait repeatability. This would have the added benefit of determining if hormone or metabolite release, rather than metabolic rate, is the proximal mechanism driving personality differences in crabs. To do this, an experiment could compare physiological stress metrics and behavioural responses of a continually stressed group (e.g., by excessive handling) and a minimally stressed group (e.g., prolonged settling time), and determine if high stress increases labile trait repeatability.

2. *Meaningful vs. superficial changes in behavioural context*

Maximal spontaneous activity in *C. irroratus* and *C. maenas* was not related to boldness or exploration measures. Since these metrics were intra-individually consistent over 10 days, but not related to each other, it suggests that a context change (i.e., shelter presence) changed behavioural expression. Although context change is a defining characteristic of behavioural syndromes (Sih et al., 2004), what specifically constitutes a context varies by study. In some studies, the same behaviour is remeasured with/without predator or conspecific (e.g., Vainikka et al., 2011). Other studies compare different behaviours that share similar motive (e.g., risk aversion; Mowles et al., 2012) or completely different behaviours (e.g., Kortet and Hedrick, 2007). If the context change is minor or superficial, some studies that report behavioural syndromes might really be doing repeated measures of the same behaviour. Future studies on behavioural syndromes should consider what determines a meaningful context change, and how behavioural motive relates to the likelihood of detecting behavioural syndromes. An experiment could compare a group with an introduced behavioural motive (e.g., starvation) to a group without (e.g., satiated). By comparing similar behaviours (e.g., exploration and activity) and introducing a motivational cue, such as food odour, one could determine whether repeatability in a group with a stronger motive to forage would have result in stronger behavioural correlation.

3. C. irroratus *and* C. maenas *interspecies differences in behaviours*

I found that *C. irroratus* were consistently less proactive than *C. maenas*, which is counter to previous studies on these same species (Saxton et al., 2020; Bélair and Miron, 2009; Breen and Metaxas, 2009; Matheson and Gagnon, 2012). I suggest that *C. irroratus* and *C. maenas* may respond differently to seasonal temperature change. While both species were collected from and maintained in similar temperature water (approximately 10 °C), they were collected in late fall when water temperatures were declining. Additionally, *C. irroratus* collected in July (Chapter 3) were less active than those collected in late October (Chapter 2), so it seems that *C. irroratus* may increase their activity in the late fall. If endogenous activity rates fluctuate seasonally, there may be an offset period during which *C. irroratus* is more active than *C. maenas*. Additionally, if *C. irroratus* are indeed better adapted to cold temperatures, it may explain why they are outcompeting *C. maenas* in Iceland (Gislasen et al., 2014), and could represent a considerable invasion threat to northern coasts. Determining the extent to which location, season, temperature, and other environmental factors influence behaviour is an important consideration for future comparative behaviour studies on crabs. A first step in elucidating the effect of seasonality on endogenous activity of *C. irroratus* and *C. maenas* would be to conduct repeat activity measures of crabs collected at monthly intervals. Additionally, by conducting parallel studies on these species at different latitudes, one could also determine the extent to which seasonality interacts with temperature fluxes to drive behavioural differences.

4. *Use of hemolymph protein density for feeding ecology*

I found that hemolymph protein density (HD) could predict feeding rates in *C. irroratus*: crabs in good nutritional condition (high relative HD) did not eat enough to maintain their HD level even when a high quality diet was offered (mussel). One potential explanation is the adaptation to an opportunistic scavenger foraging style. Food intake in crabs is constrained by stomach size, and larger meals take longer to be gut cleared from the gut (McGaw and Curtis, 2013). For *C. irroratus*, it can take about 15 h to clear the foregut (Penney et al*.,* 2016), which limits food intake for that period even if a better food source is encountered. For crabs in good nutritional condition, it may therefore be beneficial to forego filling their gut with a mediocre diet to increase the chances of eating more should a more beneficial diet be encountered. This would imply that despite mussel being the overwhelmingly preferred food type, it was still not sufficiently beneficial for crabs in high nutritional state to digest or fill their gut with. If food intake suppression is not a stress-related effect, this could mean that the *C. irroratus* natural diet contains a food type that is more preferable than mussel, such as polychaetes, amphipods or bryozoans (Stehlik, 1993; Hudon and Lamarche, 1989). Additionally, because HD was higher in stronger crabs and those in better nutritional state, it might be representative of foraging efficacy or competitive ability. First one would need to assess the relationship between hemolymph protein density and stomach contents. Subsequently, measuring HD of different populations of crabs could be used to determine food availability in different locations; if proven reliable, HD could eventually be a simple metric used to determine the effect of environmental changes on crab foraging.

5. *Habituation to lab vs. habituation to repeat measures*

My experiment on the effect of long-term holding on labile traits was carried out on the same individuals over time (repeat measures). This means that it is highly likely that crabs became habituated to the experimental chambers, and changes associated with holding may have instead been changes associated with habituation to experimental procedures. This is especially likely since after the longest interval between measures (1 month), the RMR increased, and the spontaneous activity decreased (similar to first time measures). To determine which is more likely, measurements on crabs held in the lab but only subjected to experimental conditions once should be done.

Significance Statement

In this thesis I experimentally tested the largely theoretical framework of the pace-of-life syndrome hypothesis that links physiology and behaviour. My results add to the growing list of animals that are found to have consistent individual differences in behaviour (i.e., personality), and raise questions about what constitutes context change when assessing behavioural syndromes. I add to the recent body of work that shows that the link between metabolic rate and behaviour is, at the very least, complicated (Royauté et al., 2018; Niemelä and Dingemanse, 2018). Nonetheless, using similar species proved valuable in determining that differences in behaviour can occur without differences in metabolic expenditure. I then showcased how measuring labile traits in the lab can be affected by experimental procedure, and make suggestions for holding and settling times based on empirical data. I demonstrate that crabs can be successfully stored for at least 3 months without experiencing excessive physiological or behavioural degradation. I also show that providing a diversified diet can increase feeding rates by accounting for food preference, and corroborate previous studies on mussel preference in *C. irroratus*. I also demonstrated how hemolymph protein density (HD) can be used as a proxy for energetic stores of crabs, and suggest ways in which it can be a useful metric in the future research.

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Appendix Tables

Table A.1. Overview of holding and pre-experiment settling times in laboratory experiments involving adult crabs (papers published between 2000 and 2020). The holding time refers to the duration that animals were maintained in the lab before experiments were started. In some cases the holding time might also include acclimation time (see below). The pre-experiment settling times refer to the time between introducing the animal into an experimental apparatus and the time of starting the experiment and gathering data. The settling time is not to be confused with acclimation time, which gives animals time to respond to a set of physico-chemical variables, and for biological parameters to reach new stable levels. Articles where either holding or settling times were not applicable to the study (i.e. experiments were carried out immediately post collection, or crabs were not transferred to experimental chambers) are labelled "NA". Where time is not mentioned is labelled "NM". Papers where length of holding or settling/acclimation time is based on a peer-reviewed study are labelled "Y" and where it is not cited are left blank. Times where no peer reviewed study was cited, but justification was included in the methods are labelled N*. There was a holding range of 1-270 days, and 2 of the holding times were cited. There was a settling/acclimation period range of 10 minutes to 48 hours, and 5 studies included citations or justification. References can be seen in "Appendix References" below.

Citation	**Holding Time (days)**	**Holding Time Cited?**	**Pre-Experiment Settling Time (hours)**	**Settling Time Cited?**	**Crab Species**
Almerao et al., 2009	90		NA	*NA*	*Aegla platensis*
Belgrad and Griffen, 2016	14		NA	*NA*	*Callinectes sapidus*
Bell et al. 2009	1		1.5		*Callinectes sapidus*
Bernatis et al. 2007	7		0.25		*Cancer magister*
Bridger et al, 2015	NA	*NA*	24		*Pagurus bernhardus*
Capparelli et al., 2016	3		12		*Uca rapax*

De Carvalho et al., 2016	7		24		*Acanthonyx scutiformis*
Christiansen et al., 2015	135		24		*Paralithodes camtschaticus*
Cumillaf et al., 2016	21		18	Y	*Hemigrapsus crenulatus*
Dissanayake et al. 2009	2		0.25		*Carcinus maenas*
Dodd et al., 2015	71		1.5		*Panopeus herbstii*
Forsström et al., 2015	NM	*NM*	7		*Rhithropanopeus harrisii*
Furtbauer 2015	60		NM	*NM*	*Carcinus maenas*
Gaitán-Espitia et al. (2014)	30		24		*Petrolisthes violaceus*
Giomi and Pörtner, 2013	60		20		*Carcinus maenas*
Hamilton et al., 2016	8.5		NM		*Pachygrapsus crassipes*
Jacobo et al., 2016	7		NM	*NM*	*Cancer antennarius*
Jiang et al, 2012	7		NM	*NM*	*Eriocheir sinensis*
Jungblut et al.,2018	1		3	N*	*Carcinus maenas*
Kelley et al. 2011	70		NA	*NA*	*Carcinus maenas*
Lardies et al., 2010	14		NM	*NM*	*Cyclograpsus cinereus*
Lewis and Ayers, 2014	14		NA		*Cancer borealis*
Lord et al.,2015	NM	*NM*	24		*Carcinus maenas*

Martins de Lima et al., 2015	15		NM	*NM*	*Neohelice granulata*
Matazzo et al., 2011	12		7		*Carcinus aestuarii*
McGaw and Van Leeuwen, 2017	7		3		*Cancer magister*
Metzger et al. 2007	49		24		*Cancer pagurus*
Mills et al., 2016	NM	*NM*	6		*Eriocheir sinensis*
Nagaraju et al., 2011	7		NA	*NA*	*Carcinus maenas*
Oliveira et al., 2015	NM	*NM*	48		*Pachygrapsus transversus*
Penney et al., 2015	14		5		*Carcinus maenas & Cancer irroratus*
Pennoyer et al. 2016	10.5		0	Y	*Carcinus maenas*
Qiu et al, 2011	14	*NA*	NA	*NA*	*Eriocheir sinensis*
Rastrick et al., 2014	15		NM		*Necora puber*
Robertson et al 2002	28	Y	12		*Carcinus maenas*
Rossong et al.,2011	7		0.16		*Carcinus maenas*
Salierno et al 2003	4	*NM*	0.16		*Cancer irroratus*
Seibel et al., 2017	0.5	*NA*	NM		*Pleuroncodes planipes*
Siddiqui 2015	7		1		*Barytelphusa gureini*

Simonik and Henry 2014	7		1		*Carcinus maenas*
Sneddon et al. 2000	7		0.25		*Carcinus maenas*
Strover et al. 2013	11.5		NM	*NM*	*Callinectes sapidus*
Sun et al.,2017	14		12		*Portunus trituberculatus*
Tanner et al. 2011	7	Y	NA	*NA*	*Carcinus maenas*
Tepolt and Somero 2014	24.5		1	N*	*Carcinus maenas*
Theuerkauff et al., 2018	3		NM	*NM*	*Neosarmatium meinerti*
Toscano 2017	NM	*NM*	0.25		*Panopeus herbstii*
Toscano and Monaco (2015)	NM	*NM*	0.25		*Panopeus herbstii*
Urbina et al., 2013	70		0		*Lithodes santolla*
Valverde et al. 2009	270		NM	*NM*	*Maja brachydactyla*
Wang et al, 2018	84		24	N*	*Cancer pagurus*
Watts et al., 2015	7		NA	*NA*	*Carcinus maenas*
Wu et al., 2017	14		2		*Charybdis japonica*
Average	**28.9**		**9.7**		
Median	**13.0**		**4.0**		
Std. Dev.	**46.11**		**11.46**		

Table A.2. Summary of food types offered to a variety of crab species that were held and fed in the laboratory. Data was gathered from articles published during the past 2 decades (2000-2020). A summary of the most common feed types and whether the animals were given a mixed or single diet is also included at the end of the table.

Citation	Food Given	Diet Mixed?	Crab Species
Belgrad and Griffen (2016)	mussel, fish, OR seaweed	N	*Callinectes sapidus*
Bell et al. 2009	squid OR shrimp	Y	*Callinectes sapidus*
Bernatis et al. 2007	fish	N	*Cancer magister*
Curtis and McGaw 2012	fish	N	*Cancer magister*
Curtis et al. 2009	fish	N	*Cancer magister*
Dissanayake et al. 2009	cockle	N	*Carcinus maenas*
Furtbauer 2015	fish	Y	*Carcinus maenas*
Gaitán-Espitia et al. (2014)	algae and commercial shrimp food	Y	*Petrolisthes violaceus*
Kelley et al. 2011	mussel	N	*Carcinus maenas*
Lewis and Ayers 2014	mussel	N	*Cancer borealis*
de Lima et al., 2015	beef	N	*Neohelice granulata*
Matozzo et al. 2011	mussel	N	*Carcinus aestuarii*

McGaw 2001	algae	N	*Hemigrapsus nudus*
McGaw 2007	fish	N	*Cancer gracilis*
McGaw et al. 2009	fish	N	*Cancer productus*
Metzger et al. 2007	mussel and cockle	Y	*Cancer pagurus*
Penney et al. 2015	fish	Y	*Carcinus maenas & Cancer irroratus*
Pennoyer et al. 2016	fish	N	*Carcinus maenas*
Robertson et al 2002	squid and fish	Y	*Carcinus maenas*
Rossong et al. 2012	mussel	N	*Carcinus maenas*
Salierno et al 2003	mussel and commercial diet	Y	*Cancer irroratus*
Simonik and Henry 2014	mussel and squid	Y	*Carcinus maenas*
Sneddon et al. 2000	fish	N	*Carcinus maenas*
Strover et al. 2013	shrimp and squid	Y	*Callinectes sapidus*
Tanner et al. 2011	fish	N	*Carcinus maenas*
Tepolt and Somero 2014	commercial feed	N	*Carcinus maenas*
Toscano 2017	clams	N	*Panopeus herbstii*
Toscano and Menaco (2015)	clams	N	*Panopeus herbstii*

Valverde et al. 2009	mussels	N	*Maja brachydactyla*
Almerao et al., 2009	commercial food	N	*Aegla platensis*
Urbina et al., 2013	fish and mussel	Y	*Lithodes santolla*
Christiansen et al., 2015	commercial feed and fish	Y	*Paralithodes camtschaticus*
Cumillaf et al., 2016	bivalve molluscs	N	*Hemigrapsus crenulatus*
Dodd et al.,2015	shrimp	N	*Panopeus herbstii*
Forsström et al., 2015	chironomid larvae	N	*Rhithropanopeus harrisii*
Giomi and Pörtner, 2013	mussel and clam	Y	*Carcinus maenas*
Hamilton et al., 2016	squid and fish	Y	*Pachygrapsus crassipes*
Jacobo et al., 2016	fish	N	*Cancer antennarius*
Lardies et al., 2010	mussel	N	*Cyclograpsus cinereus*
Lord et al.,2015	mussel	N	*Carcinus maenas*
McGaw and Van Leeuwen, 2017	fish	N	*Cancer magister*
Mills et al., 2016	fish	N	*Eriocheir sinensis*
Nagaraju et al., 2011	shrimp	N	*Carcinus maenas*
Qiu et al, 2010	commercial feed	N	*Eriocheir sinensis*

Rastrick et al., 2014	mussel	N	*Necora puber*
Siddiqui 2015	goat and oats	Y	*Barytelphusa gureini*
Sun et al.,2017	mussel	N	*Portunus trituberculatus*
Wang et al, 2018	mussel	N	*Cancer pagurus*
Watts et al., 2015	mussel	N	*Carcinus maenas*
Wu et al., 2017	mussel	N	*Charybdis japonica*
Table Summary	Bivalves	46%	
	Fish	33%	
	Commercial Feed	12%	
	Squid	10%	
	Shrimp	8%	
	Algae	6%	
	Other	6%	
	Mono diet	**73%**	
	Mixed diet	**27%**	

Table A.3. Sample sizes for oxygen uptake, spontaneous activity, hemolymph protein density, crab mass, and claw strength taken at each sampling week, and separated by diet treatment. A total of 60 *Cancer irroratus* crabs were used, with 30 fed a mixed diet and 30 a mono diet. Twenty crabs (10 from each diet treatment) were used for oxygen uptake and another 20 for spontaneous activity. All crabs used for oxygen uptake and activity, as well as an additional 20 (10 in each diet, maintained under the same conditions but not used in experiments) were used to measure hemolymph protein density, mass, and claw strength. The numbers shown in each column represent the number of animals with usable data gathered at each time period. The missing data could be due to the death of an animal or corruption of an experimental data set.

	Mixed Diet			**Mono Diet**		
~Weeks Since Collection	*Oxygen Uptake*	*Activity Level*	*Hemolymph Density, Mass, Strength*	*Oxygen Uptake*	*Activity Level*	*Hemolymph Density, Mass, Strength*
0	8	8	30	8	8	30
1	8	10	30	8	9	28
2	6	10	30	8	9	27
4	10	10	30	9	9	26
6	10	10	30	8	9	25
8	8	5	N/A	5	5	N/A
10	9	8	28	7	9	23
15	9	7	25	7	8	22

Table A.4. Tukey HSD post hoc test for pairwise comparisons of Resting Metabolic Rate (RMR) (mg O2 kg/h) measured at regular intervals during the 15-week experimental period. Significant differences between pairs are bolded.

Week Pair	**estimate**	**SE**	**df**	**t-ratio**	**p.value**
0 - 1	15.403	4.082	93	3.774	**0.007**
0 - 2	20.416	4.247	93	4.807	**<0.001**
0 - 4	21.875	3.838	93	5.7	**<0.001**
0 - 6	23.416	3.952	93	5.925	**<0.001**
0 - 8	22.061	4.224	93	5.223	**<0.001**
0 - 10	19.603	4.076	93	4.809	**<0.001**
0 - 15	14.42	4.35	93	3.315	**0.027**
1 - 2	5.013	4.312	93	1.162	0.94
1 - 4	6.472	3.911	93	1.655	0.716
1 - 6	8.013	4.025	93	1.991	0.494
1 - 8	6.658	4.331	93	1.537	0.785
1 - 10	4.2	4.156	93	1.011	0.972
1 - 15	-0.983	4.429	93	-0.222	1
2 - 4	1.459	4.091	93	0.357	1
2 - 6	3	4.198	93	0.715	0.996
2 - 8	1.645	4.487	93	0.367	1
2 - 10	-0.813	4.322	93	-0.188	1
2 - 15	-5.996	4.581	93	-1.309	0.893
4 - 6	1.541	3.767	93	0.409	1
4 - 8	0.186	4.079	93	0.046	1
4 - 10	-2.272	3.903	93	-0.582	0.999

4 - 15	-7.455	4.181	93	-1.783	0.633
6 - 8	-1.355	4.174	93	-0.325	1
6 - 10	-3.814	4.004	93	-0.952	0.98
6 - 15	-8.996	4.275	93	-2.104	0.42
8 - 10	-2.458	4.286	93	-0.574	0.999
8 - 15	-7.641	4.544	93	-1.682	0.699
10 - 15	-5.183	4.384	93	-1.182	0.935

Table A.5. Tukey HSD post hoc test for pairwise comparisons of spontaneous activity (total distance travelled per 20 h) as a function of time. Significant differences between pairs are bolded.

Week Pair	estimate	SE	df	t-ratio	p-value
0 - 1	-0.34	0.1673	107	-2.04	0.46018
0 - 2	-0.58	0.1673	107	-3.48	**0.01596**
0 - 4	-0.95	0.1673	107	-5.66	**0.00000**
0 - 6	-1.04	0.1673	107	-6.24	**0.00000**
0 - 8	-1.03	0.2034	107	-5.07	**0.00005**
0 - 10	-1.31	0.1726	107	-7.60	**0.00000**
0 - 15	-0.92	0.1789	107	-5.17	**0.00003**
1 - 2	-0.24	0.1578	107	-1.53	0.79026
1 - 4	-0.61	0.1578	107	-3.83	**0.00508**
1 - 6	-0.70	0.1578	107	-4.45	**0.00055**
1 - 8	-0.69	0.1937	107	-3.56	**0.01246**
1 - 10	-0.97	0.1631	107	-5.95	**0.00000**
1 - 15	-0.58	0.1694	107	-3.44	**0.01797**
2 - 4	-0.36	0.1578	107	-2.31	0.30042

2 - 6	-0.46	0.1578	107	-2.92	0.07862
2 - 8	-0.45	0.1937	107	-2.32	0.29478
2 - 10	-0.73	0.1631	107	-4.47	**0.00050**
2 - 15	-0.34	0.1694	107	-2.02	0.47373
4 - 6	-0.10	0.1578	107	-0.61	0.99861
4 - 8	-0.08	0.1937	107	-0.44	0.99985
4 - 10	-0.37	0.1631	107	-2.24	0.33586
4 - 15	0.02	0.1694	107	0.13	1.00000
6 - 8	0.01	0.1937	107	0.06	1.00000
6 - 10	-0.27	0.1631	107	-1.65	0.72085
6 - 15	0.12	0.1694	107	0.70	0.99679
8 - 10	-0.28	0.1969	107	-1.43	0.84317
8 - 15	0.11	0.2019	107	0.53	0.99948
10 - 15	0.39	0.17	107	2.24	0.33722

Appendix Figures

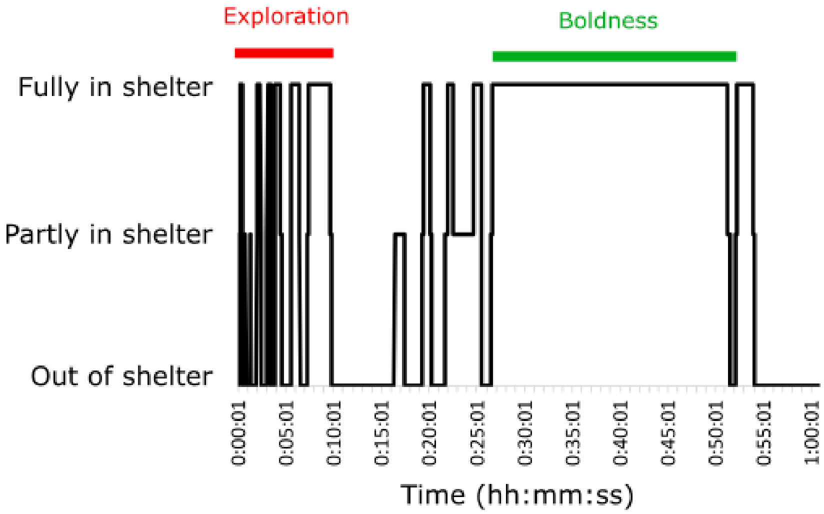

Figure A.1. Distinction between boldness and exploration behaviours. This is a sample record from one crab (CrabID=18) in a 1-hour behavioural trial. The exploration behaviour is seen as frequent entrances and exits into the shelter, seemingly to learn about the new environment. The boldness behaviour is distinguished by prolonged time spent inside the shelter without re-emerging.

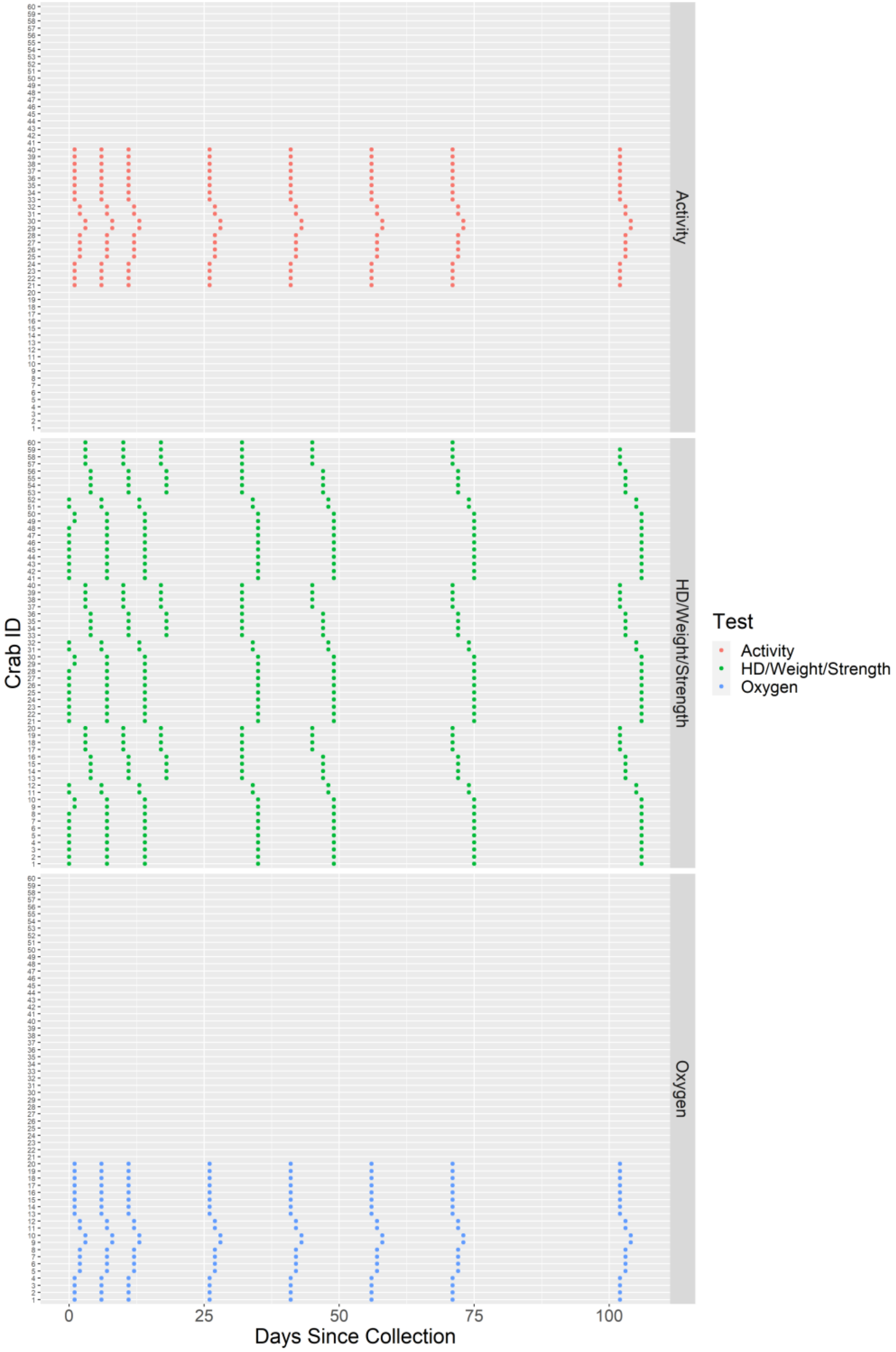

Figure A.2. Data collection schedule for Chapter 3. Sixty *C. irroratus* were stored in the lab for 3 months, and their activity, hemolymph protein density (HD), mass (i.e., weight), and oxygen uptake were repeatedly measured at varying intervals.

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