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Morphology and stable isotope ecology of *Pleuroncodes planipes* adult life stages and their vulnerability to climate change stressors

A Dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy

in

Marine Biology

by

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The Dissertation of Summer Janille Webb is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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Dedication

To Sofie – I never knew how much I needed you,

to Stuart – my unconditional support,

and to my parents – for always believing in me.

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Abstract of the Dissertation

Morphology and stable isotope ecology of *Pleuroncodes planipes* adult life stages and their vulnerability to climate change stressors

by

Summer Janille Webb

Doctor of Philosophy in Marine Biology

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Professor Jennifer Taylor, Chair

Like many in Southern California during the 2015/16 El Niño event, I was struck by the presence of thousands of bright red tuna crabs (*Pleuroncodes planipes*) abundant at sea and washing ashore. Their sudden prevalence inspired me to learn more about these fascinating animals. Despite drawing so much attention, basic details related to their feeding behaviors and life history remain unknown. *P. planipes* have long been thought, but never confirmed, to

experience a unique life history among crustaceans during which they undergo a sequential habitat shift from pelagic to benthic as adults. In this dissertation research, I applied contemporary methods of stable isotope ecology in combination with ecomorphology to examine aspects of their life history relevant to their pelagic and benthic life stages. We further assessed their vulnerability to the climate change stressors of ocean acidification and ocean warming through a long-term experiment. Through this work, we uncovered morphological differences and an ontogenetic diet shift between pelagic and benthic adult stages as well as significant impacts of temperature, but not pCO_2/pH on molting and growth in pelagic adults. These results provide the first evidence in support of the hypothesis that *P. planipes* adult pelagic and benthic stages are distinct and yield important insight into how this transition could be impacted as the oceans continue to change.

Chapter 1: Synthesis of the biology and life history of *Pleuroncodes planipes*

The bright red tuna crabs, *Pleuroncodes planipes* (Crustacea: Decapoda: Munididae) (Figure 1.1), capture the attention of news outlets and the public alike when they occasionally show up in large swarms and strand by the thousands along beaches in Southern California (Figure 1.2). Tens of thousands of live crabs in surface waters get transported onto the shore by wave action and spread across the sand where they eventually are either eaten by birds or other predators, perish and decay, or are sometimes carried back out to sea. These events receive heavy attention by the public and the media while the crabs strand on the beaches, and their occurrence has been tied to El Niño events. In addition to the strandings, large numbers of individuals can also be observed at sea (Figures 1.3 & 1.4) and have seemed to remain in local waters longer than previously observed. Until recently, no long-term data set outside of anecdotal observations had been compiled to track this occurrence and the actual mechanism behind these episodic strandings and abundance in Southern California remained unknown. The mass stranding events of *P. planipes* and observations of their high abundance at sea off the Southern California coast that began in 2015, preceded by the warm water blob in the Eastern Pacific, inspired the work in this dissertation.

Figure 1.1: An adult tuna crab, *P. planipes*. Credit: Summer Webb.

Figure 1.2: Mass stranding of *P. planipes* in Ocean Beach, San Diego. Image credit: Jim Grant.

Figure 1.3: *P. planipes* filling a trawl net during RV Robert Gordon Sproul student research cruise off La Jolla, CA in November 2016. Credit: Summer Webb.

Figure 1.4: *P. planipes* observed aggregating on the sea floor off La Jolla, CA as captured on scuba. Credit: Phil Zerofski.

Geographic range and presence in Southern California

The range of *Pleuroncodes planipes* is historically considered to span 16°N to 37°N along the coast of North America (Boyd 1962), but the main center of their distribution is off the Pacific coast of southern Baja, Mexico, in Bahia Magdalena (Boyd 1962). Large populations have also been found off Punta Eugenia, north of Bahia Magdalena (Gómez-Gutiérrez et al. 2000). During the most recent El Niño event (2015/16), they were observed as far north as the Oregon coast, whereas previous events only transported them as far north as Monterey Bay, CA. A 2013 study estimated their total biomass along their range to be 611,525 metric tons (De Anda-Montañez et al. 2013). Though *P. planipes* are effective swimmers in the vertical direction, their distribution can be affected by horizontal advective transport and eddies (Gómez-Gutiérrez et al. 2000). In the northern Baja region, strong upwelling events cause eddies to form, which may select for a semi-permanent resident population (Gómez-Gutiérrez et al. 2000). It has been proposed that *P. planipes* takes advantage of tidal streams and currents for feeding and relocation (Robinson and Gomez-Aguirre 2004).

P. Planipes undergo a seasonal bathymetric migration as adults, moving to deeper and cooler waters during the summer months (Aurioles-Gamboa and Pérez-Flores 1997), and back to shallow waters in the winter, where they feed and reproduce (Aurioles-Gamboa and Pérez-Flores 1997). Though it has been hypothesized that *P. planipes* are eurythermal because they can be found in waters ranging from 11°C to 20°C, field sampling suggests that they have a clear optimal temperature range of 13-16°C (Aurioles-Gamboa 1992). Their biomass and occurrence are strongly tied to water temperature, which is closely linked to other oceanographic factors, like upwelling, to which their life cycle is also associated (Aurioles-Gamboa 1992).

Nearshore between 24°N and 28°N off the western Baja California coast is considered the main nursery area for *P. planipes* (Gómez-Gutiérrez and Sánchez-Ortíz 1997). After hatching in these shallow, coastal waters, Ekman transport may carry the newly hatched larvae offshore, but can be potentially shut off by eddies or disrupted by anomalous advection (Gómez-Gutiérrez et al. 2000). The Davidson Current transports some larvae and juvenile *P. planipes* north (Poore et al. 2011). The early post-larval stages (young juveniles) are carried far southwest to open ocean (130°W) by the California Current (Gómez-Gutiérrez et al. 2000). Juveniles occur in large numbers between 30-100 km offshore in the outer shelf and slope region. As they mature, they move to more neritic areas where they ultimately remain associated with the continental shelf and settle as adults (Gómez-Gutiérrez et al. 2000; Poore et al. 2011). The exact benthic settlement cues remain unknown for *P. planipes*, though shelf recruitment of the sister species, *P. monodon*, is associated with seasonal development of a sulfur bacteria that indicates relaxation of the upwelling regime (Gallardo, Canete, and Enriquez-Briones 1994).

When benthic, *P. planipes* inhabit the continental shelf in muddy/sandy bottom habitats at highest abundance from 75 m down into the deep sea to 300 m (Boyd 1962). It was thought that tuna crabs engage in daily vertical migration due to differential day and night catch in surface waters (upper 25 m) (Boyd 1962), but this is not a rule. This phenomenon is only sometimes observed and tuna crabs can be found in surface waters during the daytime (Blackburn 1977) and at depth during the night (Aurioles-Gamboa 1992). Throughout their life stages, tuna crabs are exposed to a broad range of oceanographic conditions over both daily and seasonal time scales.

Impact of *Pleuroncodes planipes* in California

When tuna crabs reach Southern California in high numbers, they can disrupt local trophic systems. For example, during the 1982-1983 El Niño, *P. planipes* were documented in the waters off the northern Channel Islands. They appeared on the beaches of San Nicolas Island where they served as vital prey for a starving gull population (Stewart et al., 1984). The influx of tuna crabs also provided an important food source for many key players in the California Current Ecosystem that would otherwise experience low food availability during El Niño conditions (Stewart et al., 1984). Having an influx of *P. planipes* as a food source benefits all these organisms as El Niño conditions can decrease the availability of food. In addition to sea birds, myriad other ecologically important organisms consume *P. planipes*, including whales, sea turtles, sharks, sea lions, migratory and kelp forest fish, and sea otters (Aurioles-Gamboa & Perez-Flores, 1997). *P. planipes* are considered the most important food item for blue sharks and juvenile loggerhead turtles off the coast of Baja Mexico (Nichols, 2003, Escobar-Sánchez et al., 2011).

Aside from observations as important prey, the impact of *P. planipes'* episodic abundance in Southern California has not been quantified. Competitive interspecific interactions with Southern California native species have not been investigated, though it is likely they outcompete local grazers and have an impact in the meiofaunal populations of the continental slope due to their sheer biomass. Many local natural historians believe their presence has persisted longer than any previous episodic influx.

Oceanographic factors driving distribution

Until recently, it was thought that conditions from El Niño drove abundance and mass stranding events in Southern California, without a specifically understood mechanism. Essentially,

it was hypothesized that a strengthening of the Southern California Countercurrent and the Davidson Countercurrent brought them northward (Boyd 1962), and that any individuals caught in nearshore surface waters have an ultimate destination of the shore due to onshore winds and receding tides (Kato 1974). This was sometimes found to be the case, but evidence for this has remained inconsistent. In 2021, anomalous advection was identified using transport analysis as the mechanism that carries *P. planipes* northward (Cimino et al. 2021). This advection is often, but not always, associated with El Niño (Cimino et al. 2021).

The El Niño phenomenon is a large-scale ocean-atmosphere climate cycle marked by an increase in sea surface temperature in the central and eastern Pacific (Beckdorf, 2015). In the Pacific, trade winds blow from east to west along the equator causing warm water to "pile up" in the western equatorial Pacific (Beckdorf, 2015). During an El Niño, these trade winds weaken and allow that "pile" of warm water to flow eastward (Beckdorf, 2015). This depresses the thermocline in the eastern Pacific and therefore reduces upwelling. El Niño affects the California Current System in many ways, including reducing upwelling and advection of warm water into the region from the south (Jacox et al., 2016). This is notable because tuna crab reproduction and abundance have been closely linked to upwelling, where abundant phytoplankton can be guaranteed food for the larvae (Aurioles-Gamboa, 1992, De Anda-Montañez 2012).

As the warm water events of recent years have subsided, large numbers of adult *P. planipes* are still present along the coast of Southern California. The reasons for the persistence of these tuna crabs in the region remain unclear. It is not known whether the individuals in Southern California are continuing to be transported there or whether they are breeding and recruiting in the region.

Life history

Based on what is known from *P. planipes* in waters off Mexico, they become sexually mature at approximately 14 - 15 mm carapace length (CL), which occurs at about 12 months of age (Boyd, 1962), when they still occupy the pelagic zone. More recent estimates of size at maturity suggest that 50% of females and males reach sexual maturity at around 23 and 26 mm CL, respectively (Rodríguez-Jaramillo et al. 2018). Tuna crabs breed in the winter, with January and February projected to be the most intense period based on back-calculated ages/abundance of larval and post-larval forms (Gómez-Gutiérrez and Sánchez-Ortíz 1997). Females have been found with eggs primarily from December to March (Boyd, 1962), but there is evidence that reproduction can occur at any time of the year as gravid females and larvae can be found year-round (Poore et al., 2011). Females brood eggs like most crustaceans and are capable of carrying upwards of 3000 eggs (Boyd and Johnson, 1963), with larger females being more fecund (Rodríguez-Jaramillo et al. 2018). Both pelagic and benthic adults are reproductive (Longhurst and Seibert 1971), and it is speculated that there is some interaction between pelagic and benthic individuals during the mating season.

After hatching, *P. planipes* pass through five larval stages before molting into the adult morphology (Boyd and Johnson, 1963). The larvae advance through stages 2-4 with only one molt per stage. The fourth larval stage is the only one in which multiple molts occur, but the morphology does not change between these intra-stage molts (Boyd and Johnson, 1963). Larvae that have been hatched and raised in the laboratory to the fifth stage take between 98-127 days, and the time between each stage can vary with temperature; higher temperature shortens the time between larval stages and the total time to reach the final stage (Boyd and Johnson, 1963). It takes approximately one year to rear *P. planipes* from the first larval stage to maturity in the lab (Boyd and Johnson,

1963). Based on growth curves calculated by Boyd (1960), it appears that tuna crabs can live at least 3 years and reach a maximum size of 32 mm SCL (standard carapace length; Boyd 1960). However, animals exceeding that previously reported maximum size, up to 37 mm SCL (max size 32 mm SCL), were collected during the work on this dissertation and a 5 year life span is reported in a recent study (Cimino et al. 2021).

Pelagic and benthic stages

P. planipes was originally thought to be solely pelagic until 1960 when adults were found in benthic trawls in large numbers but absent from pelagic collections (Boyd 1962). Since then, adult *P. planipes* have been found consistently in benthic trawls (Aurioles-Gamboa and Pérez-Flores 1997; De Anda-Montañez et al. 2016; Gómez-Gutiérrez and Sánchez-Ortíz 1997). The larval and juvenile stages of *P. planipes* are both strictly pelagic. Early larval forms have little control over their vertical distribution in the water column and are found primarily at the surface (~ 10 m) (Poore et al., 2011). Late larval stages and juveniles have a wide vertical distribution in the water column (Poore et al., 2011).

It is likely that *P. planipes* evolved from a benthic ancestor, as it is one of only two described galatheid species known to exist in the pelagic habitat as adults (Boyd, 1962). As reproductive adults, *P. planipes* have a mix of pelagic and benthic phases, making them unique among crustaceans (Longhurst, 1967). The pelagic stage is thought to occur between 18 – 26 mm in SCL. In this so-called pelagic adult phase, they do not remain solely in the water column, but rather migrate between the pelagic and benthic zones, presumably to feed and escape predators (Boyd 1967). Once animals reach an average size of 27.9 mm, they are fully benthic (Boyd 1967). The maximum benthic size found in benthic samples reported in Boyd (- 1967) was 32.0 mm SCL

and has been miscited across the literature as the starting size of the benthic phase. Subsequent samplings suggest that pelagic-associated adult size ranges between 17 - 20 mm in SCL (Uncited in Robinson and Gomez-Aguirre 2004), 10 - 30 mm SCL (Gómez-Gutiérrez and Sánchez-Ortíz 1997), 18 - 26 mm in carapace length (Boyd, 1962), or 18 - 30 mm SCL (Robinson and Gomez-Aguirre 2004).

While in the pelagic phase, *P. planipes* play an integral role in converting primary production into usable energy for organisms at higher trophic levels, similar to sardines or anchovies (De Anda-Montañez, 2015). They do not remain solely in the water column, but rather migrate between the pelagic and benthic zones, presumably to feed and escape predators (Boyd 1967).

By the time animals have reached approximately 28 mm carapace length (mean benthic collected size 27.9 mm SCL as reported by Boyd, 1962), they are thought to be strictly benthic for the remainder of their lives (Boyd, 1962, Poore et al., 2011). This transition most likely occurs within a size range (e.g., 25 - 28 mm SCL) rather than a precise size, and it is further complicated by the fact that the size of *P. planipes* found in nature can be correlated with ocean temperature. Specifically, larger crabs have been found in colder water (Boyd 1962). Based on field and laboratory calculated growth curves, animals at the assumed benthic transition size are estimated to be at the end of their second year of life (Boyd 1962).

Pelagic and benthic habitats pose distinct challenges for the tuna crabs that occupy them. To remain in the pelagic zone, adult *P. planipes* must actively swim. Indeed, they are effective swimmers and are able to vertically migrate within the water column over a very short period, less than one hour (Robinson and Gomez-Aguirre 2004). Like other natantian crustaceans, *P. planipes* use a forceful tail-flip to propel rapidly upward in the water column and to escape predators. When

not actively swimming, they sink due to their negative buoyancy. Animals slow their descent by spreading out their legs and long chelipeds, which are covered with dense setae that help increase surface area and drag (Poore et al., 2011). During this highly mobile pelagic stage, tuna crabs need to be able to swim effectively to capture prey and evade predators. Pelagic crustaceans in general benefit from having relatively thin, lightweight exoskeletons compared to benthic species (Pütz and Buchholz, 1991). Thin, less mineralized exoskeletons aid in buoyancy and this highly maneuverable lifestyle, but comes at the expense of heavy exoskeletal armor that is more effective at resisting predatory attacks.

While inhabiting the benthic zone, *P. planipes* must navigate across soft substrates and around hard objects while avoiding predators. Sufficient exoskeleton armor is necessary for defense, as *P. planipes* often occur in aggregations and face pressures in the form of cannibalism, predation, and competition for resources and space on the seafloor. While living among the benthos, *P. planipes* are thought to shift trophic levels, from primary consumers to secondary consumers/scavengers (De Anda-Montañez, 2015), and no longer swim up into the water column for food. However, the evidence for this trophic shift is very limited.

Molting and exoskeleton construction

For crustaceans to grow, they must shed their exoskeleton and secrete a new one through the process of molting. Molting governs nearly every aspect of life for crustaceans, including metabolism, behavior, reproduction and sensory perception (Passano, 1960). Molting is also sensitive to environmental conditions; temperature and food availability in particular can greatly influence molting. Molting activity generally increases with increased temperature, implying that animal metabolism is accelerated (Passano, 1960). Excess food can also increase molting

frequency and increment (the size increase after molting) by providing extra energy (Passano, 1960). Sensitivity of the molting process to temperature and food availability can make growth more variable in species like *P. planipes* that encounter differences in these conditions as they migrate vertically and move with currents over significant distances. Laboratory determined intermolt duration for adult *P. planipes* (15 - 20 mm – pelagic stage) was approximately 35 days and followed a typical pattern for crustaceans after maturity, in that their molt frequency and molt increment (amount of growth between molts) decrease (Boyd 1962).

Molting also provides an opportunity for crustaceans to reconstruct the exoskeleton, which replenishes its mechanical integrity. The exoskeleton, or cuticle, is a complex material that is highly versatile despite being fairly conserved. It is hierarchical in nature, multi-layered, and consists of a protein-chitin matrix embedded with mineral. All four cuticle layers are secreted by the underlying hypodermis, including the calcified outer layers (epicuticle, exocuticle, endocuticle) and inner non-calcified membranous layer (Roer and Dillaman, 1984, Raabe et al., 2005). The outer layers are hardened with calcium carbonate as either crystalline calcite or amorphous calcium carbonate, but may also contain small amounts of magnesium and phosphorus (Roer and Dillaman, 1984). Each of the two inner calcified layers (exocuticle and endocuticle) is characterized by a unique arrangement of chitin-protein fibers arranged in lamellae, which are rotated in parallel sheets (Roer and Dillaman, 1984). The stacks of these helicoidally rotated sheets form what are known as Bouligand structures (Raabe et al., 2005). The lamellae are generally found closer together in the exocuticle compared to the endocuticle (Roer and Dillaman, 1984) and help to confer greater hardness in the exocuticle and greater toughness in the endocuticle (Chen et al. 2008).

The structure of the cuticle is relatively conserved among crustaceans, with some variations in layer thickness, lamellae stacking density, or mineral density. Even within an individual, there is variation in cuticle morphology and composition among various body regions to support different functions of the exoskeleton (Raabe et al., 2005). For example, some crustaceans have increased mineral content and cuticle thickness in the claw, which increases hardness and improves effectiveness in defense and prey capture (Chen et al. 2008, Boßelmann et al. 2007). In contrast, the carapace must be elastic enough to withstand bending and movement and is typically less mineralized (Boßelmann et al. 2007). Crustaceans can tune multiple aspects of exoskeleton morphology to optimize the structure (Raabe et al., 2005).

Molting and exoskeleton versatility provide mechanisms for crustaceans to adapt to new habitats and respond to environmental change. Adjustments to molting (frequency, molt increment) and exoskeleton morphology (composition, structure, and mechanical properties) can take place over short times scales and are known to occur when environmental parameters change, including dissolved oxygen (Wei et al. 2009), salinity (Jantrarotai et al. 2002), pCO_2/pH (Long et al. 2013) and temperature (Kuhn and Darnell 2019), among others. The responsiveness of the exoskeleton and molting to the environment can be either beneficial or detrimental, an issue that is commanding attention in climate change stressor research. For *P. planipes*, the pliancy of these traits may be a critical element of adult niche shift from the pelagic to the benthic zone. Broadly, these traits, in combination with strong physiological regulation, have facilitated the expansion of crustaceans into habitats spanning intertidal zones to deep sea, freshwater to rainforest.

Dissertation research

An impressive amount of work on *P. planipes* was done by C. M. Boyd for his dissertation research in 1962 at Scripps Institution of Oceanography, which inspired the work in this dissertation. There have been studies scattered throughout the 1990's and early 2000's focused on aspects of their distribution, biology, and abundance. More contemporary studies focused on their distribution and abundance, tolerance to low oxygen, and quality as aquaculture feed. Despite these informative studies, there is still so much to learn about these important and fascinating organisms. Aspects of their basic life history remain unresolved, including details about their adult pelagic and benthic life stages, their trophic role, and how they might be impacted as the oceans continue to change as a result of anthropogenic climate change. The major objectives of this dissertation were to investigate the pelagic and benthic life stages more deeply and to gain insight into how this species may be impacted by changing ocean conditions. I address these issues in three separate data chapter studies in this dissertation, preceded by an introductory section synthesizing what is known about the biology and lift history of *Pleuroncodes planipes*.

In Chapter 2, I address the question: Do benthic and pelagic phases of tuna crabs exhibit morphological differences related to feeding or defense that reflect disparate ecological niches? I used morphometric techniques and inductively coupled plasma mass spectrometry to look for differences in allometry and exoskeletal composition between these groups.

In Chapter 3, I used bulk stable isotope analysis to help determine whether adult pelagic-associated and fully benthic tuna crabs are ecologically distinct, as has been previously hypothesized. Evidence to support this notion is very limited, yet this idea is reinforced in citations throughout the literature. There has not yet been an effort to quantitatively determine whether these phases are distinct.

Finally, in Chapter 4, I ascertain how the survival, growth, molting and calcification of the exoskeleton in adult *Pleuroncodes planipes* responds to ocean acidification and warming conditions. I performed a long-term experiment where CO_2/pH and temperature were manipulated to simulate projected future ocean conditions.

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CHAPTER 2: Benthic vs. pelagic forms: ecomorphology clues about niche use in *Pleuroncodes planipes*

Abstract

Ecomorphology can be used to characterize relationships between organisms and their environment. This is especially relevant for *P. planipes*, as their complex life history and habitat use remain unresolved. It is thought that adults go through a pelagic stage followed by a benthic stage after reaching a larger body size. Though the relative time spent in these habitats is unknown, aspects of their morphology relevant to habitat use can be assessed to help determine whether these stages have habitat-specific specialization. In this study, we used an ecomorphological approach to test the hypothesis that pelagic and benthic *P. planipes* are distinct. Pelagic and benthic habitats impose different pressures on crustaceans, particularly related to feeding, locomotion and defense, so we examined multiple structures relevant for these functions (rostrum, chelipeds, mandibles and carapace) across juveniles and a broad size range of adult individuals using morphometric measurements and trace elemental analysis. Results showed that morphological differences exist in cheliped allometry and compositional differences of the exoskeleton between the juveniles and pelagic and benthic adults, yet the mineralization patterns were opposite to expectation. Benthic animals had relatively longer chelipeds, wider and longer chelae and less mineralized exoskeleton structures than juvenile and pelagic tuna crabs. The cheliped morphology of benthic tuna crabs correlates with greater force production, facilitating a diversified diet, yet the more mineralized exoskeleton of juvenile and pelagic animals confounds hypotheses about maintaining position in the water column. This study demonstrates for the first time that morphological and compositional differences are present between the adult pelagic and benthic life stages of *P. planipes,* which supports the standing hypothesis that they have distinct habitat use or specialization.

2.1 Introduction

Ecomorphology

Ecomorphology aims to elucidate relationships between morphological traits and environment or habitat. This field of study originated around the 1950s when ecologists found it practical to use basic morphology to address questions related to species niche, habitat use, and other related ecological situations (Bock 1994). The morphology of an organism can often provide clues about its ecological role (Bock 1994). For example, in labrid fishes, fin morphology can be directly related to swimming speed observed in the field and is also linked to their feeding mode and where they spend time on the reef (Wainwright, Bellwood, and Westneat 2002). Ecomorphology is dependent on findings of functional morphology, as understanding how structure relates to function is critical before applying those principles to ecology. Yet, ecomorphology is distinct in its focus on the biological role of the structure of interest, and not strictly function, and is coupled with consideration of the natural environment of an organism. In other words, not necessarily what the structure is capable of in a laboratory setting, but how it is employed in nature.

Ecomorphological studies begin with predictions about how morphological traits are adaptive to a specific environment, such as large eyes in a dark environment. These traits are then measured and compared, usually using some type of correlative measure, and then explained in the context of the analysis. Physical traits that are assessed include those relevant to feeding and habitat use. Overall, ecomorphological studies seek to determine how the physical or biological selective pressures of an environment can impact the phenotype of a species, including their feeding morphology, defensive structures, allometry, and performance.

Ecomorphological approaches have been beneficial in studying diverse species from a range of habitats and species, spanning terrestrial vertebrates, including birds and lizards, to aquatic organisms, including fish and crustaceans. In tropical freshwater fish species, morphological variation related to feeding (e.g., mouth gape, intestine tract length, mouth orientation and presence of barbels) has a significant predictive relationship with diet variation (i.e., prey size, prey type and position in water column) (Piet 1998). Ecomorphological relationships have also been demonstrated for brachyuran crabs (Marochi and Masunari 2016). Linear body measurements of the carapace, legs, eye stalks, abdomen and chelipeds of 17 different species of crabs from different habitats revealed 3 functional groups based on habitat type: Complex Substrates, Semiterrestrial, and Exclusively Aquatic. Crabs in the Complex Substrate group all had relatively shorter legs that are thought to aid in navigating uneven surfaces, and a cheliped with well-developed musculature that enables predation on hard shelled prey. Crabs in the Semiterrestrial group had relatively long eye stalks, likely for seeing predators, finding mates, and preying in a highly visual environment, and long fourth ambulatory legs that may be advantageous for predator avoidance by supporting an increased range of motion and escape velocity. The Exclusively Aquatic group had a larger body size, which is associated with a decreased risk of predation, underdeveloped pereopod muscles that are adequate for swimming and support in a buoyant medium, and relatively shorter eyestalks, since visibility is reduced underwater. Exclusively Aquatic crabs were further found to have a dorso-ventrally flattened carapace to help reduce drag while swimming.

Aquatic Niches/Habitats

Within the marine environment, there are several distinct habitat types/niches for which ecomorphological relationships are important, though they are not as well-studied as terrestrial systems. Yet, coral reefs, the deep sea, kelp forests, hydrothermal vents, and surface waters all have their own suite of physical and biological pressures that may require specific morphological traits. This is particularly apparent in considering the two broad, yet fundamental ocean habitats: pelagic and benthic zones. These habitats impose different pressures on animal locomotion, feeding, and defenses. Crustaceans are a diverse and successful group that possess a variety of different adaptations that can be suited to each environment.

Crustaceans that live either in the pelagic or nektobenthic zone rely on swimming behavior and therefore benefit from having a lightweight exoskeleton that improves mobility and increases buoyancy. Many pelagic crustaceans also have other exoskeletal features to help them maintain a position in the water column. In the case of the pelagic squat lobster, *Pleuroncodes planipes*, they are covered in dense setae that slow the rate of sinking by increasing surface area (Poore et al. 2011). In contrast, benthic crustaceans have a thicker, denser, and more heavily mineralized exoskeleton (Amato et al. 2008). This provides negative buoyancy to stay on the seafloor, as well as more stability for living in an environment subject to currents (Amato et al. 2008) and more rigidity for navigating around hard objects on the seafloor.

Feeding and prey capture differ widely between pelagic and benthic environments. The primary food available for pelagic crustaceans is phytoplankton or small zooplankton. In the benthic zone, however, food is more available as either particulate organic matter sifted from sediment on the seafloor or as scavenged organisms. Consumption of phytoplankton and organic matter suspended in the water column depends on the ability to maintain position in the water

column and an effective mechanism for capture. There are special mouthpart adaptations employed by many organisms to aid in pelagic and benthic filter/suspension feeding.

Predation pressures and, consequently, defense strategies also differ between the pelagic and benthic zones. Predators of pelagic crustaceans are diverse and can capture prey in several ways, including grasping and suction feeding, where the crustacean is ingested whole. Pelagic crustaceans have developed a suite of defenses to counter the array of predation threats including, exoskeleton modifications, miniaturization, camouflage and escape behavior. Many pelagic crustaceans, especially vulnerable larvae, are covered in spines that help increase their size beyond the gape of some predators; crab zoea can have spines up to seven times their body length (Bashevkin and Morgan 2020). To avoid visual predators, some crustacean larvae have transparent exoskeletons (Bashevkin and Morgan 2020). Additionally, pelagic crustaceans rely on swimming behavior to escape prey, either through a rapid escape swim response or through daily vertical migration to seek refuge from visual predators during the day at depth. Predators of benthic crustaceans are often more specialized and capture and consume their prey using crushing force. Benthic defenses, therefore, typically include a harder, more mineralized exoskeleton to resist crushing forces (Pütz and Buchholz 1991), as well as camouflage and specialized structures to aid in hiding under rocks or in burrows. The surface of the exoskeleton of decorator crabs, for example, is covered with complex and dense setae to which materials, like sponges, can be attached for camouflage (Wicksten 1980). Other benthic crustaceans have coloration that matches their habitat, like the green exoskeleton of grass shrimp, which matches the seagrass upon which it lives (Wägele 1989). The California spiny lobster has rostral horns that help them stay wedged underneath rocks. Critical to all these diverse defense mechanisms in crustaceans is the versatility of their exoskeleton.

Ecomorphology between these two habitats is poorly understood in crustaceans. There does, however, exist a clear relationship between rostrum length and vertical distribution. From the morphological examination of 18 decapod species, it was discovered that pelagic decapods have shorter rostrums than their benthic counterparts (Sardà, Company, and Costa 2005). This correlation is attributed primarily to the need to escape predators. The rostrum acts as a steering device for nektobenthic decapods who use the backwards tail-flip escape response to evade predators (Sardà, Company, and Costa 2005). In this group, the rostrum is long and curved upward which facilitates movement in the backwards direction. In contrast, the direction of escape in pelagic decapods is not as important, and their rostrum is typically short. Though crustaceans have historically been divided into swimmers (Natantia) and non-swimmers associated with the habitat bottom (Repantia) (Bentov, Abehsera, and Sagi 2016), ontogenetic shifts between these habitats within a single species remain understudied. Understanding an organism's distribution in the water column is necessary for understanding their ecological importance and establishing ecomorphological relationships in this context can help elucidate where an organism spends its time.

Tuna crabs, *Pleuroncodes planipes*

As adults, different crustacean species either remain in the pelagic zone or settle to the benthic zone. *Pleuroncodes planipes*, known also as tuna crabs, are an unusual crustacean in which adults are thought to have distinct pelagic and benthic stages (Boyd 1967). The so-called 'pelagic' adult stage is actually a stage where sexually mature adults use both the benthic and pelagic zones, sometimes following a diurnal pattern for feeding and predator escape (Boyd 1967; Aurioles-Gamboa 1992). After their second year of life, when reaching approximately 28 mm (estimates

vary, as reviewed in the Introduction) in standard carapace length (SCL), the tuna crabs supposedly become strictly benthic (Boyd 1967). It has been previously stated that no morphological differences exist between the two groups of tuna crabs (Longhurst 1966), however, the evidence for this is limited to decades-old observation methods and qualitative reporting of the presence of setose mouthparts and limbs only.

The possible occurrence of these distinct stages provides a unique opportunity to examine how the exoskeleton and structures related to key behaviors may reflect changes relevant to habitat in a single species, as tuna crabs must adapt to these habitats sequentially. Establishing ecomorphology relationships will also provide additional support, or even some resolution, to the long-standing hypothesis about this niche shift in adult *P. planipes*.

Major selective pressures associated with the transition from pelagic to benthic zones can be categorized into either locomotion, predation or feeding, which all differ between the pelagic and benthic zones. In the pelagic zone, tuna crabs need to maintain their position in the water column, wherein a lightweight exoskeleton is beneficial to increase buoyancy and minimize energy expenditure. In addition, pelagic tuna crabs likely depend more on swimming behavior than body armor to evade predators. In the water column, tuna crabs are subject to predation by large vertebrates approaching from all directions, and without substrate for hiding, their only option is to escape by rapid swimming. Many of these large predators also swallow tuna crabs whole, sometimes in large numbers, rendering body armor useless. Though *P. planipes* also have a presumed benthic life stage, they have special morphological adaptations to aid them in a pelagic existence, in the form of heavy setae along the margins of the flattened legs that slow sinking rate (Boyd 1962). Conversely, in the benthic zone, tuna crabs and other crustaceans benefit from having negative buoyancy and a denser exoskeleton to remain on the benthos. Tuna crabs are also subject

to more numerous and diverse predators on the benthos, including crushing and piercing fish and invertebrates.

Different modes of feeding may be favorable as *P. planipes* transitions between pelagic and benthic zones. Tuna crabs are associated with highly productive areas because they suspension-feed directly on phytoplankton (Poore et al. 2011), which happens to be the primary food source in the water column. Yet, the benthos is rich in macrofauna and debris. It is therefore thought that pelagic tuna crabs are primarily suspension feeders on phytoplankton and benthic tuna crabs are opportunistic scavengers of macrofauna that can also suspension-feed and obtain particulate organic matter from the sediment. Pelagic tuna crabs use their maxillipeds to generate a stream of water through which particles pass, whereas benthic tuna crabs likely depend more on using their long chelipeds to bring scavenged food to their mouths and their strong mandibles to break those larger food particles down. These differences in locomotion, feeding and defense pressures may correlate with changes in exoskeleton morphology as adult tuna crabs transition from the pelagic to benthic zones.

Adaptability of Crustacean Exoskeleton

Few animal materials are as malleable as the crustacean cuticular exoskeleton (or cuticle). Its complex structure permits the tuning of multiple aspects to facilitate a range of different functions (see Introduction chapter for details on exoskeleton morphology). Crustaceans have, therefore, evolved a variety of exoskeletal modifications and structures for feeding, defense and mobility that are responsive to challenges in their environment. In particular, cuticle density, thickness, and mineralization are the most commonly adjusted aspects for adapting to lifestyles in different environments (Amato et al. 2008).

Feeding

Crustaceans have a highly specialized series of mouthparts, consisting of 3 pairs of maxillipeds that are used for manipulating food, 2 pairs of maxillae that cut food into smaller pieces, and 1 pair of mandibles that chew food passed from the maxillae. Further processing of food takes place in the foregut, where the gastric mill, a unique and complex series of tooth-like structures, grinds food much like a bird gizzard. These mouthparts are functionally organized in order to optimize a particular mode of feeding, such as filter feeding (Manjulatha and Babu 1991). To aid in the capture of suspended food particles, the mouthparts are often modified with setae, which are even in larger decapod species (Riisgård 2015). The filter-feeding mechanism in *P. planipes* has been described in detail (Longhurst, Lorenzen, and Thomas 1967) and allows them to feed on phytoplankton and zooplankton. Filter feeding apparatuses also facilitate suspension feeding in benthic crustaceans, enabling them to take advantage of infauna as food (Riisgård 2015). Thus, *P. planipes* may retain their filter feeding morphology after they settle to the benthos even if they are no longer feeding in the water column.

Benthic crustaceans have a much more diversified diet that involves preying on other invertebrates, so mandibles take a prominent role. For durophagous diets, or eating other organisms with hard shells, mandibles need to be hard to effectively crush hard materials, so they are commonly modified by adding calcium phosphate or different forms of apatite that increase their hardness and resistance to wear (Bentov et al. 2016; 2012).

Defense

Exoskeletal defensive structures are common and diverse. Though the hard exoskeleton inherently provides a rigid armor, specialized structures enhance defenses against particular

threats. Especially notable are the prominent spines covering the bodies of California king crabs (*Paralithodes rathbuni*), spiny lobsters (*Panulirus interruptus*), and larval porcelain crabs (family: Porcellanidae). Among many shrimps and lobsters, a sharp rostrum extends anteriorly between the eyes and can be used to jab potential predators or other threats. Rostrums can vary in length and sharpness depending on where the animal lives in the water column. For example, rostrum length and sharpness vary within the same species of cave shrimp, depending on the presence of their predator (Jugovic et al. 2010). Longer rostrums have greater reach, but are more susceptible to buckling, and sharper rostrums are more effective at piercing soft tissues.

Powerful chelae are often the primary defense for clawed crustaceans, and their biomechanics is well-studied (reviewed in Fujiwara and Kawai 2016). Musculature, occlusive structures and chelae dimensions determine the pinch force of crustacean claws. Chela width and height (cross-sectional area) are correlated with pinch force because they reflect the muscle mass available to generate force. Larger dimensions are correlated with greater force production (Brown, Cassuto, and Loos 1979). Length of the dactyl (moveable finger of the claw) determines force output based on simple lever mechanics: longer levers, or dactyls, correlate with lower output, or pinch force. The dual functions of the chelae for prey capture and defense can pose competing demands on the evolution of claw morphology, leading to trade-offs in force production, claw gape, and energetic investment.

Locomotion

Crustacean exoskeletons can be adapted in multiple ways to optimize different types of locomotion in different habitats. Some species, for example, have evolved paddle-like structures on their fifth pereiopods (walking legs) to facilitate swimming (Spirito 1972) or longer pereiopod

segments to increase running speed (Burrows and Hoyle 1973). Carapace shape can be adapted similarly, with more hydrodynamic shapes to reduce drag in swimming species (Blake 1985) or flattened shapes to reduce toppling in tree-climbing species (Fratini et al. 2005). The shape of the carapace is also related to crustacean distribution in the water column; pelagic crustaceans were found to have a longer, flatter carapace compared to benthic and nektobenthic species, which have a more rounded carapace (Sardà, Company, and Costa 2005).

Exoskeleton morphology and composition is more readily modified and can reflect the habitat in which a crustacean lives, though this has been less studied. For example, the exoskeletons of some pelagic crustaceans have several features that are structurally distinct from their benthic counterparts. Pelagic crustaceans typically have closer stacked lamellae in the endocuticle than benthic crustaceans (Pütz and Buchholz 1991). Nektobenthic malacostracans (largest class of crustaceans), which vertically migrate within the water column, have an endocuticle with decreasing lamellae stacking density toward the membranous layer (Pütz and Buchholz 1991). However, pelagic crustaceans exhibit a thin, lightly mineralized and lightweight cuticle (Pütz and Buchholz 1991). Reduced exoskeleton density, through altered thickness, lamellae stacking density, or mineral content, could aid pelagic animals in maintaining position in the water column. However, limited analysis has been done on the cuticle of pelagic crustaceans, where buoyancy issues can affect mobility in the water column. In contrast, the cuticle of benthic crustaceans is typically thicker and more heavily calcified (Pütz and Buchholz 1991).

Objective

The main objective of this study was to determine whether there are morphological differences in the exoskeleton between the pelagic and benthic adult stages of tuna crabs. We tested

the hypotheses that: (1) pelagic tuna crabs have a comparatively lightweight exoskeleton (less calcified) that becomes more dense (more calcified) when they transition to the benthos, (2) that feeding structures (mandibles and chelae) become more mineralized, and perhaps morphologically or allometrically distinct when tuna crabs transition to the benthos, and (3) that defense structures (carapace and rostrum) become more mineralized or morphologically distinct when tuna crabs transition to the benthos to be better suited in the different habitats.

2.2 Methods

Animal Collection

Live tuna crabs, *P. planipes*, within the juvenile (8-13 mm carapace length (CL)), pelagic adult (15-26 mm CL), and benthic adult (26-33 mm CL) size ranges were collected in the winter of 2020 off the coast of La Jolla, CA using SCUBA, trawls and traps. Collection took place over a broad range of depths, from surface (juveniles) to between 30 and several hundred meters depth, as the goal was to obtain as wide a size range as possible. Upon collection, animals were brought to Scripps Institution of Oceanography (SIO) and immediately placed in a -20°C freezer for euthanization. Animals were then stored in sealed bags in a -20°C freezer until dissections and measurements were carried out in 2022.

The juvenile, pelagic adult and benthic adult groups were collected at the same time (Winter 2019), whereas the benthic L group was collected later (Summer 2022) and constituted a larger size class than the benthic group.

Morphological measurements

All morphological measurements were performed on euthanized tuna crabs to increase precision. Each tuna crab was sexed and measured for carapace length (from the notch between the supraorbital spine and rostrum to the dorsal posterior median edge of the carapace) to the nearest 0.01 mm using digital calipers (Mitutoyo Digimatic Calipers, CD-6" AX, Mitutoyo Corporation, Tokyo, Japan). Only tuna crabs in the intermolt stage were used for analysis.

The exoskeleton features (and associated functions) examined were: mandibles (food capture/handling), carapace (defense), rostrum (defense), and chelipeds (food capture/handling/defense). Morphological measurements focused on the carapace, rostrum and cheliped, whereas cuticle composition analysis was focused on the mandibles, carapace, and cheliped dactyls.

Four distinct claw measurements were used to compare allometric relationships across the size ranges collected. The four metrics were: total cheliped length, claw height, claw width and dactyl length. Chelipeds were removed at random (right or left as *P. planipes* do not have heterochely) at the coxa-basis joint using scissors and were then laid flat. Total cheliped length was measured as the distance from the distal edge of the basis to the tip of the dactyl. Claw height and width were measured at the tallest and widest portions of the propus. Dactyl length was measured from the proximal base, at the pivot point, to the distal tip. Each metric was measured three times to the nearest 0.01 mm using digital calipers and averaged.

Rostrum sharpness was measured after being excised from the carapace. Separated rostrums were positioned and imaged under a stereomicroscope (Leica M165 C, Buffalo Grove, IL, USA) equipped with a HD digital camera (Leica DFC290, Buffalo Grove, IL, USA). Sharpness

of the dactyl tip was measured as the radius of curvature. This was done from the images by fitting the largest circle possible into the tip of the rostrum using the Leica microscope software.

Cuticle Mineral Composition

Elemental trace analysis was performed on the mandible, carapace and dactyl dissected from 52 euthanized tuna crabs spanning the full size range collected. Samples of carapace (approximately 5 x 5 mm) were cut from a consistent location on the lateral posterior, dorsal region using dissection scissors. Dactyl tips were cut from randomized claws, at approximately 5 mm from the distal tip using dissection scissors. Mandibles were dissected, and due to asymmetry, the right one was used for analysis. Samples were rinsed with distilled water and any underlying hypodermal tissue was carefully removed with a small paint brush before being air dried.

Just prior to ICP-MS analysis, samples were weighed to the nearest 0.00001 g. Samples were then placed into teflon vials, dissolved in 0.5 ml of concentrated Teflon-distilled (TD) nitric acid, and set on a hot plate at 120° for >24 h. Afterwards, samples were dried down and diluted by a factor of 4000 with 2% TD HNO_3 before being transferred to pre-cleaned centrifuge tubes for analysis. Samples were doped with an indium solution in order to monitor instrumental drift. Once samples were prepared, they were run through a mass spectrometer, which provided counts for each element present in the sample. Measurements were carried out using a *ThermoScientific iCAPq* c ICP-MS (Thermo Fisher Scientific GmbH, Bremen, Germany), in standard mode. Masses of Mg, Ca and P were sequentially measured for 30 ratios, resulting in internal precision of <2% (2 s.d.). Elements were corrected for total mole fraction (n = 52 per exoskeletal structure). Total procedural blanks represented <0.3% of the measurement for Mg and Ca. Raw data were corrected off line for instrument background and drift. Samples were bracketed by internal standards of crab carapace (n = 2) and two artificial fish otolith solutions, which allowed for calculation of absolute

values. The standards yielded external precision of better than 1% for Mg and Ca (2 s.d.). This analysis was carried out at the Scripps Isotope Geochemistry Laboratory at SIO.

Data Analysis

All statistics were computed using R (v. 4.2.01). An ordinary least squares linear regression was used to determine whether there was an explanatory relationship between rostrum sharpness and body size. OLS linear regressions were performed for each of the four claw measurements against carapace length. The four claw measurements were compared using a principal component analysis. Only males were used in the adult groups for claw measurements, as there were slightly uneven sex ratios in the sample groups and sexual dimorphism in the chelipeds of this species has been noted after sexual maturity. Specifically, males have longer and wider chelae than females (Serrano 1991). This analysis was carried out in the Vegan Community Ecology package for R using the rda() function. The body length corrected values for total cheliped length were compared across the groups using an ANOVA (after being assessed for normality and homogeneity of variance using Shapiro-Wilk and Bartlett's Tests) and post hoc Tukey-Kramer Test as this appeared to drive the difference seen between the claw metrics.

ICP-MS data were corrected for body size by dividing the elemental content by carapace length of the animal. Four groups (juveniles, pelagic, benthic and benthic L) were evaluated for elemental composition across three sections of the exoskeleton: carapace, claw and mandible. The benthic L and benthic groups had some significant differences in elemental composition, so were kept separate for elemental analysis. Data were tested for normality and homogeneity of variance using Shapiro-Wilk and Bartlett's Tests. Kruskal Wallace tests with post hoc Dunn Tests were

performed to compare the elemental compositions across the groups. All data are presented as mean ± standard deviation.

2.3 Results

Morphology

Rostrum sharpness (shown as radius of curvature) showed no correlation with carapace length (OLS: N= 36, R^2= -0.0028, p = 0.8495) (Figure 2.1) and was therefore similar across all groups.

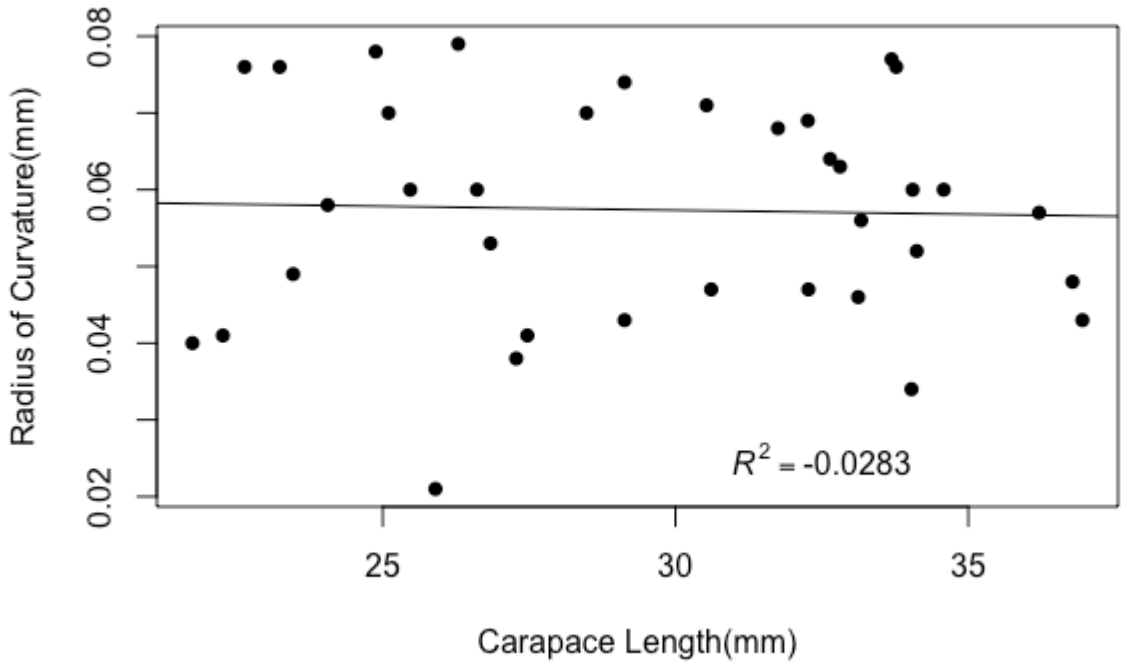

Figure 2.1: Rostrum sharpness (shown as radius of curvature) shows no correlation with carapace length for tuna crabs across the size range collected. N = 36.

Claw allometry was different across the size groups, following a trajectory from juvenile to pelagic to benthic increasing in relative length, width and height. The principal component

analysis yielded separation between all three groups of tuna crabs; benthic, pelagic and juvenile size groups all clustered apart (Figure 2.2) (Table 2.1). The first principal component, total cheliped length, explained 0.95890 of the variance.

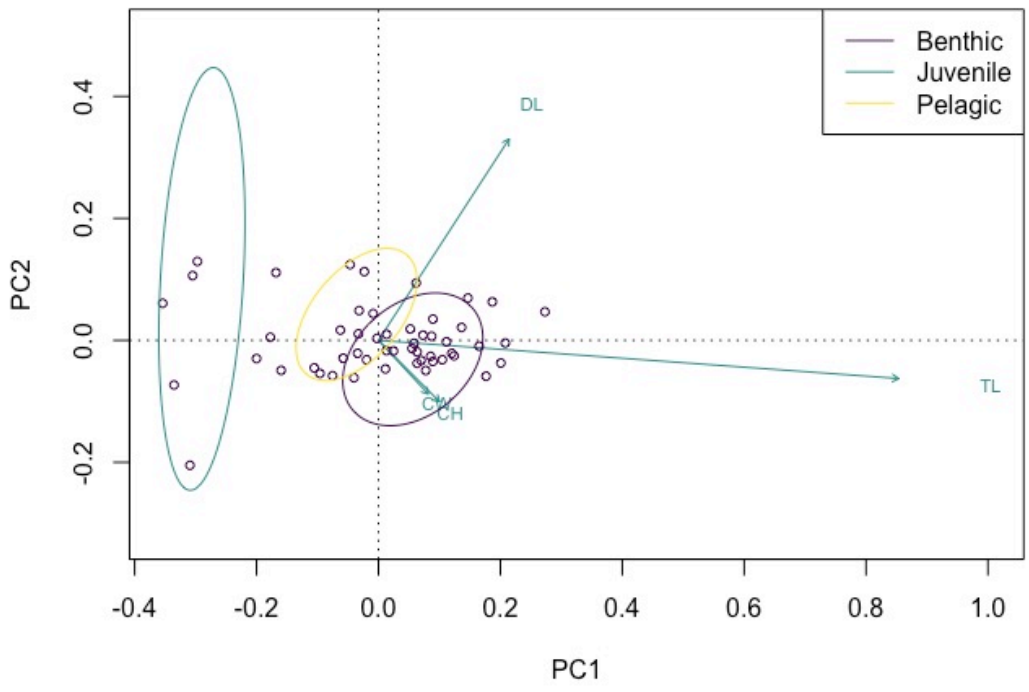

Figure 2.2: PCA of the four claw metrics. Juveniles clustered in the blue ellipse on the left of the figure. Pelagic crabs appear in the yellow ellipse in the middle. Benthic crabs are on the right side clustered in purple. DL = dactyl length, TL = total cheliped length, CH = claw height, CW = claw width.

Table 2.1: Summary values for the claw metric PCA.

Principal Component	**PC1**	**PC2**
Cheliped Length	0.9939	-0.02968
Dactyl Length	0.2496	0.15637
Claw Height	0.1168	0.08534
Claw Width	0.0952	-0.04170
Eigenvalue	0.02354	0.0006437
Proportion Explained	0.95890	0.0262243
Cumulative Proportion	0.95890	0.9851256

Tuna crab groups differed significantly in all four claw metrics (corrected for body size before comparison by dividing by carapace length): relative cheliped length (ANOVA, $F = 18.245$, $p = 0.0001$) relative dactyl length (ANOVA, $F = 6.2$, $df = 2$, $p = 0.0034$) (Figure 2.3), relative chela height (Kruskal-Wallis, chi-squared = 26.38, $df = 2$, $p = 1.87e-06$) (Figure 2.4) and relative chela width (Kruskal-Wallis, chi-squared = 26.42, $df = 2$, $p = 1.83e-06$). Post hoc significant differences were found between pelagic and benthic tuna crabs in relative cheliped length ($p = 0.001$), chela height ($p = 0.0002$), chela width (9.75e-04). Benthic tuna crabs had the greatest relative cheliped length (2.40 ± 0.15) compared to both juvenile (2.08 ± 0.07, $p = 0.00005$) and

pelagic adults (2.26 ± 0.12, p = 0.001), and a smaller, but significant difference between juveniles and pelagic (p = 0.04) (Figure 2.3).

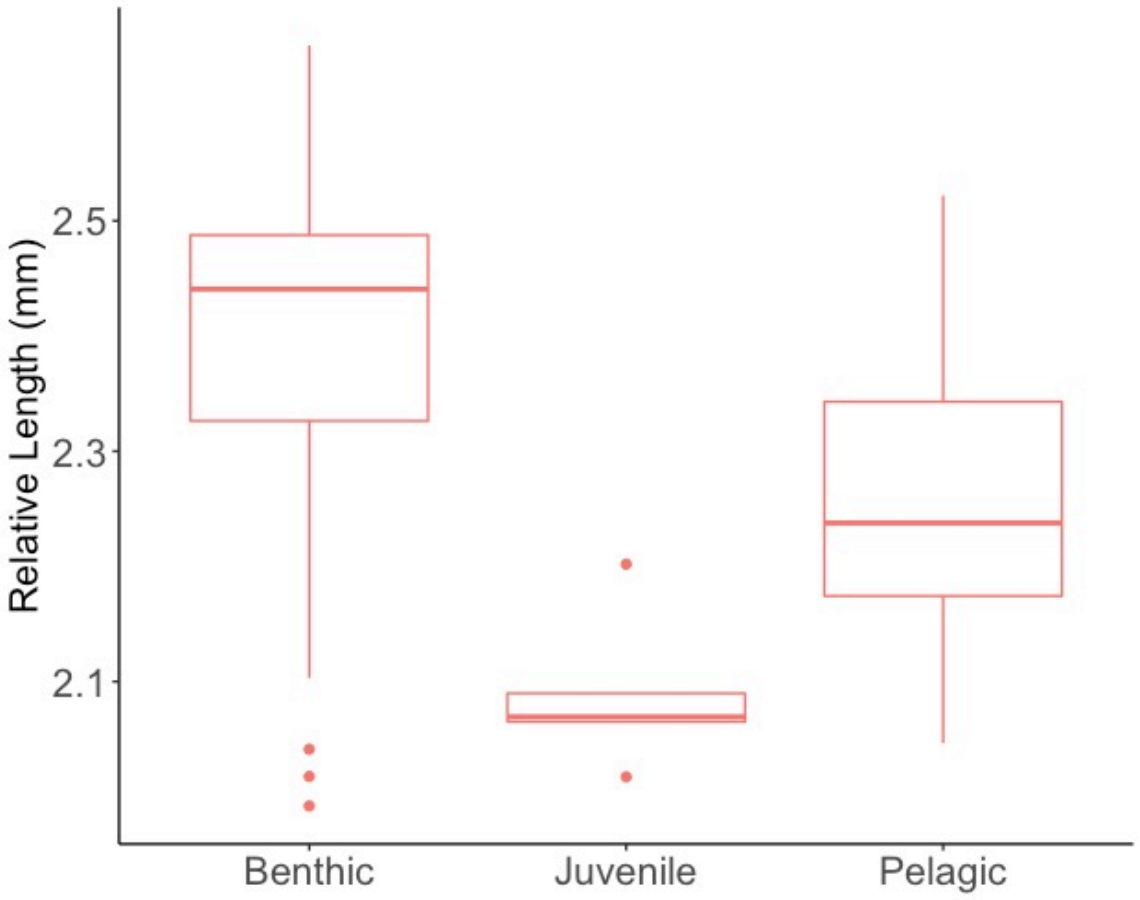

Figure 2.3: Relative cheliped length was greater for benthic (N=38) tuna crabs compared to juvenile (N=5) and pelagic (N=24) individuals. Box boundaries: 25th and 75th percentile; error bars: 10th and 90th percentile; solid line: median.

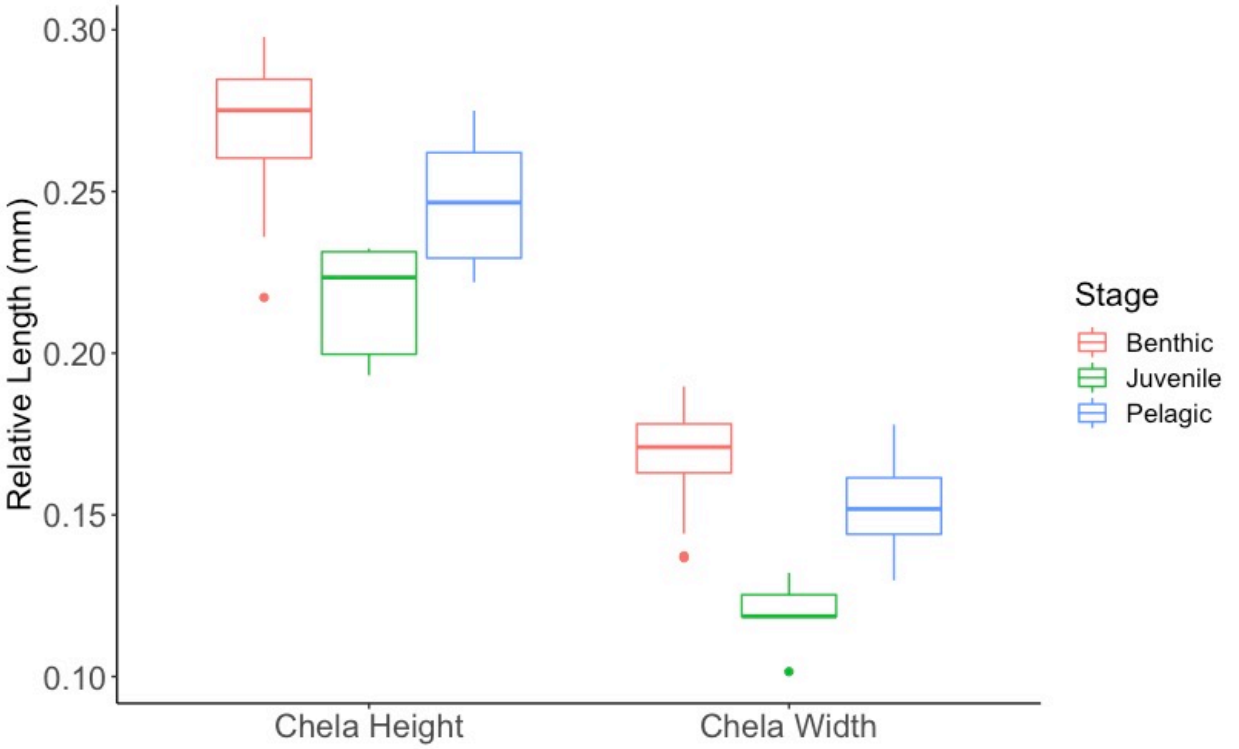

Figure 2.4: Relative chela (claw) height and width are greater for benthic (N=38) tuna crabs compared to juvenile (N=5) and pelagic (N=24) individuals. Box boundaries: 25th and 75th percentile; error bars: 10th and 90th percentile; solid line: median.

Elemental composition

Calcium content differed significantly across all body structures between the groups (carapace: Kruskal-Wallis, chi-squared = 41.35, df = 3, p = 5.519e-09, claw: Kruskal-Wallis, chi-squared = 42.45, df = 3, p = 3.221e-09, and mandible: Kruskal-Wallis, chi-squared = 43.19, df = 3, p = 2.243e-09) (Figure 2.5). For the calcium content in the carapace, the pelagic and juveniles did not significantly differ (p = 0.054), but the pelagic and benthic (p = 0.015) and the pelagic and benthicL (p = 4.91e-07) were significantly different. The juveniles had significantly higher magnesium content than the other groups across all body structures (carapace: Kruskal-Wallis chi-squared = 41.16, df = 3, p = 6.05e-09, claw: Kruskal-Wallis chi-squared = 42.56, df = 3, p = 3.05e-

09, and mandible: Kruskal-Wallis chi-squared = 43.36, df = 3, p-value = 2.06e-09) (Figure 2.6). Phosphorus content also differed significantly between groups (Kruskal-Wallis chi-squared = 39.74, df = 3, p = 1.21e-08). The patterns for phosphorus followed similar patterns to calcium and magnesium, with juveniles trending toward higher concentrations than the other groups.

The calcium to magnesium ratio (Ca:Mg) differed significantly between the groups: carapace (Kruskal-Wallis, chi-squared = 12.25, df = 3, p = 0.007), claw (Kruskal-Wallis, chi-squared = 27.58, df = 3, p = 4.46e-06), and mandible (Kruskal-Wallis, chi-squared = 25.51, df = 3, p = 1.28e-05) (Figure 2.7). For the carapace, the juvenile group had a lower Ca:Mg ratio than the other three groups (benthic: p = 0.006, benthic L: p = 0.02, and pelagic: p = 0.009). For the claw, the benthic and juvenile (p = 0.017), the benthic L and pelagic (p = 0.0002) and the juvenile and pelagic (p = 0.00014) groups differed significantly. For the mandible, all groups differed significantly except the benthic and benthic L (benthic/juvenile: p = 1.26e-03, benthic L/juvenile: p = 6.67e-05, benthic/pelagic: p = 1.19e-01, benthic L/pelagic: p = 5.01e-03, and juvenile/pelagic: p = 1.59e-01), with juveniles having a lower Ca:Mg than the other groups. Body size corrected values for these elements are summarized in Table 2.2.

Table 2.2: Body size-corrected values for calcium, magnesium, Ca:Mg and Phosphorus content (µmol/g/mm) across size groups. (Mean ± sd).

	Ca	Mg	Ca:Mg	P
Juvenile n = 5				
Carapace	1.71 ± 0.46	0.37 ± 0.10	4.72 ± 1.41	0.336 ± 0.130
Claw	4.38 ± 0.62	0.38 ± 0.06	11.49 ± 0.80	0.225 ± 0.069
Mandible	3.75 ± 1.88	0.53 ± 0.21	6.83 ± 0.63	0.258 ± 0.116
Pelagic n = 19				
Carapace	0.80 ± 0.32	0.08 ± 0.03	10.63 ± 2.60	0.101 ± 0.061
Claw	0.49 ± 0.24	0.06 ± 0.03	8.98 ± 0.82	0.032 ± 0.017
Mandible	0.45 ± 0.20	0.05 ± 0.02	10.02 ± 0.90	0.023 ± 0.013
Benthic n = 12				
Carapace	0.40 ± 0.22	0.04 ± 0.02	10.67 ± 2.52	0.033 ± 0.028
Claw	0.20 ± 0.06	0.02 ± 0.007	9.54 ± 0.90	0.011 ± 0.006
Mandible	0.18 ±0.06	0.02 ±0.006	10.92 ± 0.88	0.007 ± 0.003
Benthic L n = 15				
Carapace	0.13 ±0.03	0.01 ±0.003	10.93 ± 0.33	0.005 ± 0.001
Claw	0.092 ±0.01	0.009 ±0.001	10.37 ± 0.33	0.006 ± 0.001
Mandible	0.45 ±0.02	0.008 ±0.002	11.25 ± 0.49	0.002 ± 0.001

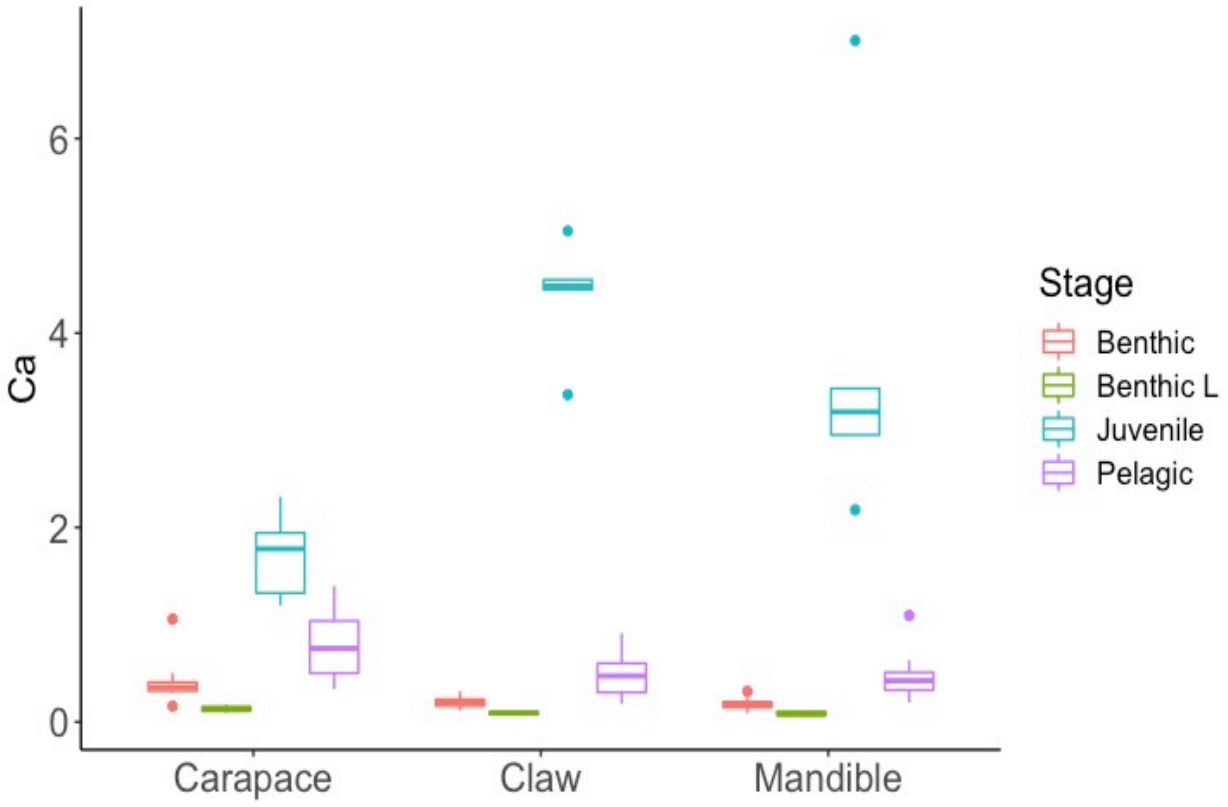

Figure 2.5: Calcium content (µmol/g/mm) in the carapace, claw and mandible of the different size groups. Both benthic groups had consistently less Ca than juvenile and pelagic groups in each structure.

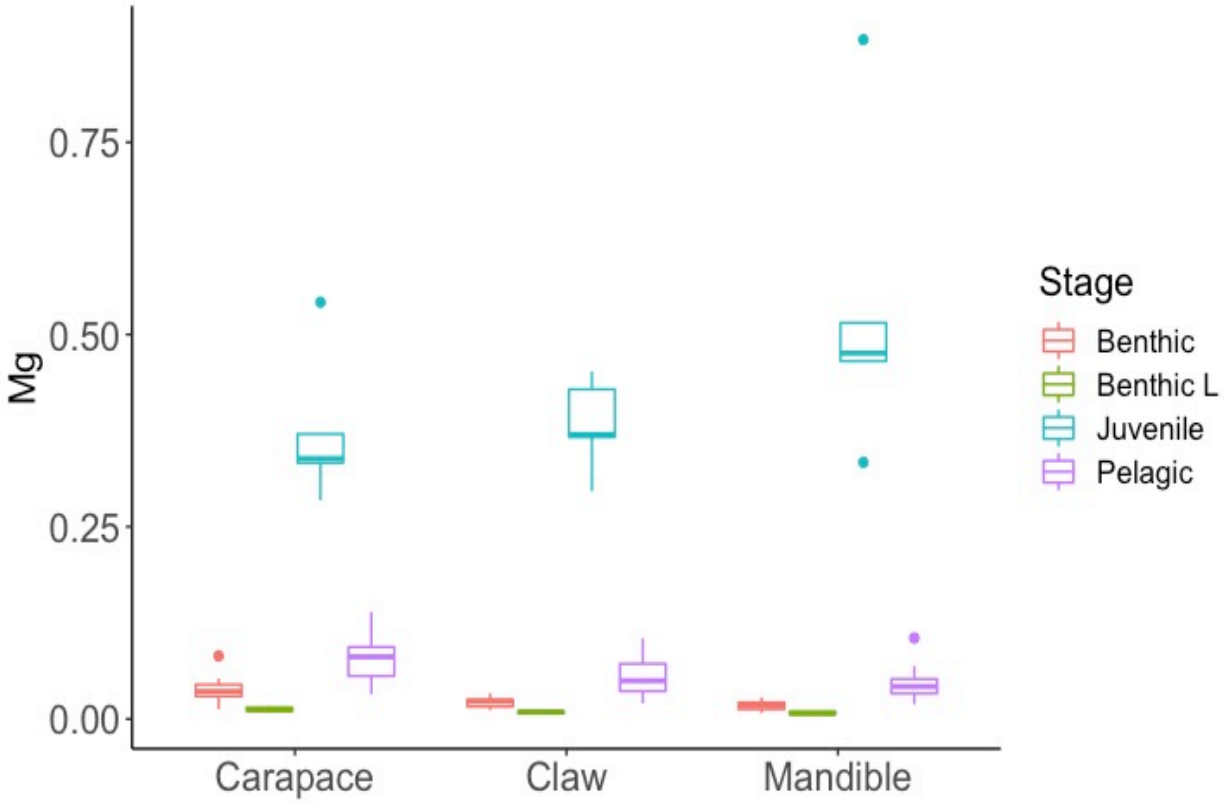

Figure 2.6: Magnesium content (µmol/g/mm) in the carapace, claw and mandible of the different size groups. The juveniles had significantly higher Mg content than the other groups across all body regions.

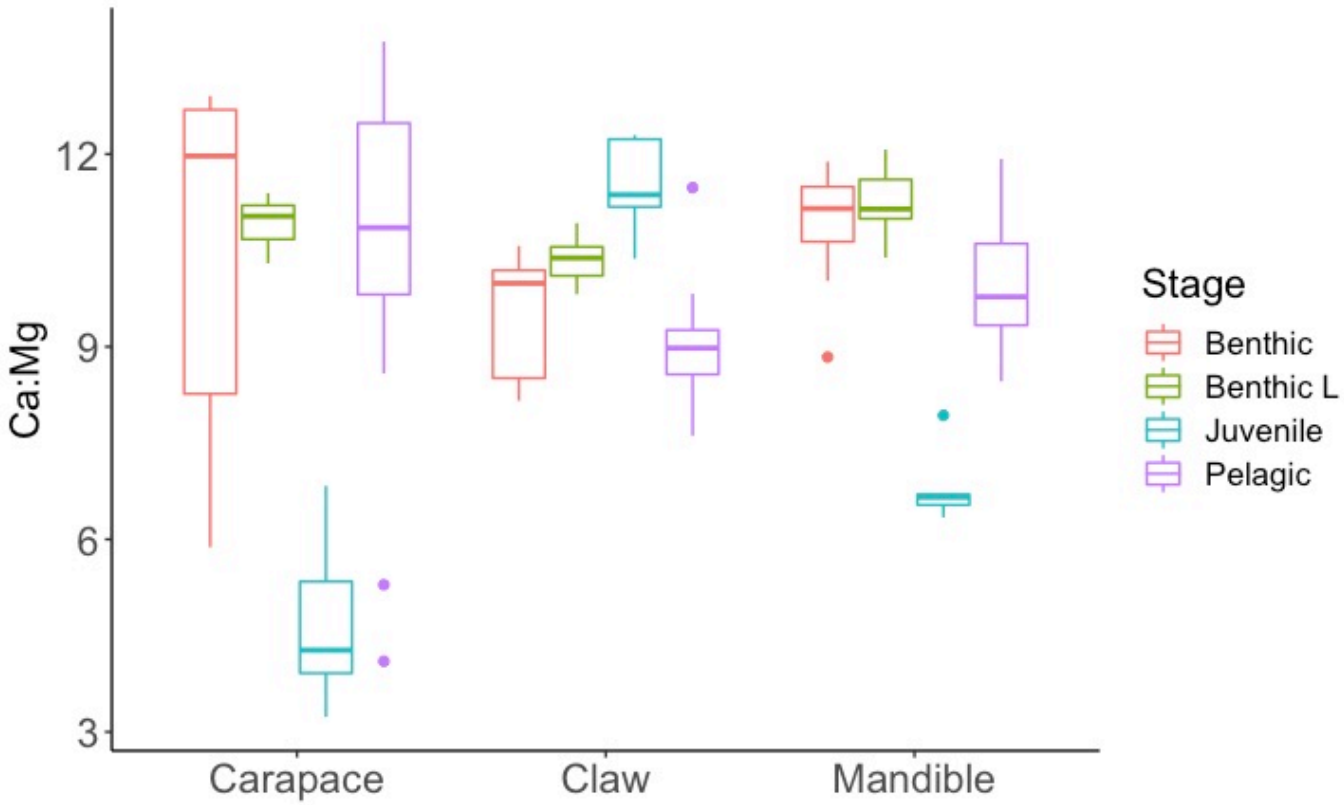

Figure 2.7: Calcium to Magnesium ratio (Ca:Mg) for the carapace, claw and mandible of the different groups. Juveniles had lower ratios in the carapace and mandible than other size groups, but not the claw.

2.4 Discussion

The postulated niche shift of adult tuna crabs, *P. planipes*, from a pelagic to a benthic existence is based primarily on their abundance in different trawl collections. Pressures associated with feeding, locomotion, and protection differ between these distinct habitats, potentially driving changes in relevant morphology. This ecomorphological analysis revealed potentially important morphological adaptations in *P. planipes* that help resolve a key uncertainty in their life history - are adult pelagic and benthic animals distinct in their feeding behaviors or habitat use?

Morphological separation between habitat

Rostrum sharpness was evaluated as one potential morphological trait that is responsive to different predation pressures and therefore might distinguish pelagic and benthic tuna crab stages. Rostrum sharpness did not, however, have a relationship with tuna crab size, and thus no difference between the size groups. This suggests that either the pelagic and benthic individuals do not have distinct morphological adaptations for defense, or that tuna crabs do not rely on their rostrum as a primary defense, though it is common in other species (Kunz et al. 2006). For pelagic tuna crabs, their main predators are fish and mammals, whose soft tissues would be subject to greater damage with sharper rostrums. On the other hand, rostrums easily break and wear as crustaceans interact with predators and navigate their environment, generating large variability in length and sharpness. It is somewhat surprising then that pelagic and benthic groups have similar rostrum sharpness, because benthic crabs most likely encounter more hard structures and predators on the seafloor. One might therefore expect to see more blunt rostrums in benthic tuna crabs that aren't as easily broken. Determining the true use of rostrums in tuna crabs would shed light on these results.

Tuna crabs use their claws for both feeding and defense, which makes parsing out the ecological function difficult. Nonetheless, there were key differences in claw morphology between size groups; claw allometry clustered apart between the juvenile, pelagic and benthic groups. There were significant differences in total cheliped length, claw height and claw width (metrics that confer pinch force) between the pelagic and benthic groups. Chelipeds become relatively longer, and larger in cross-section in relation to the rest of the body through the life stages. Essentially, as tuna crabs get larger, they also develop relatively larger chelipeds, even after reaching sexual maturity. Thus, benthic tuna crabs have relatively longer and potentially stronger chelipeds than pelagic tuna crabs. This might reflect a potential advantage of allometrically larger chelipeds once

the animals settle out to a benthic habitat. Larger chelipeds could be advantageous in the benthic habitat for defense, in the sense that they could be used to keep predators or other threats at bay from a slightly longer distance, offering more protection to important body parts. Benefits to feeding might be clearer, as it is thought that the benthic individuals are opportunistic scavengers and longer and stronger chelipeds would likely aid in prey handling and capture. Interestingly, pelagic tuna crabs group more closely with juveniles, which are known to occupy the pelagic habitat (Longhurst 1968), than with benthic tuna crabs. Sharing more similar morphological space supports the possibility that the pelagic tuna crabs are spending more time in the pelagic zone than the benthos.

Exoskeletal mineralization separates habitats, but not function

A stark pattern of differences in exoskeleton mineralization was observed between juvenile, pelagic and benthic groups of *P. planipes* that contrasted with our hypothesis. Benthic tuna crabs were hypothesized to be more heavily mineralized than pelagic animals to improve feeding, defense and locomotion on the seafloor. Interestingly, pelagic and juvenile tuna crabs had greater calcium content than benthic animals. Also, juveniles had greater Mg and P content than pelagic and benthic animals. This was consistent across all functional structures examined (carapace, mandible, and claw), revealing a general trend of the juvenile group having higher masses of the elements, followed by the pelagic and then the benthic groups. This was unexpected because, generally, benthic crustaceans have a more dense and heavily calcified exoskeleton than pelagic crustaceans (Pütz and Buchholz 1991).

Pelagic and juvenile tuna crabs had higher mineral content in the carapace than benthic crabs, suggesting one or more of several possibilities: that the differences in mineral observed

between size groups has negligible effects on buoyancy, that juveniles use other mechanisms to achieve proper buoyancy, that the dense setae covering the body, coupled with body positioning, is highly effective at slowing descent, or that juveniles simply expend more energy to swim in the water column. On the other hand, the benthic and benthic L individuals may benefit from a lighter weight exoskeleton, perhaps by affording them increased mobility, which is unsurprising considering their presumed migratory cross shelf/slope behavior and observed tidal stream use for feeding (Robinson and Gomez-Aguirre 2004; Aurioles-Gamboa and Pérez-Flores 1997). It is also possible that benthic tuna crabs cannot readily obtain the environmental Ca or Mg necessary to build a heavily calcified cuticle, though they reside above the Calcite Compensation Depth. Interestingly, the pelagic tuna crabs once again demonstrate intermediate mineral concentrations in the carapace; they are not as heavily calcified as the juveniles, but more so than the benthic groups. This supports the argument that there is some real advantage to having a more calcified cuticle in the water column relative to the benthos and that the pelagic tuna crabs are potentially relying on their exoskeleton in a different way than the benthic tuna crabs. However, it is also possible that other factors related to differential habitat use are driving differences in mineralization patterns. Specifically, there is a positive relationship between temperature and calcification in some crustaceans (Greenaway 1985) and warmer temperatures can also increase expression of cuticle/calcification genes in smaller/younger crustaceans more than adults (Stillman et al. 2020). If the pelagic tuna crabs are spending more time in the water column where it is warmer, and the benthic tuna crabs are spending more time in the cooler temperatures on the seafloor, this could also potentially explain the differences.

In line with patterns observed for the carapace, neither the mandibles nor claws exhibited mineralization patterns that fit our functional niche separation hypotheses. It was expected that

benthic tuna crabs would have more heavily mineralized claws, to defend against more diverse threats and to, along with more mineralized mandibles, support an expanded diet that includes durophagy. It is well-established that crustaceans have fine local control over the mineralization process, leading to variation across body regions and special structures, exemplified by the raptorial appendage and telson of mantis shrimp (Weaver and Milliron 2012; Taylor and Patek 2010; Currey, Nash, and Bonfield 1982). Thus, it would be a reasonable hypothesis that the mandible and claw would show different patterns compared to the carapace. That this pattern did not materialize in these structures is difficult to explain since the juveniles, which are thought to only feed on plankton in the water column, have such highly mineralized mandibles and claws relative to the benthic animals. It is possible that the benthic tuna crabs are using other minerals, like Mg or P, to harden their mandibles and claws, but these elements also show the same patterns as Ca. Further explanations for this pattern are unsatisfying and warrant a deeper look into the structure of the exoskeleton and crystal forms of these minerals.

Despite the inability of these data to satisfy our functional hypotheses, they do clearly establish a distinction between pelagic and benthic tuna crabs based on exoskeleton mineralization. Since it is known that juveniles are pelagic (Longhurst 1968), that "pelagic" adults are both pelagic and benthic, and that benthic adults are at least benthic as much of the time as pelagic (if not more), it is reasonable to think that the pelagic and benthic groups might be using the habitats differently due to these elemental differences, but not in the ways that we considered.

Conclusions

Findings from this study contrast with other studies that reported no morphological differences or distinctions between the adult pelagic and benthic life stages (Longhurst 1966).

Though rostrum sharpness did not differ, there was a difference in claw allometry between the pelagic and benthic life stages. There are also significant differences between pelagic and benthic tuna crab exoskeletal composition across multiple structures of the body.

This study aimed to determine whether the pelagic and benthic stages of tuna crabs are ecomorphologically distinct. The observational methods employed in this study have not yet been applied to tuna crabs nor have these life stages been studied together in this level of detail. This is important for resolving the unanswered questions about these life stages being distinct and provides better insights into how tuna crabs respond to the selective pressures of their environment. This study helps us better understand an organism that may only become more common locally to Southern California as ocean conditions continue to change and their range potentially expands. The life stages of tuna crabs are complex, but it is clear that there are differences between these adult stages. Such a unique life history provides an exciting opportunity to explore ecomorphological questions and certainly warrants further study.

Acknowledgements

Chapter 2, in full, is currently being prepared for submission for publication of the material with permission of the coauthors. Webb, Summer; Day, James and Taylor, Jennifer. The dissertation author was the primary author of this chapter. We would like to thank Phil Zerofski for his help with animal collection.

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CHAPTER 3: Stable isotope comparative ecology of adult pelagic and benthic *Pleuroncodes planipes*

Abstract

P. planipes, or tuna crabs, are highly efficient grazers that have episodic occurrence in large numbers off Southern California. Though their ecological importance is recognized as prey, their feeding habits are not fully understood. Adult tuna crabs are believed to go through a size-structured phase shift, from pelagic to benthic habitats as adults, and so it has been hypothesized that these life stages are ecologically distinct. In this study, we used bulk stable isotopes to compare values of δ^{15}N and δ^{13}C over a wide size range of adult tuna crabs to determine whether these populations are distinct in their trophic niches. The smaller-sized, presumed pelagic tuna crabs had significantly lower values of δ^{15}N than the larger-sized, presumed benthic tuna crabs. There were no differences in δ^{13}C between the groups and the values of δ^{13}C spanned a wide range. We propose that the larger individuals do in fact undergo and ontogenetic diet shift in the form of niche expansion, demonstrated by the higher δ^{15}N. Benthic adult tuna crabs are likely eating similar things to pelagic, but also incorporating opportunistic omnivory of higher trophic level organisms into their diet. The variable δ^{13}C values are not unexpected due to the variable nature of the diet but warrants further study.

2.1 Introduction

There are undoubtedly ecological impacts from the presence of tuna crabs, *Pleuroncodes planipes,* in Southern California, as many local organisms consume them, but also because they are voracious feeders themselves. They have been considered to be the most important prey and grazers in the southern end of the coastal upwelling region of the California Current (Longhurst, Lorenzen, and Thomas 1967), with speculation that their substantial grazing effect extends into Southern California during El Niño years (Longhurst, Lorenzen, and Thomas 1967). Their availability as prey and grazers is linked to their pelagic adult stage, which is hypothesized to be distinct from the benthic adult stage based on feeding behavior and habitat use (Gómez-Gutiérrez et al. 2000; Boyd 1967; Aurioles-Gamboa and Pérez-Flores 1997; Aurioles-Gamboa 1992; Longhurst, Lorenzen, and Thomas 1967). These attributes of tuna crabs, in combination with their cyclical abundance in Southern California, have piqued the curiosity of many people, yet questions related to their ecological role and how it might change with life stages remain unanswered.

The ecological role of *P. planipes* is challenging to clarify because adults are thought to experience a pelagic phase, during which they migrate between the water column and benthos, followed by a purely benthic phase after their second year of life, or after reaching an average estimate of approximately 28 mm in carapace length (a standard measure for crustacean body size) (Boyd 1967). These phases are considered ecologically distinct, but there is little quantitative evidence to support this hypothesis. This idea was originally developed around 1960 when no individuals over 26 mm were caught in midwater trawls, but larger individuals (mean SCL 27.9 mm and max 32 mm) were found in benthic trawls that used trap doors to close the collection nets, ensuring that no crabs were caught as they were brought up (Boyd 1967). Before these benthic trawls were conducted, it was believed that this species was only pelagic. This idea has been

reinforced in other studies that assessed the size distribution of individuals through various collection methods (Aurioles-Gamboa 1992; Gómez-Gutiérrez and Sánchez-Ortíz 1997). Having separate stages in which different habitats are being used for feeding has major implications for understanding the ecological role of a species. Additionally, this shift is rare for adult marine crustaceans. If these adult stages really are ecologically distinct, they must be considered separately when examining their effects on ecosystems.

Deciphering the ecological role of tuna crabs is valuable because they are thought to play a critical role as a "trophic shortcut" in converting primary production directly into usable energy for large predators while in the pelagic stage. In the pelagic zone, *P. planipes* are the primary prey item for juvenile loggerhead turtles and blue sharks off Baja, Mexico (Escobar-Sánchez, Galván-Magaña, and Rosíles-Martínez 2011). Pelagic tuna crabs are thought to be the most important grazers of micronekton off Baja, Mexico as well (Longhurst, Lorenzen, and Thomas 1967). Furthermore, *P. planipes* also likely play an important role in pelagic-benthic coupling with their use of both habitats.

Known feeding habits of *P. planipes*

In the pelagic stage, it is thought that *P. planipes* primarily feed on phytoplankton, though they have the capability of catching small zooplankton (Boyd 1962) and they have even been observed capturing individual copepods (Longhurst, Lorenzen, and Thomas 1967). Their capacity for filter feeding on phytoplankton has been clearly documented in previous studies (Boyd 1962; Longhurst, Lorenzen, and Thomas 1967). The third pair of maxillipeds of *P. planipes* serve as the filter feeding apparatus and are "copiously" more setose than those of other squat lobsters, such as *Galathea squamifera* and *G. dispersa* (both benthic), which is hypothesized to be an adaptation to

pelagic filter-feeding on phytoplankton (Boyd 1962). A laboratory study that aimed to quantify their grazing capacity suggested that tuna crabs are capable of significantly grazing down phytoplankton blooms, and depending on the size of cells they are eating, an individual can consume over 450,000 phytoplankton cells per day (Longhurst, Lorenzen, and Thomas 1967). We now know that studying filter feeding apparatuses by morphology and laboratory observations alone can lead to an inaccurate assessment of their use, because observational methods sometimes constrain fluid dynamics in ways unnatural to the feeding behavior of the organism (Koehl and Strickier 1981). The laboratory grazing study mentioned (Longhurst, Lorenzen, and Thomas 1967) also had a field component that assessed the gut content of pelagic individuals, and it was found that their diet can be more variable than originally thought, and typically reflected what was most abundant in the environment. However, this study was limited by some constraints, including the use of only visual identification methods for the gut contents, which could have overlooked other components in terms of prey species. In addition, no size range for the individuals considered to be "pelagic" was stated.

In the benthic stage, *P. planipes* have been observed using their maxillipeds to sift through the sediment to graze on organisms (Boyd 1962). The large chelipeds and strong mandibles also support the possibility that *P. planipes* can be scavengers, and even predators. Benthic tuna crabs have been seen handling and manipulating large food items both in aquaria and in their natural habitat, including entire krill and large chunks of fish (Kato 1974). Like many other crustaceans, *P. planipes* are highly cannibalistic both in captivity and in nature (Boyd 1962). The benthic habitat of *P. planipes* stretches into the oxygen minimum zone, likely affecting their feeding behavior in these regions. In low oxygen, it has been shown that *P. planipes* metabolism drops to one third of

their normal level (Boyd 1962). Consequently, when benthic stage tuna crabs are confined to this low oxygen zone, they may have a lower growth rate and therefore lower food demands.

In addition to feeding observations, gut content analysis has revealed further insights into the diet of benthic *P. planipes*. Diatom frustules and occasional crustacean fragments and setae have been identified in their guts, and it was originally hypothesized that the bulk of the benthic diet was also phytoplankton (Boyd 1962). The gut contents of benthic *P. planipes* were examined in two sampling periods in March and September 1990 off the coast of Baja, Mexico, at different depths (0-300 m). The largest proportion of the gut contents identified during both sampling periods was particulate organic matter (POM), then inorganic matter (mostly sediment), followed by zooplankton and then phytoplankton (Aurioles-Gamboa and Pérez-Flores 1997). POM consistently made up around 60% of the stomach contents in *P. planipes* found at all depths. However, POM was not clearly defined or investigated with identification methods beyond visual. Unfortunately, no size range was reported in this study. Nonetheless, this study does demonstrate that the benthic tuna crabs are suspension feeding on the sediments, that food composition varied with depth, and that the amount of food found varied seasonally (Aurioles-Gamboa and Pérez-Flores 1997). Specifically, in the September sample, the amount and number of species in the stomach contents were lower than those in the March sample.

The seasonal and apparent spatial heterogeneity of the tuna crab diet makes assessing the pelagic and benthic adult groups separately unsatisfying for determining whether they are ecologically distinct. Figuring out the exact diet of *P. planipes* is also complicated by the fact that some phytoplankton and organic matter become unidentifiable inside the gut. Additionally, while informative and important, gut content only provides a snapshot of feeding habits over a short period of time. This seems especially tricky given the apparent opportunistic feeding habits of both

pelagic and benthic tuna crabs. Other more contemporary methods can view diet habits over longer periods of time (on the order of months). With stable isotope analysis, for instance, diet can be examined over longer time frames. Furthermore, it permits comparison of dietary niches within the adult populations of tuna crabs to determine whether these different age classes are in fact ecologically distinct and to more broadly understand the role of tuna crabs in the Southern California food web.

Stable isotopes

Stable isotopes of Carbon (^{13}C) and Nitrogen (^{15}N) are widely used to address different ecological questions related to diet and trophic characterization. $\delta^{15}N$ can provide information about an organism's trophic position (Zanden and Rasmussen 2001) and $\delta^{13}C$ can provide information about the environment or the photosynthetic pathway of the primary producer in the system (France 1995; Perry et al. 1999). The delta value used in these comparisons comes from a comparison between the light and heavy isotopes present in the sample and their comparison to a standardized reference value. Values of $\delta^{15}N$ and $\delta^{13}C$ are present in somewhat predictable ratios in consumers in relationship to their prey. For $\delta^{13}C$ you "are what you eat", or the value increases from 0-1‰ per trophic level and for $\delta^{15}N$ there is an increase of between 2.2 to 5.0‰ from the diet to the consumer (DeNiro and Epstein 1978; Peterson and Fry 1987). This means that animals at higher trophic levels have isotope values for $\delta^{15}N$ that are more enriched, which is due to the preferential excretion of the lighter ^{14}N isotope (France 1995). It has also been observed that benthic algae are more enriched in ^{13}C than their pelagic counterparts (France 1995). These widely used values of $\delta^{13}C$ and $\delta^{15}N$ are referred to as "bulk" stable isotope values.

Ontogenetic shifts

Bulk stable isotope values can be especially useful for comparative studies that examine niche changes through life history or ontogenetic shifts. Ontogenetic shifts are well documented in size-structured populations, are thought to occur in order to improve foraging efficiency and growth (Jensen, Kiljunen, and Amundsen 2012), and can be identified using bulk stable isotope analysis. Many examples of these ontogenetic diet shifts occur in freshwater fish. Brown trout (*Salmo trutta*) change their diet from benthic invertebrates and surface insects to fish as they grow in length (Jensen, Kiljunen, and Amundsen 2012). A similar size-dependent transition to piscivory has been documented for largemouth bass (Post 2003). Ontogenetic diet shifts have also been documented in marine fish species using bulk stable isotope values. Yellowfin tuna undergo a diet shift at a specific body length, switching from primarily planktonic crustaceans to teleosts and vertically migrating midwater shrimp (Graham et al. 2007).

In many cases, ontogenetic shifts are also associated with changes in habitat use, such as, for example, a shift between pelagic and benthic habitats. Ocean sunfish (*Mola spp.*) undergo an ontogenetic diet shift from benthic prey to pelagic prey as they increase in size (Phillips et al. 2020). Differences in $\delta^{13}C$ attributed to pelagic vs. benthic sources can be reflected in isotopic values of consumers that live in coastal marine habitats (France 1995). This has been documented in several decapod crustacean species. The mangrove-dwelling portunid crab, *Scylla serrata,* undergoes an ontogenetic diet shift, and feeds on invertebrates once they reach a larger body size (Tue et al. 2012). An ontogenetic diet shift has also been documented in the freshwater anomuran crab species, *Aegla urutuayana*, which shift from being herbivore-detritivores as juveniles to omnivores as adults (Burress, Gangloff, and Siefferman 2013). A size-related ontogenetic shift was also found in an invasive freshwater shrimp, where differences in $\delta^{13}C$ and $\delta^{15}N$ reflected

changes to both diet trophic level and primary productivity sources (Mancini et al. 2021). Bulk stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ are useful for detecting ontogenetic shifts relevant to both trophic level and habitat use.

Understanding whether a species can occupy distinct niches through ontogeny is crucial because a single species can act as a different functional group at different life stages. This has implications for broader ecological understanding. Stable isotope comparisons can help elucidate an organism's ecological roles through ontogeny. The wide use of stable isotope data to address ecological questions has led to an expansion of different analyses. A hypothesis testing framework was developed in order to examine ontogenetic niche shifts using isotopic ratios (Turner, Collyer, and Krabbenhoft 2010; Hammerschlag-Peyer et al. 2011). Both bivariate and univariate approaches can be used to address questions of niche width, niche positions and niche overlap. The multivariate approach was most useful in examining the extent of niche width or overlap and the univariate approach was most useful for looking at niche expansion (Hammerschlag-Peyer et al. 2011). All of these scenarios were considered biological possibilities for *P. planipes*. A change in niche width could be possible if the benthic group is still feeding in the pelagic environment, but is also scavenging on benthic prey. A change in niche overlap could illustrate whether the pelagic and benthic groups represent different trophic guilds (Hammerschlag-Peyer et al. 2011). Differences in niche positions could indicate that there is a clear ontogenetic shift in which the benthic group is isotopically distinct from the pelagic group. Univariate analysis can help determine what factor is driving any difference seen in multivariate analysis.

Objective

The main objective of this study was to examine the isotopic niche(s) occupied by the adult stages of *P. planipes* so that we can better understand their ecological and functional role. Specifically, I tested the hypothesis that adults <28 mm CL and >28 mm CL occupy distinct ecological niches. Bulk stable isotope analysis was conducted for carbon (^{13}C) and nitrogen (^{15}N) isotopes on an expansive size range of tuna crabs to determine whether they feed from different sources and at different trophic levels. This research provides a better understanding of the role that these ubiquitous consumers play in pelagic-benthic coupling and of their overall ecological role.

3.2 Methods

Animal collection and sample preparation

A broad size range of pelagic and benthic animals (21-35 mm CL, N=79) were collected in Southern California from a range of depths (10-300 m) using SCUBA, trawls and traps. Sampling took place during the late fall of 2019 (October - December) off La Jolla, CA. Upon collection, animals were euthanized by placement in a -20°C freezer where they were stored until analysis. Individuals were measured for Standard Carapace Length (SCL) after euthanization to increase accuracy. SCL was measured from the notch between the subrostral and rostral spines to the dorsal center of the posterior edge of the carapace. Measurements were taken with digital calipers (Mitutoyo Digimatic, CD-6" AX, Mitutoyo Corporation, Tokyo, Japan).

Tuna crabs were slightly thawed to allow for tissue dissection. A portion of the muscle tissue was taken from the ventral side of the abdominal segment with care taken to avoid the digestive tube. Tissue was rinsed with distilled water and then placed in cryovials that were stored in a -20°C freezer until freeze-drying. Lipid extraction was not performed as it has not been found

to impact isotopic values of $\delta^{13}C$ or $\delta^{15}N$ for muscle tissue in a species of decapod crustacean (Bodin, Le Loc'h, and Hily 2007). Samples were freeze-dried for 24 hours (FreeZone 2.5 Liter Freeze Dry Systems freeze dryer, Labconco Corporation, Kansas City, MO) and then homogenized using a mortar and pestle and weighed to the nearest 0.0005 g. Samples were then encapsulated in Costech 5x9 mm tin capsules and stored in 96 well plates inside a desiccator until shipment.

Samples were sent to the University of California, Santa Cruz Stable Isotope Laboratory and analyzed for carbon (C) and nitrogen (N) stable isotope ratios and C and N amounts per standard procedures using a CE Instruments NC2500 elemental analyzer coupled to a Thermo Scientific DELTAplus XP isotope ratio mass spectrometer via a Thermo-Scientific Conflo III. Measurements were corrected to VPDB (Vienna PeeDee Belemnite) for $\delta^{13}C$ and AIR for $\delta^{15}N$ against an in-house gelatin standard reference material (PUGel), which was extensively calibrated against international standard reference materials. Measurements were corrected for size effects, blank-mixing effects, and drift effects. An externally calibrated Acetanilide standard reference material purchased from Dr. Arndt Schimmelmann of Indiana University was measured as a sample for independent quality control (methods per UCSC Stalbe Isotop Lab).

Statistical analysis

All statistical analyses were computed using Rv4.2.1. Both bivariate and univariate analyses were applied to the data following recommendations from Turner, Collyer and Krabbenhoft (2010). For the bivariate analysis, nested linear models and residual permutation procedure methods for evaluating differences in dispersion and central tendency were used to determine differences in niche width and niche position between the pelagic and benthic groups per Turner, Collyer and Krabbenhoft (2010). Hotelling's T-squared test for multivariate data was

also applied to determine whether benthic and pelagic groups were different. For the univariate analyses, the samples were grouped both categorically and continuously, because part of this study is to investigate whether there is an ontogenetic shift at the hypothesized size of 28 mm SCL. For categorical univariate analysis, the pelagic and benthic groups were separated as <28 mm and >28 mm SCL. To look at differences between δ^{15}N and δ^{13}C for the groups, the data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Bartlett's test. An independent samples t-test was used to determine whether there was a difference between the δ^{13}C values between the benthic and pelagic groups. A non-parametric Mann-Whitney U test was used to determine whether there was a significant difference between the δ^{15}N values between the benthic and pelagic groups. For continuous univariate analyses, linear regressions were performed on the carbon and nitrogen isotopic values to test for non-zero slope values (per recommendation from Hammerschlag et al (Hammerschlag-Peyer et al. 2011).

2.3 Results

Bivariate results

The benthic centroid occurs above the pelagic centroid, indicating that the benthic group is more enriched in nitrogen (Figure 3.1). The pelagic data cluster more tightly together, indicating less variability than the benthic group, and are overall lower in δ^{15}N. Both benthic and pelagic groups span a similar range of carbon values.

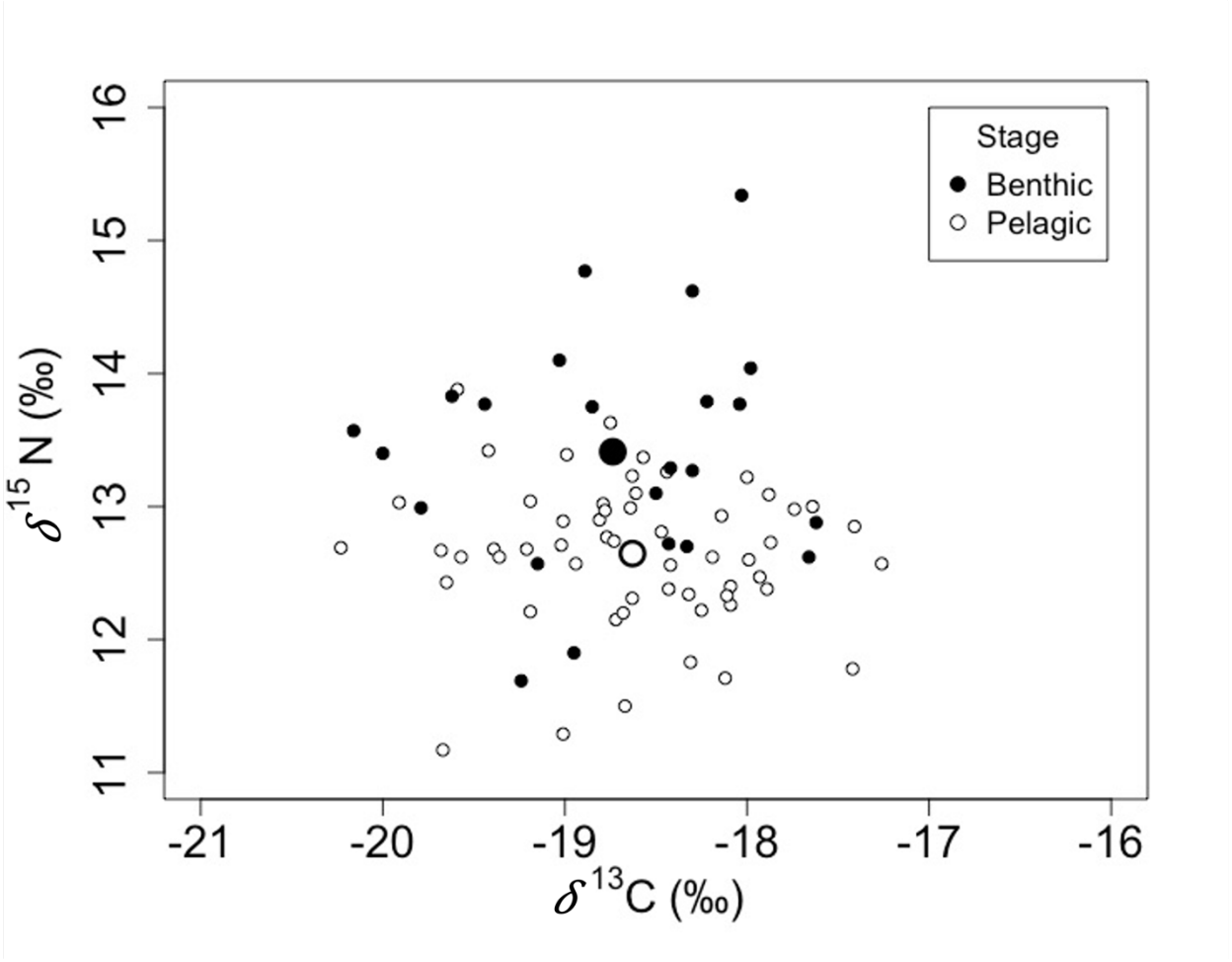

Figure 3.1: Stable isotope biplot of δ^{13}C and δ^{15}N. For benthic (n=23) and for pelagic (n=56) groups. The large open circle indicates the mean pelagic value/centroid and closed black circle indicates the mean benthic value/centroid.

For the bivariate analysis, the Euclidean distance between the centroids of the groups, a measure of niche position, was significantly different. The bivariate test for difference between means was significant (Hotelling's $T^2 = 22.8$, $p = 5.07e\text{-}05$). The mean distance of each data point in each group to their respective centroid, a metric used to determine niche width, differed significantly between pelagic (0.75) and benthic (1.02) groups ($p = 0.017$). The mean nearest neighbor (distance between data points in each group) and eccentricity (a measure for whether

the groups have different covariates) values did not significantly differ (p = 0.357, 0.926, respectively).

The mean $\delta^{13}C$ values for pelagic and benthic groups were -18.6 ± 0.66 and -18.7 ± 0.73, respectively, and mean $\delta^{15}N$ values for pelagic and benthic were 12.6 ± 0.54 and 13.4 ± 0.87), respectively. Both the $\delta^{13}C$ and $\delta^{15}N$ data passed the Shapiro-Wilk test for normality (p = 0.538, 0.0531, respectively). Only the $\delta^{13}C$ data passed Bartlett's test for homogeneity of variance (p = 0.618). There was no significant difference between the carbon stable isotope values for benthic and pelagic (Independent Samples t = -0.61471, df = 37.951, p = 0.542). There was a statistically significant difference in the median $\delta^{15}N$ isotope values between the benthic and pelagic groups (Mann Whitney U Rank Sum, W = 1004.5, p = 0.0001) (Figure 3.2).

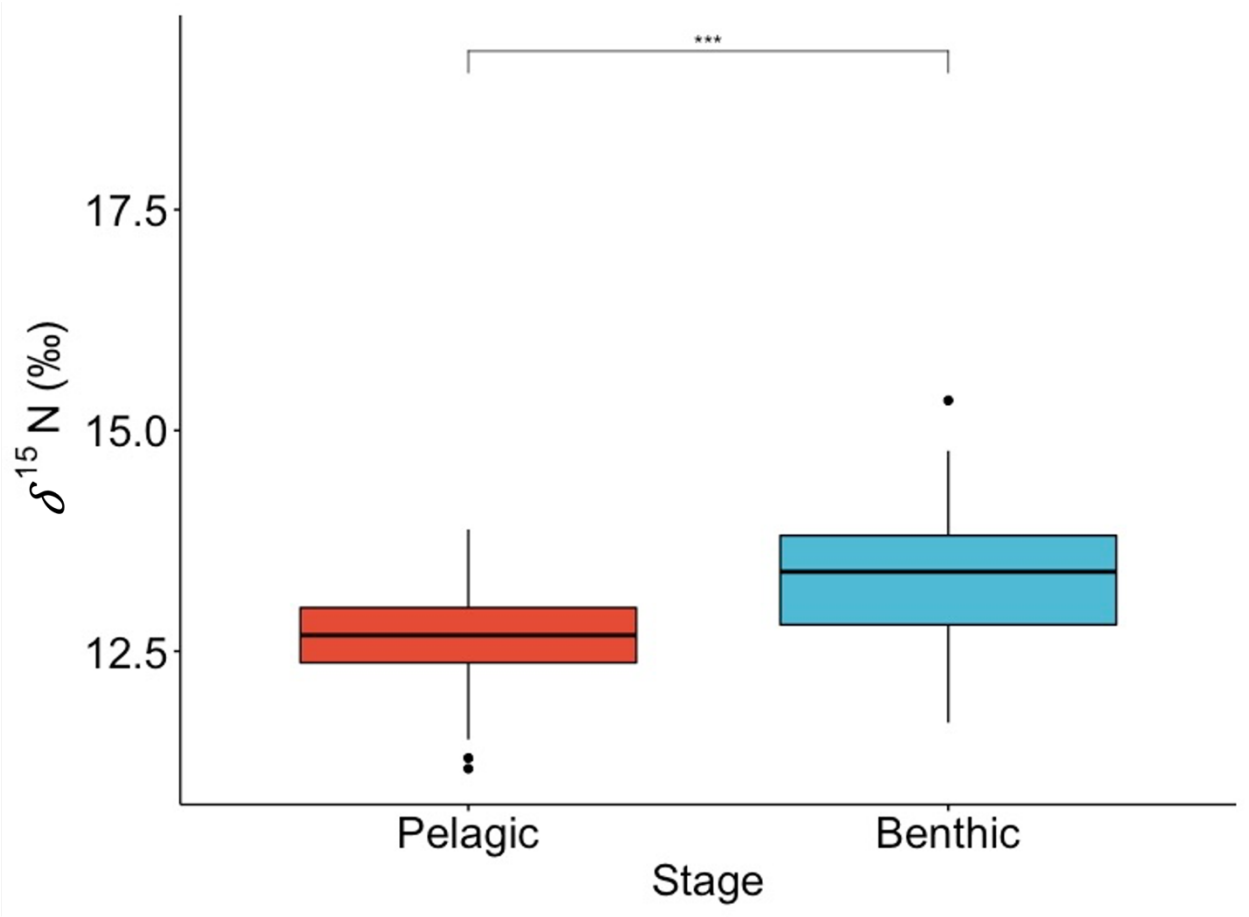

Figure 3.2: Mean values of δ^{15}N (‰) for pelagic and benthic groups. Pelagic tuna crabs are significantly lower in δ^{15}N than benthic tuna crabs. *** indicates a statistical significance level of <0.0001.

Univariate results

A statistically significant relationship between carapace length (mm) and δ^{15}N was determined (ordinary least squares: $R^2 = 0.3179$, ANOVA, F = 35.89, p = 6.27e-08) (Figure 3.3), whereas no statistically significant relationship was found between carapace length (mm) and δ^{13}C (OLS: $R^2 = 0.008$, ANOVA, F = 0.63, p = 0.429).

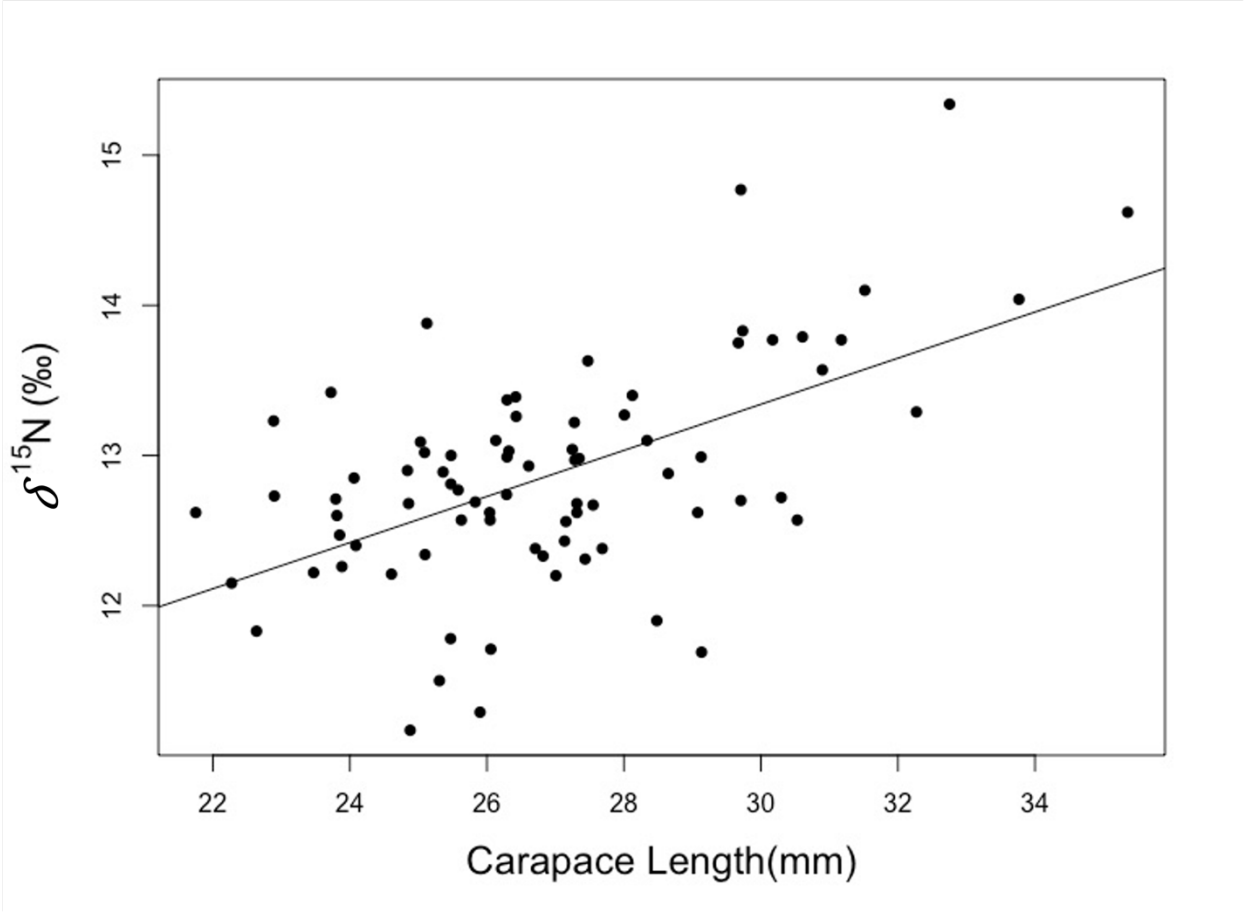

Figure 3.3: Carapace length is significantly correlated with δ15N (‰) across benthic and pelagic tuna crab size range. $R^2 = 0.3179$

3.4 Discussion

In this study, bulk stable isotopes were used to assess whether pelagic and benthic adult tuna crabs, *P. planipes*, are ecologically distinct. Tuna crabs were separated by size using a proposed 28 mm cutoff from exchange with the pelagic environment, as literature estimates vary by several mm from the original 26 mm cutoff proposed by Boyd (see introduction for more information). Setting the pelagic group at <28 mm and the benthic group at >28 mm yielded significant results, though the transition between these stages likely occurs over a range of body sizes rather than a specific cutoff and probably varies within individual tuna crabs.

Pelagic and benthic groups of tuna crabs varied significantly in measures used to compare niche width and niche position. The benthic group was more widely dispersed than the pelagic group, demonstrating a greater niche width. The benthic group was also at a higher trophic position relative to the pelagic group, as the distance between their centroids was significantly different, and the benthic centroid was above the pelagic centroid in the $\delta^{15}N/\delta^{13}C$ biplot. These differences observed between the groups were driven by differences in $\delta^{15}N$. Pelagic and benthic groups overlapped in their values for $\delta^{13}C$. The significant difference in $\delta^{15}N$, combined with the overlap in $\delta^{13}C$ values between the groups, suggests that there is indeed an ontogenetic shift that occurs, and it is in the form of niche expansion. Benthic tuna crab's enrichment in nitrogen is likely due to a highly variable existing diet that begins to incorporate more opportunistic scavenging in the seafloor habitat. It is most plausible that the benthic tuna crabs are eating many of the same things as the pelagic tuna crabs, and that those things are highly variable, but it is also likely that they are scavenging on higher trophic level prey on the seafloor as well.

A logical hypothesis for this stage transition is that at a certain body size, the cost of swimming up into the water column outweighs the benefit energetically. Additionally, larger individuals are also better equipped for capturing and handling larger prey items at higher trophic levels than small particles. This opportunistic benthic scavenging likely becomes incorporated for several reasons, including spending more time in this habitat. Benthic tuna crabs may also be more likely to engage in suspension feeding within the sediments on the seafloor if they are not venturing to the water column to filter feed on phytoplankton.

While one previous study found a relationship between $\delta^{13}C$ and distance from shore (Miller, Brodeur, and Rau 2008), this would be difficult to assess in *P. planipes* due to their high mobility and known cross-slope migratory behavior (De Anda-Montañez et al. 2016). It is possible

that the benthic tuna crabs can acquire pelagic carbon sources that have sunk to the seafloor. It is also possible that they are getting some combination of pelagic and benthic carbon sources. One review postulates that coastal consumers with $\delta^{13}C$ values below -20‰ likely depend on phytoplankton, whereas dependence upon benthic algae would yield values above -19‰ (France 1995). While the tuna crabs in this study had $\delta^{13}C$ values of -18.7 and-18.7‰, they were highly variable and did not separate by group. Some tuna crabs had $\delta^{13}C$ values of around -20‰ whereas others had values of around -17‰. It is possible, given this wide range of carbon values, that the carbon sources themselves are highly variable and potentially even come from both habitats. Additionally, the residence time for $\delta^{13}C$ has been estimated to be almost twice that of $\delta^{15}N$ in muscle tissue in another decapod crustacean, *Macrobrachium borelli* (Viozzi, del Rio, and Williner 2021), so there could be a lag or mismatch in the observed difference in the data.

It has been long proposed that the pelagic group converts primary production into energy for larger organisms and fulfills the role of primary consumer, whereas the benthic group competes with other benthic scavengers (secondary consumers) for space and food, which likely affects the meiofaunal population. It has also been stated that the pelagic and benthic groups represent distinct ecological roles (Boyd 1967), but stable isotope analysis has not previously been used to investigate this, nor has any other quantitative method been used to examine tuna crabs across a broad size range in a single sampling event. This study demonstrates a niche difference between pelagic and benthic tuna crabs for the first time, and that this niche difference occurs across the same habitat and collection areas.

Conclusions

Understanding whether adult tuna crabs fulfill multiple ecological roles as adults is critical to understanding their ecology. They are vital prey for many organisms and important grazers. Tuna crabs and other organisms commonly included as prey items in larger isotopic analyses are often grouped together, with a small number of samples. This study highlights the importance of taking life history of prey organisms into account when sampling, as these ontogenetic shifts in invertebrates may be more common than is currently thought. Since *P. planipes* move between pelagic and benthic zones in their life history, they likely contribute significantly to benthic-pelagic coupling in coastal systems. A sampling effort over an even broader size range, and potentially over multiple seasons, would help further clarify this complex system. Also, comparing the isotopic signatures of tuna crabs to other local grazers would help elucidate intraspecific competitive interactions.

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CHAPTER 4: *Pleuroncodes planipes* more sensitive to temperature than pH under long-term exposure

Abstract

Tuna crabs, *P. planipes*, are exposed to a broad range of pH and temperatures through their unique life history that includes a size-structured phase shift from pelagic to benthic zones as adults. This makes them potentially vulnerable, but also potentially adaptable to climate change stressors like ocean acidification and ocean warming. In this study, we exposed adult pelagic stage tuna crabs to a full factorial combination of increased CO_2/decreased pH and increased temperature conditions for 10 months to simulate OA-like conditions. Survival, mineral composition of the exoskeleton, molting and growth were assessed. Survival was significantly affected by exposure, but this effect decreased over time. There was no measurable effect of the exposure on mineral content of the exoskeleton. Generally, tuna crabs were sensitive to temperature but not pH. Regardless of pH, tuna crabs in the warm temperature treatments molted more frequently than those in cooler treatments and had a smaller growth increment over a single molt, but did not achieve larger size than the other treatments by the end of the experiment. These short-term effects on growth could potentially delay pelagic-benthic transitions in tuna crabs, but only temporarily. Our findings highlight the importance of observing multiple molt cycles for crustacean OA experiments while providing the first insight into how the life stages of tuna crabs may be impacted as ocean conditions continue to change.

4.1 Introduction

Tuna crabs (also known as pelagic red crabs), *Pleuroncodes planipes,* (Decapoda; Family: Munididae) are unique among crustaceans in that they are thought to experience a size-structured life stage shift during adulthood, from pelagic to benthic habitats (Boyd 1967; Aurioles-Gamboa 1992; Longhurst 1966). The so-called "pelagic" adults occasionally settle onto the continental shelf but are thought to primarily feed in the pelagic zone. In contrast, "benthic" adults are thought to no longer venture into the pelagic zone and feed exclusively in the benthic zone. The transition between these life stages is poorly understood, but it appears to depend primarily on size. Once tuna crabs reach approximately 28 mm in carapace length, or after their second year of life, it is thought that they settle into the benthic zone and assume an ecologically separate existence from their pelagic-associated counterparts (Boyd 1967). This transition is dependent on molting and growth, which makes them potentially vulnerable, but also potentially adaptable, to the pressing stressors of ocean acidification (OA) and ocean warming (OW).

Crustaceans are generally thought to be resistant to the changes in carbonate chemistry associated with OA due to a combination of factors, such as their strong acid-base regulatory capacity (Whiteley 2011), their use of bicarbonate for calcification (Whiteley 2011), and even their high mobility (Siegel et al. 2022; Kroeker et al. 2013). Despite these attributes, a variety of crustacean species show measurable sensitivities to elevated pCO_2/reduced pH associated with OA, particularly in terms of growth and calcification (Whiteley 2011; Siegel et al. 2022). For example, juvenile red king crabs (*Paralithodes camtschaticus*) and tanner crabs (*Chionocetes bairdi*) both experienced slower growth (i.e., longer intermolt, smaller mass and carapace width) at seawater pH levels of 7.8 and 7.5, respectively (Long et al. 2013). Decreased growth has also been observed in larval American lobsters (*Homarus americanus*) (Keppel, Scrosati, and Courtenay 2012) and

adult marine shrimp (*Palaemon pacificus*) (Kurihara et al. 2008) under pCO_2/pH levels reflecting those predicted for the years 2100 and 2300, respectively. Both of these species exhibited reduced molt frequency and increment (smaller carapace length or total body length). Thus, OA conditions can decrease growth in some crustacean species by altering multiple aspects of the molt process, which may be consequential for important life transitions.

Calcifying a newly constructed exoskeleton after each molt is a critical cap to the process that is also altered by elevated pCO_2/reduced pH conditions for some species of crustaceans. Despite having physiological mechanisms that help buffer the process against changes in external pH conditions (Whiteley 2011), net increases in calcification (i.e., calcium and/or magnesium exoskeleton content) have been documented in crabs (Coffey et al. 2017; Dickinson et al. 2021), shrimp (Ries, Cohen, and McCorkle 2009; J. R. A. Taylor et al. 2015), mantis shrimp (deVries et al. 2016), and lobsters (Ries, Cohen, and McCorkle 2009), while decreased calcification has been observed in larval European lobsters *Homarus gammarus* (Arnold et al. 2009), juvenile California spiny lobsters *Panulirus interruptus* (Lowder et al. 2022), and adult tanner crabs *Chionocetes bairdi* (Long et al. 2013). It is apparent from the growing number of OA crustacean studies that calcification effects should be assessed in a species-specific manner.

Altered calcification is noteworthy because it can directly affect animal function. Observed changes in mineralization in response to elevated pCO_2/reduced pH are often presumed to disrupt the mechanical integrity of the exoskeleton. A recent meta-analysis (Siegel et al. 2022) confirms that crustacean exoskeleton microhardness and strength generally decrease under elevated pCO_2/reduced pH levels, though a consistent relationship between mineral content and biomechanics is obscure (Coffey et al. 2017). Beyond the material properties, mineral content also contributes to animal density, and therefore the efficiency with which an animal can navigate the

water column. This aspect is less relevant for most juvenile and adult decapod crustaceans that are typically benthic, but it is pertinent to species like *P. planipes* that occupy both pelagic and benthic habitats.

Habitat is thought to be an indicator of species sensitivity to OA conditions (Bednaršek et al. 2021), but it is not fully explanatory, which makes predicting ecological impacts of changing ocean conditions more challenging. For example, shallow water habitats are dynamic, and it is a reasonable hypothesis that species in these dynamic habitats are robust to environmental stressors because they are adapted to variable conditions. In four closely related species of porcelain crabs, exoskeleton cation composition was found to be impacted differently by increased pCO_2/decreased pH (Page et al. 2017). The habitat in which they were found seemed to drive the difference in responses: mid-intertidal crabs were not as impacted by reduced pH as low intertidal crabs (Page et al. 2017), suggestive of the former being more adaptable to a more variable environment. Regardless, all species showed some level of response to increased pCO_2/decreased pH. Species in deep, cold-water habitats are hypothesized to be more susceptible to environmental stressors because of the stability of the environment to which they are adapted. Yet, for two deep water crab species off of Alaska, red king crabs (*P. camtschaticus*) and tanner crabs (*C. bairdi*) (Long et al. 2013), the early effects they experienced from exposure to increased pCO_2/decreased pH as juveniles subsided over time, demonstrating an adaptive capacity to environmental stressors.

Across ocean habitats, temperature is increasing in parallel to rising pCO_2 levels (IPCC, 2014), and can exert its own effects on crustacean molting and growth. Increases in temperature have been associated with increased growth rate and increased molt frequency, but smaller overall body size (Maszczyk and Brzeziński 2018; Atkinson 1994). Growth rate in crustaceans continually rises with temperature, until high enough temperatures are reached to result in mortality (Hartnoll

2001). The reason behind this relationship between growth rate and temperature is most likely that metabolic processes and those involved with preparation for molting also increase with temperature (Hartnoll 2001). Increased growth rates manifest as either a shortened intermolt period, an increase in molt increment or a combination of both. Based on experimental studies, many crustaceans experience a reduction in molt increment when exposed to increased temperature, though the effects are slightly variable (Hartnoll 2001). Despite a decrease in molt increment that suggests slower growth, the intermolt period is often disproportionately shorter, which ultimately still results in a higher overall growth rate at increased temperatures (Hartnoll 2001). Though these general patterns exist, the effects of temperature on growth and molting in crustaceans are complex, especially when combined with other environmental factors.

In combination, increases in temperature and pCO_2 can have either synergistic, neutral or mitigating effects on crustaceans. Though the combined effects of increased temperature and elevated pCO_2/decreased pH on crustaceans remain less studied than effects of elevated pCO_2 alone, the results are again wide-ranging and species-specific. A 2013 meta-analysis found that overall, the combined effects of increased pCO_2 and temperature on crustacean growth, survival, calcification, development and abundance were insignificant (Kroeker et al. 2013). However, recent studies that have examined elevated pCO_2 and temperature in combination to assess impacts on growth and calcification in crustaceans have yielded significant and variable results. In the grass shrimp, *Hippolyte californiensis*, an increase in growth was observed under a combination of reduced pH and increased temperature, but not under reduced pH alone (Lowder et al. 2017). In the tropical mantis shrimp, *Neogonydactylus bredini*, the raptorial appendage had higher magnesium content under reduced pH alone, showing an impact on calcification, but not in the combined reduced pH/increased temperature treatment, which suggests temperature has a

mitigating effect (deVries et al. 2016). Combined changes in pH and temperature have the potential to alter growth and calcification, thereby affecting key size-dependent life stage transitions, like the one undergone by tuna crabs. If this life stage transition is accelerated, delayed or otherwise compromised by OA and OW, then their distribution and availability as a food source in the pelagic stage could pose a problem for the many organisms that rely on them as a key food source. Understanding how their growth, molting and exoskeleton are impacted by pCO_2/pH and temperature is critical for determining how this species may fare under changing ocean conditions, specifically OA and OW.

Objective

The main objective of this study was to determine how decreased pH and increased temperature affect the growth, molting and exoskeleton of *P. planipes*. Specifically, a long-term experiment was conducted to test the hypothesis that under decreased pH and increased temperature, tuna crabs will experience both increased growth rates and increased calcification. These changes would accelerate the timing of their settlement to the benthos, thereby reducing the duration of the transient pelagic phase upon which so many organisms depend for food.

4.2 Methods

Animal collection and maintenance

Adult *Pleuroncodes planipes* (23-28 mm carapace length) were collected off La Jolla, California using deep water benthic traps in February, 2018. Tuna crabs were brought to the experimental aquarium at Scripps Institution of Oceanography (SIO), University of California, San Diego (UCSD), where they were held together in one large tank for approximately 2 weeks

until the start of the experiment. This holding tank received flow-through sea water pumped from the SIO pier at ambient conditions. Animals were fed a diverse diet that included frozen cod, frozen krill, fish pellets and fish flakes. Sixty individuals that appeared healthy and had all appendages intact were selected for the experiment.

Experimental design and maintenance

Tuna crabs were assigned to one of four treatments semi-randomly, to ensure equal sex and size distribution across treatments. Temperature and pH targets were set to reflect ambient parameters at the collection location and projected values for the year 2100 under the business as usual scenario, estimated as a decrease of 0.3-0.4 pH units and an increase of 2-3°C (IPCC, 2014). *P. planipes* have a thermal preference of 13°-16°C, based on frequency of occurrence during sampling (Aurioles-Gamboa 1992), so the increased temperature target was set at 2°C above the upper end of this range, at 18°C, while the ambient temperature was set at the local temperature at which they were collected, 12°C. Target pH was set at 7.60, reflecting 0.4 units below ambient. The treatments were: ambient pH/ambient temperature (pH 8.1, 12°C), decreased pH/ambient temperature (pH 7.60, 12°C), ambient pH/increased temperature (pH 8.1, 18°C), and decreased pH/increased temperature (pH 7.60, 18°C). Treatments are herein referred to as: control, low pH, low pH/high temp, and high temp, respectively.

The experiment was conducted in an OA experimental aquarium system in the Hubbs Experimental Aquarium facility at SIO. The system consisted of four large header tanks (150 L) that each received flow-through filtered seawater from the SIO pier (3-4 m depth, 300 m offshore). Each header tank supplied flow-through seawater to 15 experimental tanks grouped by treatment (1.6 L) that each housed a single tuna crab, for a total of 60 individuals. Header tanks were adjusted to experimental pH and temperature treatments. Target temperatures were achieved by mixing

chilled and warm seawater supply lines, and decreased pH was accomplished by the bubbling of 100% CO_2 gas delivered through an air stone and administered by a solenoid. Addition of CO_2 was controlled with an Apex Lite aquarium controller with Apex Neptune pH and temperature probes (0.01 pH accuracy; 0.1°C temperature accuracy; Neptune Systems, Morgan Hill, CA, USA), which logged temperature and pH of the header tanks every 20 minutes throughout the duration of the experiment.

Water chemistry

Daily pH and temperature measurements were recorded for the header tanks and each experimental animal tank using a portable probe (HQ40d, probe PHC201, accuracy 0.01 pH, 0.1°C temperature, Hach, Loveland, CO, USA). All probes were calibrated with NBS buffer solutions (Fisher Scientific, Fair Lawn, NJ, USA) once per month.

Water samples were taken at the midpoint of the experiment from each header tank and one experimental tank from each treatment (N=8) to determine absolute pH and total alkalinity, per standard operating procedures (Dickson et al. 2007) and used to correct the portable pH probe values to pH_{total}. Water samples were submitted to the Dickson laboratory at SIO for analysis of pH_{SWS}, density-based salinity, and total alkalinity (A_T) at 25°C. Other carbonate parameters, including pCO_2, carbonate and aragonite saturation states, and concentrations of carbonate and bicarbonate were calculated using CO_2Sys 2.01 (Pierrot et al. 2006). Water samples were used to calculate the offset from the Hach pH probe readings, and these corrected pH values were averaged for each treatment.

Experimental pH and temperature were gradually increased over 7 days to minimize the risk of acute stress. Tuna crabs remained in these experimental conditions for sufficient time to

observe a minimum of 2 molts (294 days or approx. 10 months). During that time, tuna crabs were fed a mixed diet *ad libitum* consisting of frozen krill, frozen cod and flake food. Uneaten food was removed from tanks after 24 hours to prevent fouling. Tuna crabs were checked daily for molts and recorded, and exuviae were promptly removed. Animals were measured for carapace length and weighed after each molt.

Growth

Prior to the start of the experiment, individuals were sexed and measured for carapace length (CL), carapace width (CW), buoyant mass (in 500 mL of seawater), and wet mass. CL and CW were each measured with digital calipers (Mitutoyo Digimatic, CD-6" AX, Mitutoyo Corporation, Tokyo, Japan) three times and then averaged.

To evaluate growth, each molting event was recorded. Animals were measured after each molt and at the end of the experiment for CL, CW, buoyant mass and wet mass using the procedures described above. Growth was assessed as both intermolt duration (amount of time between molts) and molt increment (percent change in CL, CW, buoyant mass, and wet mass between molts). Potential changes in morphology were assessed by comparing the CW:CL ratio across the treatments.

Calcification

At the end of the experiment, tuna crabs were anesthetized and euthanized by brief placement in a -20°C freezer. Only individuals estimated to be in intermolt, showing no signs of apolysis, were used for exoskeleton analysis (N=17). The carapace was carefully dissected and cleaned of epidermis using a small paint brush. Samples of exoskeleton were cut from the dorsal, posterior region of the carapace and then freeze-fractured with liquid nitrogen to obtain a clean

cross-section. Fractured specimens were then placed in a critical point dryer, secured to a double 90° SEM mount revealing the cross-section, sputter-coated with iridium, and examined using a scanning electron microscope (SEM) (Phillips XL30 ESEM, FEI Co., Hillsboro, OR, USA) equipped with an energy dispersive x-ray analysis system (EDX) (Oxford Instruments, UK) at the Nano3 Facility at UCSD.

SEM imaging of the cuticle cross-section was performed at 10 kV acceleration voltage. Only sections with clean breaks and smooth surfaces were used for EDX analysis of elemental composition. The cuticle image was magnified to fill the screen and spectra were collected at 20 kV acceleration voltage and a minimum of 5,000 counts per second. Nine elements were consistently detected in cuticle cross-sections: C, O, Na, Mg, P, Al, K, Cl and Ca. The amount of calcium and magnesium in each sample was calculated as the weight percent (wt%) and atomic percent (at%) relative to all detected elements, excluding the Ir coating.

Statistical analyses

Kaplan-Meier survival curves were generated and compared between treatments using a Gehan-Breslow Wilcoxon test. The number of molts (molt frequency) was compared across treatments using a Fisher's Exact test. Mean growth increment (as percent change for CL, buoyant and wet body mass), calcium content (%Ca), magnesium content (%Mg), calcium/magnesium ratio (Ca:Mg), and CL:CW (a metric used to assess morphological differences) were compared across treatments using either Kruskal Wallis or ANOVA and post hoc Dunn or Tukey Kramer analyses with Bonferroni adjusted alpha values where appropriate. All data were tested for normality using the Shapiro-Wilk Test and for homogeneity of variance using Bartlett's Test. Statistical analyses were computed using R (v.4.2.1).

4.3 Results

Water chemistry

Water parameters for the duration of the experiment are shown in Table 4.1. Actual pH was slightly lower in the high temp treatment than in the control treatment, but all pH values were relatively stable over the course of the experiment. Temperature in the two high temperature treatments showed slightly more variation over the duration of the experiment, likely due to seasonal fluctuations to the ambient water supply line.

Table 4.1: Water parameters for the duration of the experiment as mean ± sd. Sample size in parentheses. pH was calculated by applying the offset between discrete water samples and portable probe readings to the daily probe readings. TA is measured directly from water samples, while pCO_2, HCO_3^-, and calcite saturation state are all calculated using CO2Sys.

Treatment	pH	Temp (°C)	TA (µmol/kg SW)	pCO_2 (µatm)	HCO_3^- (µmol/kg SW)	Ω Calcite
Control	8.13 ± 0.16 (N=4946)	13.00 ± 0.94 (N=4946)	2233 ± 1.40 (N=2)	308 ± 7.82 (N=2)	1655 ± 9.71 (N=2)	4.11 ± 0.05 (N=2)
Low pH	7.62 ± 0.16 (N=4686)	11.59 ± 0.80 (N=4686)	2232 ± 0.40 (N=2)	1122 ± 18.9 (N=2)	2020 ± 3.30 (N=2)	1.38 ± 0.01 (N=2)
Low pH/high temp	7.57 ± 0.13 (N=4258)	18.85 ± 1.47 (N=4258)	2233 ± 1.23 (N=2)	1322 ± 5.23 (N=2)	2037 ± 1.81 (N=2)	1.74 ± 0.01 (N=2)
High temp	7.99 ± 0.13 (N=4296)	19.11 ± 1.57 (N=4296)	2240 ± 8.40 (N=2)	445 ± 14.6 (N=2)	1781 ± 15.88 (N=2)	4.16 ± 0.05 (N=2)

Survival, growth & molting

At the end of the 10-month exposure (294 days), mortality occurred in each treatment (N = 1 in low pH, N = 8 in low pH/high temp, N = 5 in high temp, and N = 3 in control) (Figure 4.1).

The effect of mortality was significant between treatments (Gehan-Breslow Wilcoxon 9.389, p = 0.025), but post hoc pairwise comparisons failed to detect significant differences.

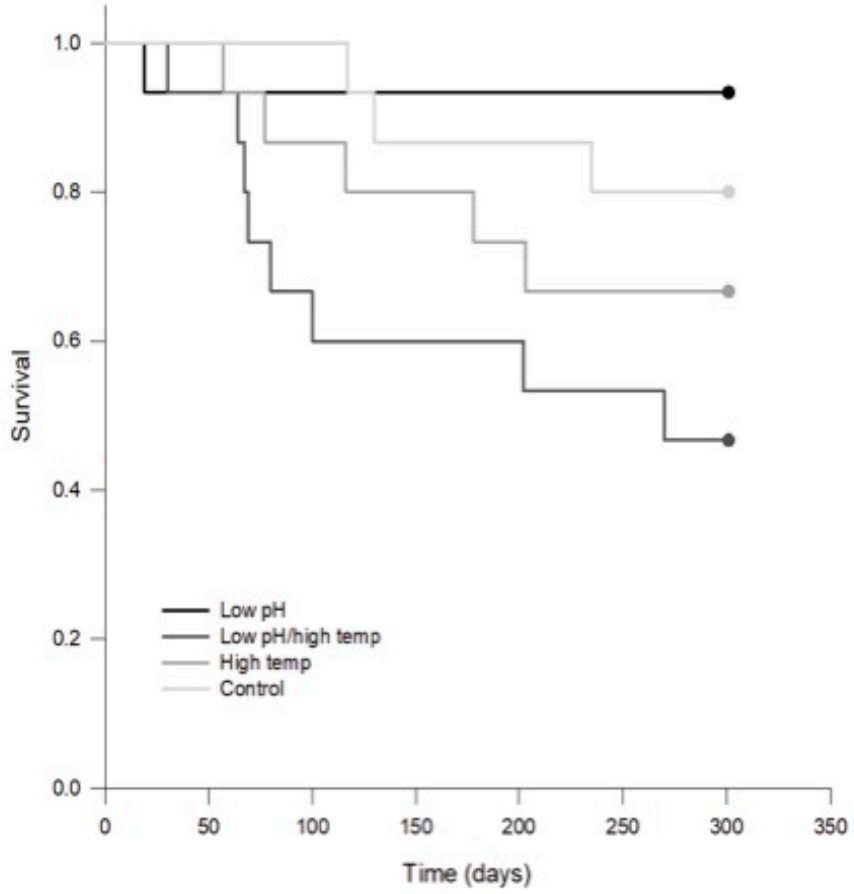

Figure 4.1: Survival curves for each treatment over the course of the experiment. Probability of survival differed between treatments, but were not detected in post hoc tests.

One animal from the low pH treatment, three from the low pH/high temp, four from the high temp, and no animals from the control died before reaching molt 1. All remaining animals reached a first molt: 14 in the low pH, 12 in the low pH/high temp, 11 in the high temp and 15 in the control. The time to reach molt 1 from the start of the experiment (T_0) did not differ between treatments (Kruskal-Wallis, chi-squared = 5.53, p = 0.1366). By the end of the experiment (T_F),

many tuna crabs molted twice (N = 26), and a few molted a third time (N = 4). The number of experimental animals that molted twice were: N = 5 in low pH, N = 9 in low pH/high temp, N = 9 in high temp, and N = 4 control. Three animals from the low pH/high temp and one from the high temp treatment reached a third molt. The number of molts reached differed significantly between the groups based on their temperatures (Fisher's Exact, p = 0.000257) (Figure 4.2). Specifically, the low pH/high temp treatment had significantly more second and third molts than the low pH treatment (p = 0.02833, 0.02833) and the control (p = 0.02833, 0.03750) (p-values listed respectively). The high temp treatment had significantly more second molts than the low pH treatment (p = 0.04636) and the control (p = 0.04121).

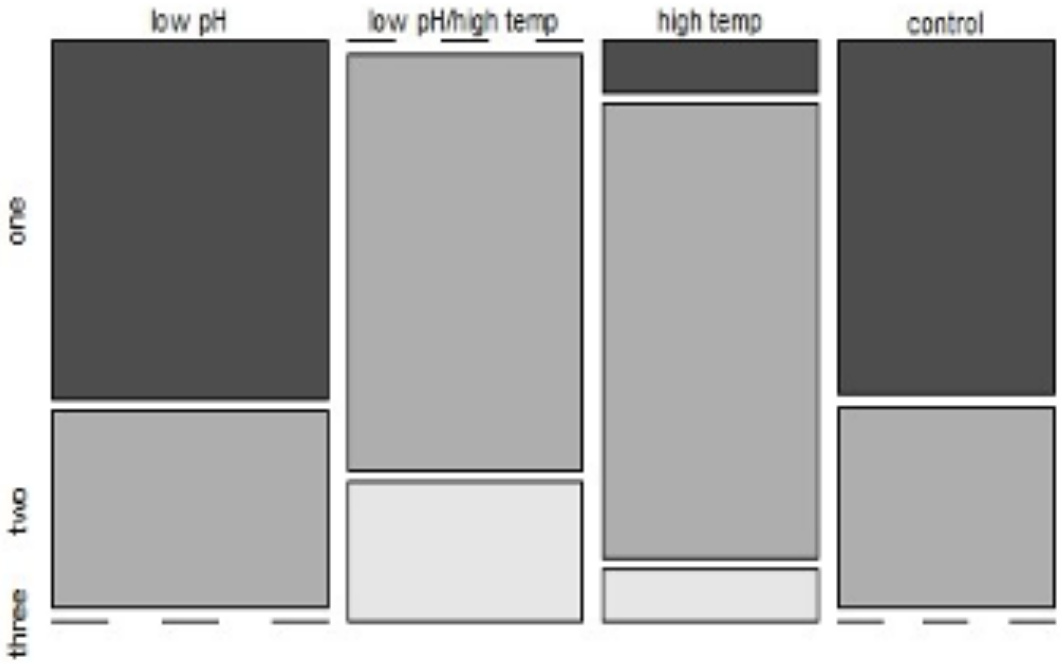

Figure 4.2: Fisher's Exact test plot. Color indicates how many tuna crabs that molted that number of times: dark gray: 1 molt, medium gray: 2 molts, and light gray: 3 molts. The low pH/high temp treatment had more second and third molts than the low pH and control treatments. The high temp treatment had more second molts than the low pH and control treatments.

Molt increment, as percent growth in CL between T_0 and molt 1 differed significantly (Kruskal Wallis, chi-squared, 17.795, p = 0.000485) between the low pH & low pH high temp (p = 0.0005), low pH/high temp & control (0.02884), and low pH & high temp (p = 0.0479). Molt increment between molts 1 and 2 were also significantly different among treatments, with groups separating significantly by temperature (e.g. warm vs cool) (Kruskal Wallis, W = 13.545, df = 3, p-value = 0.003595). Specifically, tuna crabs in the control and low pH treatments had significantly greater percent growth in carapace length than those in both the high temp and low pH/high temp treatments (Bonferroni adj. p = 0.012; Figure 4.3A). However, when total growth was considered over the duration of the experiment (T_0 - T_F), there were no significant differences between treatments (ANOVA, F (3, 39) = 1.56, p = 0.214) (Figure 4.3B). Essentially, all groups grew the same amount in carapace length over the course of experiment, but the treatments with warmer temperatures molted more. Molt increment values are summarized in Table 4.2. There were also no differences in the ratio of carapace width to carapace length between treatments at the end of the experiment (ANOVA, F(3, 39) 0.851 , p-value = 0.474).

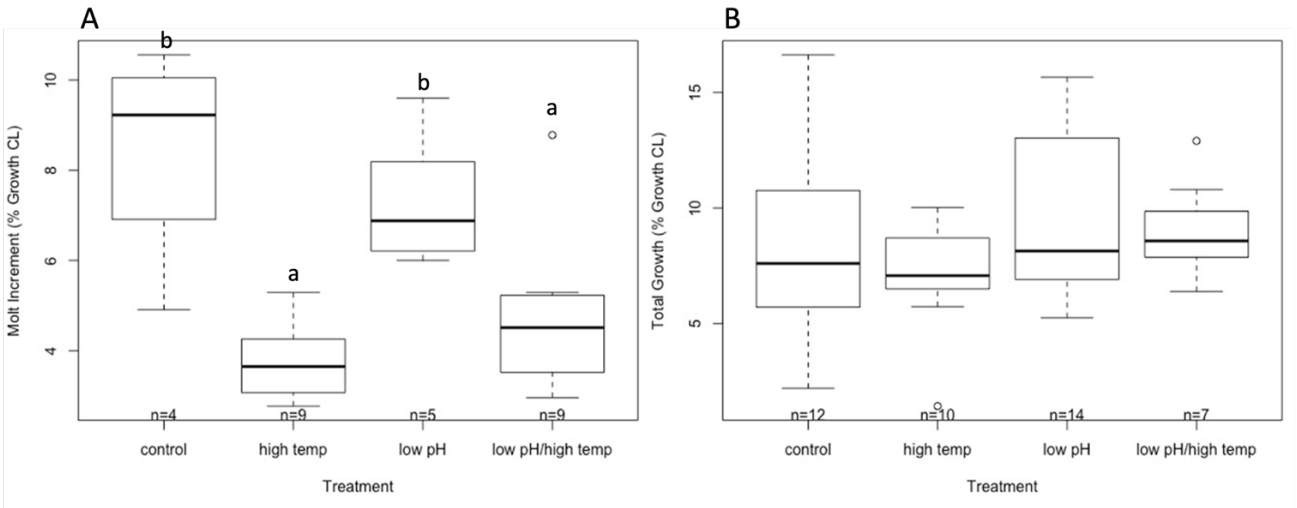

Figure 4.3: Mean percent growth in carapace length. Molt increment (A) was significantly less in both high temperature treatments (A), but total growth over the duration of the experiment (B) was the same across all treatments. Box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentile, solid line = median.

Table 4.2: Growth in CL (Molt Increment) expressed as percent growth for different time points. T_0-M_1 represents the time from the start of the experiment until the first molt, M_1-M_2 represents the time between molts 1 and 2, and T_0-T_F represents the total duration of the experiment. (Mean ± sd)

Treatment	Control	High temp	Low pH	Low pH/high temp
T_0 - M_1	5.08 ± 3.04 n=15	3.70 ± 1.45 n=11	6.12 ± 1.79 n=14	2.95 ± 1.40 n=12
M_1 - M_2	8.48 ± 2.48 n=4	3.74 ± 0.82 n=9	7.38 ± 1.51 n=5	4.74 ± 1.75 n=9
T_0 - T_F	8.25 ± 4.05 n=12	6.97 ± 2.34 n=10	9.89 ± 3.73 n=14	9.04 ± 2.17 n=7

The time from the start of the experiment until the first molt ($T_0 – M_1$) did not differ between treatments (ANOVA, $F(3, 49) = 2.6$, $p = 0.063$), but intermolt duration, the time between molt 1 and molt 2, did (ANOVA, $F(3, 23) = 11.72$, $p = 7.33e\text{-}05$). Tuna crabs in both colder temperature treatments (low pH and control) had significantly longer intermolt durations than those in the warm water treatments regardless of pH (low pH/high temp and high temp) (Figure 4.4). Intermolt duration from molt 1 to molt 2 averaged 203.4 ± 37.7 days in low pH, 222.8 ± 8.9 days in control, 137.6 ± 26.6 days in low pH/high temp, and 146.1 ± 34.8 days in high temp treatments (mean ± sd).

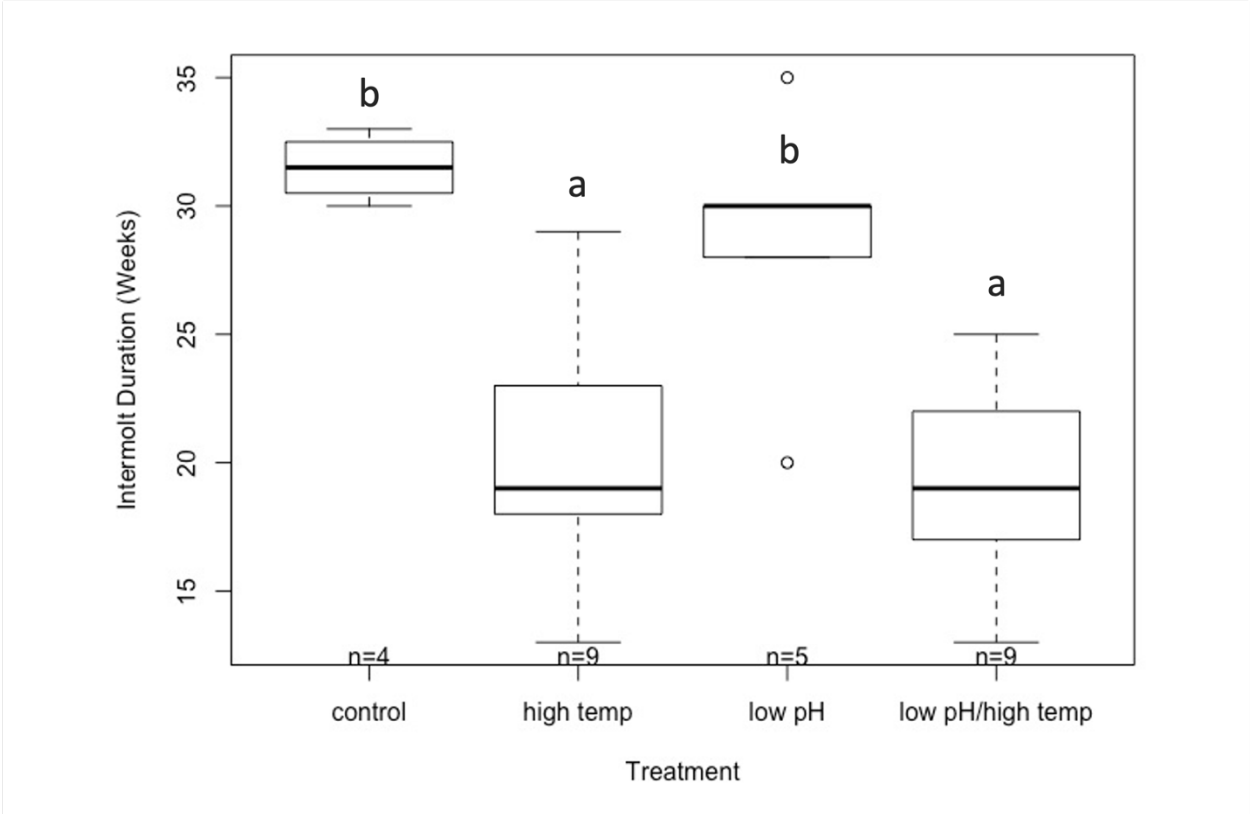

Figure 4.4: Mean intermolt duration from molt 1 to molt 2 across treatments. Tuna crabs in cold water temperature treatments (low pH and control) had significantly longer intermolt durations than those in warm water treatments (low pH/high temp and high temp). Box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentile, solid line = median.

Buoyant mass was not affected by either pH or temperature over the duration of the experiment (Kruskal Wallis, chi-square = 5.141, df = 3, p = 0.162) (Table 4.3). However, wet body mass over the course of the experiment (T_0 - T_F) was significantly lower in the high temp vs the low pH/high temp treatment, but different not among any other treatments (Kruskal Wallis, chi-square = 11.106, df = 3, p = 0.0112, Bonferroni adj. p = 0.0126) (Figure 4.5). This difference was also present from T_0 to molt 1 (Kruskal Wallis, chi-square = 13.942, df = 3, p = 0.003, Bonferroni adj. p = 0.0279) along with a significant difference between the control and low pH/high temp treatments (Bonferroni adj. P = 0.0223).

Table 4.3: Growth in buoyant and wet mass expressed as percent growth for different time points related to molt cycle. T_0-M_1 represents the time from the start of the experiment until the first molt, M_1-M_2 represents the time between molts 1 and 2, and T_0-T_F represents the total duration of the experiment. (Mean ± sd)

Treatment:	Control	High temp	Low pH	Low pH/high temp
Buoyant Mass (% growth)				
T_0 - M_1	20.13 ± 14.45 n=13	10.71 ± 9.85 n=10	17.92 ± 9.71 n=14	9.87 ± 5.72 n=11
M_1 - M_2	31.50 ± 27.46 n=3	9.13 ± 6.10 n=8	20.72 ± 10.86 n=3	9.97 ± 5.98 n=8
T_0 - T_F	38.63 ± 21.52 n=11	24.64 ± 10.14 n=9	34.35 ± 11.59 n=14	35.21 ± 6.42 n=7
Wet Mass (% growth)				
T_0 - M_1	17.52 ± 8.59 n=13	9.80 ± 4.55 n=10	17.26 ± 8.07 n=14	8.91 ± 3.90 n=11
M_1 - M_2	26.11 ± 12.15 n=3	6.27 ± 5.19 n=8	18.71 ± 14.03 n=3	13.24 ± 5.75 n=8
T_0 - T_F	36.05 ± 20.38 n=11	19.56 ± 7.20 n=9	30.83 ± 12.46 n=14	34.01 ± 4.92 n=7

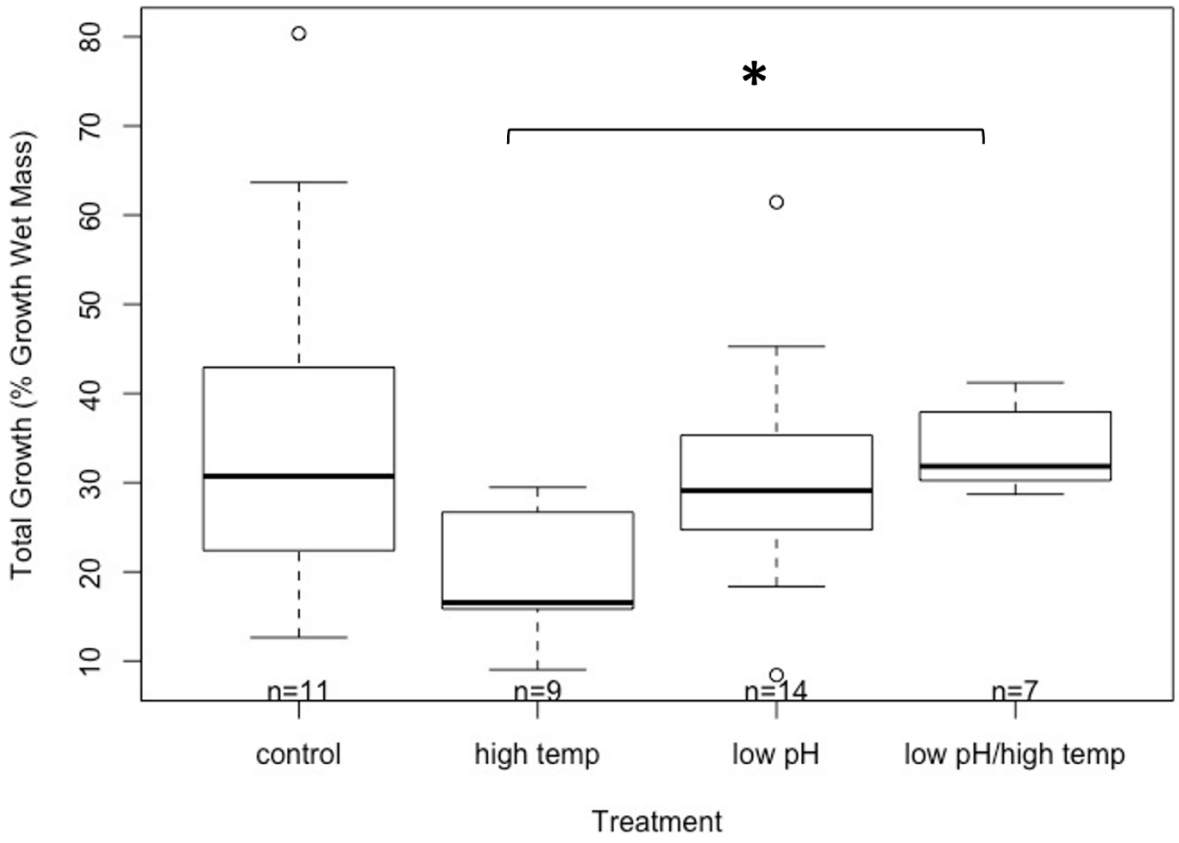

Figure 4.5: Mean percent growth in wet body mass over the duration of the experiment. Tuna crabs in the high temp treatment had a significantly smaller molt increment than those in the low pH/high temp treatment. Box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentile, solid line = median.

Exoskeleton composition

No qualitative differences were noted in exoskeleton morphology across treatments; no signs of cuticle damage or major differences in relative layer thickness were apparent. EDX analysis revealed no significant differences in the percent calcium (ANOVA, $F(3, 13) = 1.89$, $p = 0.181$) (Figure 3.6A), percent magnesium (ANOVA, $F(3, 13) = 2.18$, $p = 0.138$) (Figure 3.6B), or the calcium to magnesium ratio (ANOVA, $F(3, 13) = 2.78$, $p = 0.083$) (Figure 3.7) among the treatments. There appears to be a trend for lower %Ca in the control treatment as well as a lower

Ca:Mg in the high temp treatment, though they are not statistically significant from other treatments.

Table 4.4: Values for %Ca, %Mg and %Ca:%Mg presented listed as mean ±sd.

Treatment	%Ca	%Mg	%Ca:%Mg
control (n=4)	21.2 ±8.30	1.7 ±0.83	13.2 ±1.66
high temp (n=4)	42.3 ±21.31	3.9 ±2.09	11.3 ±1.34
low pH (n=4)	34.0 ±10.10	2.5 ±0.84	14.1 ±1.47
low pH/high temp (n=5)	27.2 ±10.42	2.2 ±0.96	12.7 ±1.07

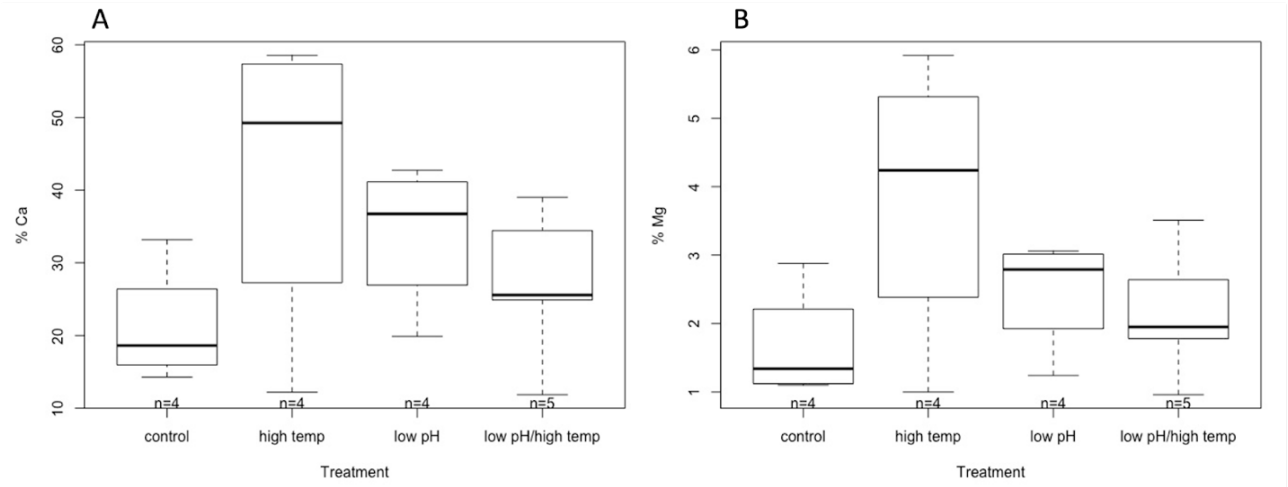

Figure 4.6: Percent calcium (A) and percent magnesium (B) for all treatments. There were no differences in either the percent calcium or percent magnesium between treatments.

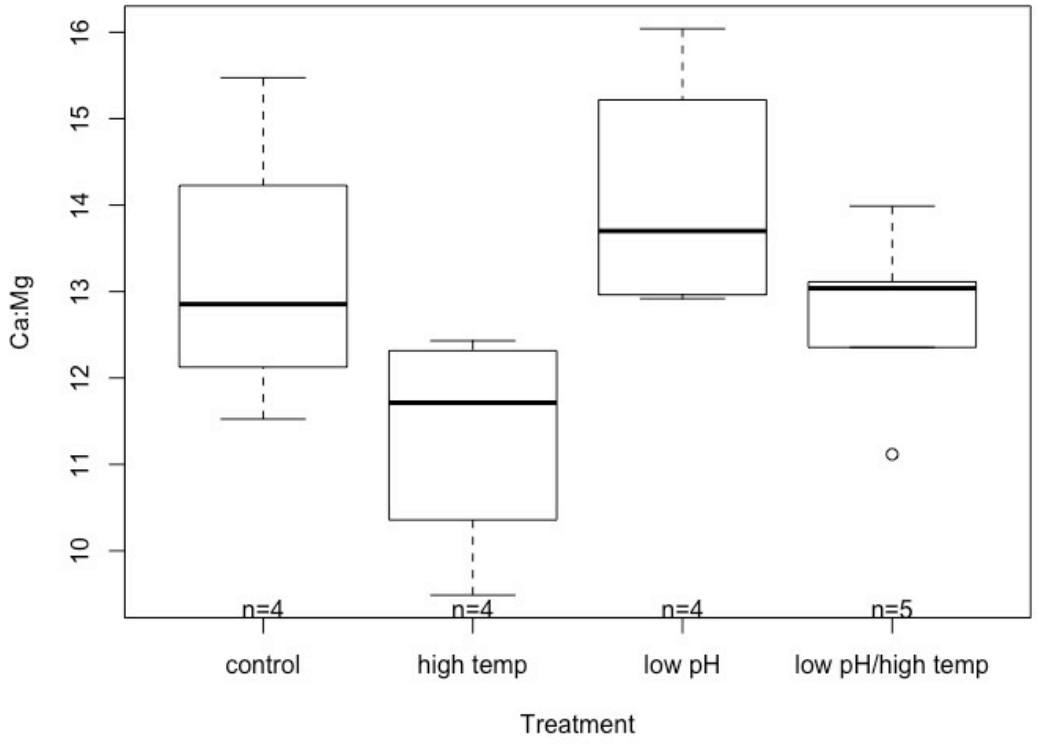

Figure 4.7: Calcium to magnesium ratio in the carapace exoskeleton of tuna crabs acquired from EDX. Ca:Mg did not differ among treatments. Box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentile, solid line = median.

4.4 Discussion

Ocean acidification (OA)-like conditions are known to affect the growth and calcification of crustaceans in a species-specific manner. For adult tuna crabs, *P. planipes*, such impacts could be highly consequential for their niche shift from pelagic to benthic habitats, as well as for the numerous species that rely on their pelagic phase as a critical food source. Here we tested the hypotheses that increased pCO_2/reduced pH would slow growth and enhance calcification rates, and that these effects would be mitigated when combined with increased water temperature. We exposed adult *P. planipes* of the pelagic phase size range to reduced pH and increased temperature

reflective of near-future ocean conditions for 10 months. After this long-term exposure, we found that our experimental pH levels (7.6) had no effect on growth and calcification, and that growth was sensitive to temperature regardless of pH. Differences in molt increment and intermolt duration that were initially observed between animals in the high versus low water temperature treatments did not translate into differences in growth by the end of the experiment, when all *P. planipes* exhibited similar total growth. Though mortality differed significantly between the treatments, the post hoc testing did not detect any differences. What is driving the significance is likely the reduction in survival in the low pH/high temp treatment compared with the low pH treatment early on. However, this effect appears to level off with exposure time. This is in agreement with findings in the blue crab, wherein longer exposure times over multiple molt cycles resulted in decreasing negative effects in terms of survival (Long et al. 2017).

Temperature, not pH, influential on growth

Though contrary to our hypotheses, it is unsurprising that reduced pH has no measurable effect on *P. planipes* growth and molting, while seawater temperature does. Environmental temperature is strongly correlated with crustacean growth and molting, whereby temperatures warmer than optima tend to increase molt frequency and increment while colder than optima temperatures increase intermolt duration (Hartnoll, 1982; Hartnoll, 2001; Skinner, et al., 1985). Environmental pH effects are less consistent based on experiments conducted on a range of crustacean species (Long et al. 2017; Lowder et al. 2017; J. R. A. Taylor et al. 2015; Bednaršek et al. 2021). Like the estuarine blue crab, *Callinectes sapidus* (Hillary L. Glandon and Miller 2017), the intertidal porcelain crab, *Petrolisthes cinctipes* (Carter et al. 2013), and the coral reef mantis shrimp, *Neogonodactylus bredeni* (deVries et al. 2016), tuna crabs also incur no effects on linear

growth (percent increase in carapace length) under pH levels as low as 7.5. This is interesting given that a pH of 7.75 was determined to be the threshold for which juvenile and adult crustacean species generally experience pH effects on growth (Bednaršek et al. 2021). The robustness of the aforementioned species to increased pCO_2/reduced pH lends support to the hypothesis that species living in habitats with strong diel fluctuations in pH are generally more adaptable to changing conditions. *P. planipes* is not a shallow water species, however, the pelagic juvenile and adult stages can vertically migrate over significant depths (Boyd 1967; Aurioles-Gamboa 1992), potentially exposing them to strong pH gradients.

Over the duration of the experiment, tuna crabs in the high temperature treatment had a smaller percent increase in wet mass than those in the reduced pH/high temperature treatment, which is consistent with the general trend for crustaceans (Hartnoll 2001). Not only does this demonstrate that both linear dimensions and mass should be accounted for in molt increment, but also that CO_2 may have some mitigating effects on high temperature-induced growth, since there was no impact on growth in the combined treatment. The mechanism for this is unclear, as increased pCO_2/reduced pH generally reduces growth in crustaceans, at least for juveniles (Bednaršek et al. 2021). In the present experiment, tuna crab wet mass was measured consistently within 2-3 days of molting to try and minimize impacts of a still soft exoskeleton on mass and to minimize handling stress to the animal, but it may be that calcification occurs faster or that water retention is prolonged under reduced pH conditions, both of which could lead to greater wet mass after molting. If reduced mass increment in high temperature conditions can be minimized, then there may be some physiological advantage to being exposed to concurrent low pH and high temperatures compared to high temperatures alone. This has not been observed in crustacean species studied to date.

These complex effects on crustacean growth convey the importance of using combined factors in OA research and project a need for more studies at the cellular physiological level to help understand the underlying mechanisms. Regardless of pH, temperatures above and below the thermal preference of *P. planipes* affected growth in predictable ways. Individuals in high temperature treatments had shorter intermolt durations (e.g., greater molt frequency) with smaller molt increments compared to those in low temperature treatments, similar to what has been observed in other crustaceans, like *C. sapidus* (Cunningham and Darnell 2015). However, by the end of the 10-month exposure, the overall growth in carapace length was the same across all treatments despite differences in molt frequency. This is a critical point, because impacts observed over short time scales may dissipate as animals continue to molt and adapt to OA-like conditions. Molting is not only a mechanism for growth, but it is also an opportunity for crustaceans to repair and respond to environmental conditions. Shrimp, for example, can modify their gill morphology during a single molt in response to reduced dissolved oxygen conditions in their environment (Peruzza et al. 2018). Duration of exposure is an essential factor in how crustaceans respond to OA-like conditions (Bednaršek et al. 2021). Long-term studies that encompass multiple molt cycles are necessary for assessing how crustaceans will respond to both current and near-future changes in ocean conditions, though such lengthy experiments are challenging and have therefore only been conducted to a limited extent (Long et al. 2017).

Calcification robust to pH and temperature

No significant differences were observed in exoskeleton structure and calcium and magnesium content among treatments over the duration of the experiment, suggesting that *P. planipes* can tolerate a wide range of pH and temperature conditions without compromising the

process of building and calcifying their exoskeleton. There are several aspects of the crustacean calcification process that protect it from changes in environmental pH conditions, including the fact that many use bicarbonate during calcification (Whiteley et al. 1999), which gives them an advantage compared to other calcifiers in a high pCO_2/lower pH environment. Maintaining a stable calcification process under changing environmental conditions is beneficial to *P. planipes* as they undergo both vertical and horizontal migration over great distances. This may come at a metabolic cost, however, and compromise other essential physiological functions (Bednaršek et al. 2021; Whiteley 2011).

Our data show a slight trend of less calcium in the control treatment and smaller exoskeleton calcium-magnesium ratios in animals in the high temperature treatments. Though they are not statistically significant, it could potentially emerge as such if sample sizes were greater. This would coincide with observations in other crustaceans that experience increased calcium content at lower pH conditions (Taylor et al. 2015) and decreased calcium content at higher water temperatures (Hillary Lane Glandon et al. 2018). Increased temperature speeds up molting (Hartnoll 2001), and any change to the timing of the molting process has the potential to impact calcification. Increased respiratory activity that can occur in high temperatures and internal buffering that occurs in response to low pH can both contribute to internal dissolution of the exoskeleton (Bednaršek et al. 2021; Truchot 1979; Cameron 1985; Hans et al. 2014). These conditions thereby possess the potential to have a direct impact on calcification.

Implications for adult niche shifts

It was hypothesized that increased temperature conditions, including when combined with reduced pH, would accelerate the growth process in *P. planipes* and ultimately their time to settle

to the benthos. Our results indicate that over the short term, one molt cycle, *P. planipes* located higher in the water column or in warmer regions will grow less at each molt. For individuals near the size that triggers niche shift to the benthos (approximately 28 mm CL), the smaller molt increment could delay this transition, keeping them in the water column for months longer, where they are exposed to greater predation risk. Increased molt frequency enables tuna crabs in warmer water to 'catch up' in growth in as little as 2-3 molt cycles. Consequently, tuna crabs in warm water would be able to reach the niche shift size in approximately the same time frame as those in cold water, thereby neither accelerating or delaying their transition to the benthos. Increased molt frequency would come at a cost though. Molting is energetically expensive, increases predation risk, and compromises locomotor efficiency (Maszczyk and Brzeziński 2018).

In the context of *P. planipes* life history and distribution, there could be significant impacts due to the differences in growth observed across the temperature range of 12-18°C used in this study. In the short term, warm water events (El Niño, The Eastern Pacific warm water "blob" of 2015) and shifts in currents could expose *P. planipes* to temperatures above their optimal range, resulting in increased molt frequencies and mortality. As the oceans continue to warm, it could be more challenging for tuna crabs to find suitable habitat. They may experience increased mortality or undergo further range shifts. Persistently warm ocean temperatures and corresponding changes in currents and mixing may have longer term effects on molt frequency, mortality, and distributions among tuna crab populations. In this experiment, we used a temperature just above their optimal range, one that they already experience occasionally, and it was sufficient to yield significant effects on growth. Temperatures that exceed their optima impact tuna crabs, and they quite likely will encounter these higher temperatures more regularly in the future. This could be problematic for many organisms at the southern end of the California Current that rely on the

pelagic stage for food (Aurioles-Gamboa and Pérez-Flores 1997). Additionally, the transient pelagic adult phase is how tuna crabs are transported into different areas. A change to growth rate could therefore have both cascading trophic effects and alter where tuna crabs are found and transported.

Effects on growth can extend beyond just body size and potentially impact other important life history traits. For example, the size and age at reproductive maturity was delayed in the amphipod *Amplesica brevicornis* under reduced pH levels (Bhuiyan et al. 2022). *P. planipes* reproduction is often coupled with seasonal upwelling (Aurioles-Gamboa 1992; De Anda-Montañez et al. 2013). This specifically timed event that depends on the proper oceanographic conditions could be interrupted if they reach sexual maturity at a different size or time/season.

Conclusions

This study reveals that ocean temperature affects molt frequency, but increased pCO_2/reduced pH has no measurable effects on growth or calcification. These results provide insight into how different oceanic conditions could impact the life history of *P. planipes*. Tuna crabs can be transported over long distances, and adults can end up in different habitats that vary greatly in pH and temperature. With their transient pelagic phase being a major food resource, and so little known about their niche shift or sensitivity to environmental changes, this research is essential for understanding how tuna crabs may continue to impact the Southern California ecosystem. This study also adds to the growing body of multistressor studies demonstrating ranging effects on crustaceans.

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Chapter 4, in full, is currently being prepared for submission for publication of the material with permission of the coauthors. Webb, Summer, Sebright, Zoe, Taylor, Jennifer. The dissertation author was the primary researcher and author of this material. We would like to thank Phil Zerofski for his help with animal collection and aquarium assistance. We would also like to thank the many volunteers who helped keep this experiment running.

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Chapter 5: Conclusions

The major objectives of this dissertation were to investigate the pelagic and benthic adult life stages of *Pleuroncodes planipes* and to gain insight into how they may be impacted by changing ocean conditions. These objectives were addressed in three separate studies in this dissertation, preceded by an introductory chapter that is a thorough review of existing data regarding their biology and life history.

In Chapter 2, morphometric techniques and inductively coupled plasma mass spectrometry were used to look for differences in allometry and exoskeletal composition between the pelagic and benthic adult size groups. Results from chapter 2 showed that morphological differences exist in cheliped allometry and compositional differences of the exoskeleton between the juveniles and pelagic and benthic adults. Interestingly the compositional patterns were opposite to expectation wherein benthic tuna crabs had less mineralized exoskeleton structures than juvenile and pelagic tuna crabs. Benthic animals had relatively longer chelipeds, with greater chela height and width (metrics related to claw force production). This study demonstrates for the first time that morphological and compositional differences are present between the adult pelagic and benthic life stages of *P. planipes,* which supports the idea of distinct habitat use or specialization.

In Chapter 3, bulk stable isotope analysis was used to determine whether adult pelagic-associated and fully benthic tuna crabs may be ecologically distinct. The smaller-sized, presumed pelagic feeding tuna crabs had significantly lower values of δ^{15}N than the larger-sized, presumed benthic feeding tuna crabs. We proposed that the larger tuna crabs do in fact undergo and ontogenetic diet shift in the form of niche expansion, demonstrated by the higher δ^{15}N. This study examined the feeding habits of the pelagic and benthic sizes together and demonstrated a quantitative difference indicative of different feeding habits between the adult size groups for the

first time. Results from this study support the hypothesis that the adult populations of tuna crabs may have distinct trophic roles.

Finally, in Chapter 4, the survival, growth, molting and calcification of the exoskeleton was assessed in adult *Pleuroncodes planipes* in response to ocean acidification-like conditions. We performed a long-term experiment where pH and temperature were manipulated to simulate future-like ocean conditions. We found that survival was significantly affected by exposure, but this effect decreased over time. There was no measurable effect on mineral content of the exoskeleton. Generally, tuna crabs were sensitive to temperature and not pH. Regardless of pH, tuna crabs in the warm temperature treatments molted more frequently and had a smaller growth increment over a single molt than those in cooler treatments, but achieved the same amount of growth by the end of the experiment. These short-term effects on growth could potentially delay pelagic-benthic transitions in tuna crabs, or interrupt other important aspects of their growth and survival as there is likely an increased metabolic cost to more frequent molting. Our findings from this study highlight the importance of observing crustaceans over multiple molt cycles for OA experiments. Results from this study also provide the first insight into how the life stages of tuna crabs may be impacted by climate change-relevant stressors.

Taken together, results from the studies undertaken in this dissertation demonstrate morphological, exoskeletal, and trophic differences between adult tuna crabs that are presumed to have distinct and sequential pelagic and benthic stages. Though disentangling habitat use is difficult to accomplish with certainty for any species, the data collected provide evidence for specialization to these habitats, which has not been previously demonstrated. The data collected in chapter 4 are also helpful for providing context for the results obtained in chapter 2. Specifically, differences in mineralization between the pelagic and benthic groups found in chapter 2 could

potentially be attributed to environmental conditions like pH, however the experiment in chapter 4 exposed adult tuna crabs to high pCO_2/decreased pH and no differences in exoskeleton mineral content were observed. This supports the notion that there is some habitat-specific specialization driving the difference observed in mineralization between the groups, rather than environmental forcing. Additionally, morphological results from chapter 2 help bring context to the stable isotope results in chapter 3. The shift to higher trophic levels at larger size is supported by differences seen in the chelipeds and mandibles, which are feeding relevant structures. The data and results in this dissertation highlight the complexities associated with the life history of *Pleuroncodes planipes*, but also provide support for the long-standing but untested hypothesis that the adult size classes are ecologically distinct and may use different habitats.