Prevalence and effects of rhizocephalan (Cirripedia: *Briarosaccus callosus)* parasitism and assessment of post-capture survival using the reflex action mortality predictor (RAMP) method on the deep water Porcupine crab (*Neolithodes grimaldii*) captured on the Labrador Shelf in the Northwest Atlantic.

By Greg Furey

A Thesis submitted to the school of graduate studies

in partial fulfillment of the requirements for the degree

Masters of Science

Department of Biology

Memorial University of Newfoundland

December 2016

St. John's, Newfoundland and Labrador

Abstract

The porcupine crab (*Neolithodes grimaldii)* is a deep water (>800m) species of king crab (Lithodidae) and little is known about its biology, ecology, and distribution. Historically, high bycatch rates of porcupine crab have been observed in gillnet fisheries targeting Greenland halibut (*Reinhardtius hippoglossoides)* in the northwest Atlantic. As there is currently no directed fishery for the porcupine crab in the north Atlantic the information collected in this study will be of interest to science, industry, and fisheries management. The results of this project will provide a basis to make informed decisions at the forefront on the sustainability of a potential fishery for this renewable resource. This thesis collected data on the prevalence of the rhizocephalan parasite (*Briarosaccus callosus*) infesting the porcupine crab which was found to be high (21%) compared to other species of open water king crab. Morphological and reproductive effects of *B. callosus* on the porcupine crab were also observed. The parasite sterilized male and female porcupine crabs. The parasite correlated with reductions in size of chelae height, length of the first and second merus, and carapace length in infested males, however, females had increased carapace and merus length. The post-capture survival of porcupine crab captured incidentally in gillnets targeting Greenland halibut was also determined using the reflex action mortality predictor (RAMP) method. The survival rate was high (74%) for crabs when removed from gillnets without extensive trauma and held 5-9 days in holding tanks aboard a commercial vessel. The survival rate was greater for crabs that were removed without extensive trauma (74%) and crabs that were selected in the second year that had incurred trauma from gear or rough handling (28%). Using the RAMP method revealed stark differences between porcupine crab captured in gillnets and previous studies conducted on bottom trawl captured king crab. Porcupine crab appear to be in a heightened state during capture and removal from gillnets reducing the ability to predict mortality using the RAMP method as they failed few reflex actions. Analyzing these results revealed that mortality could not be predicted with a high amount of confidence as a low amount of variability was explained by the models used (5-15%).

Acknowledgments

I would like to acknowledge Memorial University of Newfoundland, the Centre for Sustainable Aquatic Resources at Marine Institute, the Government of Nunavut, Arctic Fisheries Alliance, Baffin Fisheries Coalition, Northern Research Corporation, and the Qikiqtaaluk Corporation for the resources necessary to complete this research. I would like to acknowledge, the help of Mike Hurley and Rennie Sullivan in collecting and processing samples. And I would like to thank my supervisor Scott Grant for support and guidance throughout the course of the project. I would also like to thank my supervisory committee consisting of Paul Winger and Bob Hooper for feedback and support. Finally, I would like to thank my family for ongoing support in completing my masters research.

Table of Contents

List of Tables

List of Figures

Co-authorship Statement

The author of this thesis contributed to the design of the experiments, collected/organized all of the data, and analyzed and wrote all of the subsequent manuscripts. Dr. Scott Grant contributed significantly to research proposals, experimental designs, and discussions of ideas and also provided editorial reviews of all chapters. Mike Hurley and Rennie Sullivan contributed significantly to the data collection for Chapter 2 and 3.

Mike Hurley and Dr. Scott Grant are second and third authors for Chapter 2, respectively.

Mike Hurley and Dr. Scott Grant are second and third authors for Chapter 3, respectively.

It is anticipated that Chapters 2 and 3 will be submitted as separate manuscripts to scientific journals.

Chapter 1: 1.1 Introduction

A thorough understanding of the baseline biological information of a targeted species is important for sustainable fisheries (Alverson et al. 1994). As such, fisheries management is most effective when scientific information is available on the biology of the target species to increase confidence in management decisions. In the Pacific Ocean, the sharp declines in commercial landings of red king crab (*Paralithodes camschatica)* since the 1980s highlight the need for fisheries that are well regulated and for sufficient scientific knowledge to be accessible for effective fisheries management (Zaklan 2002). The complexity of king crab ecology has made it clear that both anthropogenic and environmental effects on populations should be considered to prevent population declines such as those seen in the Pacific red king crab (Loher et al. 1998).

The current study is being undertaken to obtain a better understanding of porcupine crab (*Neolithodes grimaldii)* populations on the Labrador Shelf. The main goal of this study is to increase baseline biological information of this species to obtain the best information required for science, industry, and fisheries management. As there is currently no directed fishery for porcupine crab, this study will aid in the understanding of the commercial potential and sustainability of this species at the forefront of fishery development.

The porcupine crab is a deep sea (>800m) king crab (family Lithodidae, see Table 1.1) found along continental shelves on both sides of the North Atlantic (Macpherson 1988; Squires 1990). In the northwest Atlantic it has been observed off the coast of North Carolina northward to Baffin Bay, while in the northeast Atlantic it has been observed from Cape Verde northward to the coasts of Iceland and the United Kingdom (Macpherson 1988; He 2005; Cartes et al. 2013; Bungay et al. 2014). The deep sea habitat of porcupine crab is a main factor that distinguishes them from other commercially harvested king crab species which are usually encountered from the intertidal zone to ~500 m (Zaklan 2002). The porcupine crab is characterized by its fire truck red exoskeleton and long spines that protrude from its carapace and limbs (Figure 1.1, 1.2). Little is known about the life history of porcupine crabs although the males and females can be distinguished by observing the abdomen on the ventral side which is small and v-shaped for males and large with lobes and pleopods to provide a cavity for incubating eggs in females (Figure 1.3, 1.4).

Although past exploratory fisheries targeting porcupine crab were unsuccessful, this species is frequently caught in bottom trawl multispecies surveys and as bycatch in the deep sea gillnet and otter trawl fisheries for Greenland halibut (*Reinhardtius hippoglossoides)* (DFA 2000; He 2005; Cartes et al. 2013; Bungay et al. 2014). In the northwest Atlantic the amount of porcupine crab bycatch captured in deep sea gillnet fisheries is historically high (He et al. 1994; He 2005; Winger 2013; Bungay et al. 2014). In 1996, log books for the Greenland halibut gillnet fishery revealed an estimated 767,000 kg of porcupine crab were captured incidentally, while the total catch of Greenland halibut was ~3,872,000 kg (He 2005).

The current study collaborated with a commercial Greenland halibut vessel to sample porcupine crab captured in monofilament gillnets in NAFO Division 0B. Sampling was conducted to increase biological knowledge of this king crab species. Two biological factors key to effective fisheries management will be assessed in this study, which are the presence and effects of *Briarosaccus callosus* on porcupine crab (Chapter 2), and post-capture survival using the RAMP method (Chapter 3).

The prevalence of rhizocephalan barnacle (Cirripedia) parasites such as *B. callosus* are an important factor in the sustainable management of commercial King crab stocks because of their negative effects on king crab hosts, such as reductions in growth, prevention of maturity, and reproductive sterility (Sloan 1985; Hoggarth 1990). The parasite *B. callosus* composes of two parts the interna which penetrates the body cavity and the externa (Figure 1.5) which can be observed by viewing the ventral side of porcupine crab and examining the cavity underneath their abdomen (Figure 1.6). Previous studies have observed these effects on several commercially important king crab species such as red (*Paralithodes camtchatica*), blue (*Paralithodes platypus*), and golden (*Lithodes* aequispina) king crab in the Pacific Ocean (Table 1.2). The rhizocephalan species *B. callosus* is distributed worldwide and has been observed infesting over 19 king crab hosts in almost all major ocean bodies (Atlantic, Pacific, Indian, and Antarctic) (Zaklan 2002). The broad distribution of *B. callosus* and the large number of hosts it infests highlights the importance of investigating the abundance and negative effects of this parasite on king crab hosts.

The presence of *B. callosus* on the porcupine crab host in the northwest Atlantic was first observed from a single male crab collected in 1986 (Pohle 1992a,b). Although *B. callosus* was observed at this time there was not a large enough sample size to determine prevalence or the morphological and reproductive effects on porcupine crab. The current study therefore collected large samples of porcupine crab over two successive years which allowed for a more thorough understanding of the prevalence and effects of *B. callosus* on porcupine crab.

Another goal of this thesis was to assess the post-capture survival of porcupine crabs captured incidentally in the Greenland halibut gillnet fishery. To date studies assessing survival of animals captured or encountered by fishing gear have organized these occurrences based on the number of mortalities and the time it takes for them to occur. In this way, the survival of individuals can be organized into those that are immediately affected by capture methods (observed mortality) and those that may be indirectly affected (unobserved mortality) (Broadhurst et al. 2006). A main factor in the mortality of animals that encounter or are captured in fishing gear is the type of fishing gear used and the method utilized (Broadhurst et al. 2006).

The current study aims to quantify the survival of porcupine crab captured in gillnets. Previous studies have been conducted on reducing the amount of king crab captured incidentally in gillnet fisheries but these studies focused on gear components and not survival (Godøy et al. 2003). Differences may exist in the interaction that active versus passive gear may have on the survival of bycatch species such as king crab. This study will aid future studies by providing comparison and context based on previous results such as baseline information on gillnet capture survival of porcupine crab, as well as, whether differences in the removal process of crabs from fishing gear can result in different levels of stress and injury. This study offers realistic field conditions in that it was conducted aboard a commercial vessel during fishing procedures, and thus differs from other studies in which the main focus of the trip was post-capture survival research. However, being limited to working on a commercial vessel during fishing operations constrained this study to shorter time frames of onboard holding for assessments of post-capture survival. There were also potential differences in handling between the crabs captured in passive gillnets and those from other studies conducted aboard research trips utilizing active gear such as bottom trawls. The differences between active gear and passive gear can be quite substantial. In terms of the time taken to deploy and retrieve gear, most studies using bottom trawls utilized fifteen minute tows before retrieval whereas the gillnets used in this study were left to soak for five days or more before retrieval.

Studies to quantify post-capture survival of crustaceans have been carried out in many regions of the world using a variety of methods such as holding animals in situ in sea cages or pots (Grant 2003), as well as, holding animals in onboard tanks after being captured to testing reflex actions (Davis and Ottomar 2006; Davis 2007; Stoner et al. 2008; Stoner 2009; Rose et al. 2013; Hammond et al. 2013), or to analyze haemolymph components in search of chemical cues indicating stress (Leland et al. 2013). Chapter three of this thesis includes onboard holding and reflex actions of porcupine crab to provide insights into the post-capture survival of porcupine crab captured as bycatch in gillnet fisheries. Developing a survival assessment for porcupine crab is important as there is currently no knowledge on its survivability, yet a substantially high number of porcupine crab have been historically captured incidentally in the Greenland halibut gillnet fishery. As this is the first time this type of survival assessment has been conducted on this animal, it will provide baseline data for further research on the biology of porcupine crab. The reflex action mortality predictor (RAMP) method tested during this study has been used for other king crab species captured in bottom trawls but before this study this methodology had not yet been tested on porcupine crab or on gillnet captured king crab (Rose et al. 2013).

Understanding the effects on mortality that gillnet fisheries have on porcupine crab is important for several reasons. For example, the number of vessels allocated annually to capture Greenland halibut with gillnets and the amount of porcupine crab captured historically suggest the potential for long term effects on the conservation of this species. In addition, the study of reflex actions to predict mortality in crustaceans is relatively new and little work has been done on gillnet captured species of king crab such as porcupine crab. This baseline information on the predictability of mortality through use of reflex actions can be used to assess its viability in gillnet fisheries when compared to other studies where active gear such as bottom trawls are used to capture king crab. This study will also provide baseline information on a deep water species of king crab which could be used to compare results with species captured in shallower water.

1.2 References

Ahyong, S.T. and Dawson, E.W. (2006) Lithodidae from the Ross Sea, Antarctica, with descriptions of Two New Species (Crustacea: Decapoda: Anomura), *Zootaxa*, vol. 1303, pp. 45-68

Alverson. D.L., Freeberg, M.H., Murawski, S.A. and Pope, J.G. (1994) A global assessment of fisheries bycatch and discards, FAO *Fisheries Technical Paper* 339, 233 pp. Food and Agriculture Organization of the United Nations, Rome, Italy.

Abello, P., & Macpherson, E. (1992). Epibiosis and Rhizocephalan infestation patterns in relation to the reproductive biology of Lithodes ferox (Filhol, 1885) (Anomura: Lithodidae). Journal of Crustacean Biology, 12(4), 561–570.

Ahyong, S.T. and Dawson, E.W. (2006) Lithodidae from the Ross Sea, Antarctica, with descriptions of Two New Species (Crustacea: Decapoda: Anomura), *Zootaxa*, vol. 1303, pp. 45-68

Arnaud P.M., Do-Chi, T. (1977). Données biologiques et biométriques sur les lithodes *Lithodes murrayi* (Crustacea: Decapoda: Anomura) des îles Crozet (SW océan Indien). Mar Biol 39:147–159.

Boschma, H. (1930). *Briarosaccus callosus*, a new genus and new species of rhizocephalan parasite of *Lithodes agassizii* Smith. Proc. U.S. Natl. Mus. 76:1-8.

Boschma, H. (1970). Notes on rhizocephala of the genus *Briarosaccus*, with the description of a new species. Proceedings Koninklijke Nederlandse Akademievan Wetenschappen, Series C 73:233-242.

Boschma, H., and E. Haynes. (1969). Occurrence of the rhizocephalan *Briarosaccus callosus* Boschma in the king crab *Paralithodes camtschatica* (Tilesius) in the Northeast Pacific Ocean. Crustaceana 16:97-98.

Bower, S.M., and N.A. Sloan. (1985). Morphology of the externa of *Briarosaccus callosus* Boschma (Rhizocephala) and the relationship with its host *Lithodes aequispina* Benedict (Anomura). J. Parasitol. 17:455-463.

Broadhurst, M. K., Suuronen, P., & Hulme, A. (2006). Estimating collateral mortality from towed fishing gear. *Fish and Fisheries* (Vol. 7).

Bungay, T., Grant, S.M., Winger, P., and Moret, K. (2014). Mapping catch statistics for porcupine crab (*Neolithodes grimaldii*) in the Northwest Atlantic. *Centre for Sustainable Aquatic Resources, Marine Institute of Memorial University of Newfoundland.* Technical Report P-431: 25 p.

Cañete, J. I., Cárdenas, C. A., Oyarzún, S., Plana, J., Palacios, M., & Santana, M. (2008). Pseudione tuberculata Richardson, 1904 (Isopoda: Bopyridae): un parásito de juveniles de centolla *Lithodes santolla* (Molina, 1782)(Anomura: Lithodidae) en el estrecho de Magallanes, Chile. Revista de biología marina y oceanografía, 43(2), 265-274.

Cartes, J. E., Papiol, V., Frutos, I., Macpherson, E., González-Pola, C., Punzón, A., Serrano, A. (2013). Distribution and biogeographic trends of decapod assemblages from Galicia Bank (NE Atlantic) at depths between 700 and 1800 m, with connexions to regional water masses. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *106*(iv), 165–178. doi:10.1016/j.dsr2.2013.09.034

Chace, F.A.J. (1939). Reports on the scientific results of the first Atlantis expedition to the West Indies, under the joint auspices of the university Havana and Harvard University. Preliminary descriptions of one new genus and seventeen new species of decapod and stomatopod crustaceans. Memorias de al sociedad Cubana de Historia Natural 13:31-54.

Davis, M. W. (2007). Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish. *ICES Journal of Marine Science*, *64*(8), 1535–1542. doi:10.1093/icesjms/fsm087

Davis, M. W., & Ottmar, M. L. (2006). Wounding and reflex impairment may be predictors for mortality in discarded or escaped fish. *Fisheries Research*, *82*(1-3), 1–6. doi:10.1016/j.fishres.2006.09.004

DFA (2000). Experimental fishery – porcupine crab with pots. Fisheries Diversification Program, Department of Fisheries and Aquaculture, St. John's NL. FDP Project No. 151, 7 p. http://www.fishaq.gov.nl.ca/research_development/fdp/fdp_151.pdf

Faxon, W. (1895). Reports on an exploration off the west coasts of Mexico, Central and South America and off the Galapagos Islands, in charge of Alexander Agassiz, by the U.S. Fish Commission Steamer "Albatross" during 1891, Lieut. Commander Z.L. Tanner, U.S.N. commanding. XV. The stalk eyed Crustacea. Mem. Mus. Comp. Zool. Harvard College 18:1-292.

Godøy, H., Furevik, D., & Løkkeborg, S. (2003). Reduced bycatch of red king crab (*Paralithodes camtschaticus*) in the gillnet fishery for cod (*Gadus morhua*) in northern Norway. *Fisheries Research*, *62*(3), 377–384. doi:10.1016/S0165-7836(02)00281-3

Grant, S. M. (2003). Mortality of Snow crab discarded in Newfoundland and Labrador's trap fishery: at-sea experiments on the effect of drop height and air exposure duration. *Canadian Technical Report of Fisheries and Aquatic Sciences*. 2481: vi + 28 p.

Hammond, C. F., Conquest, L. L., & Rose, C. S. (2013). Using reflex action mortality predictors (RAMP) to evaluate if trawl gear modifications reduce the unobserved mortality of Tanner carb (*Chionocetes bairdi*) and snow crab (*C. opilio*). *ICES Journal of Marine Science*, *70*(7), 1308–1318.

Hawkes, C. R., Meyers, T. R., & Shirley, T. C. (1985a). Parasitism of the Blue King crab, Paralithodes platypus, by the Rhizocephalan, Briarosaccus callosus. Journal of Invertebrate Pathology, 45, 252–253.

Hawkes, C.R., Meyers, T., & Shirley, T. C. (1985b). Larval biology of Briarosaccus callosus Boschma (Cirripedia: Rhizocephala). Proceedings of the Biological Society of Washington, 98(4), 935–944.

Hawkes, C. R., Meyers, T. R., & Shirley, T. C. (1986a). Length-weight relationships of blue, Paralithodes platypus, and golden, Lithodes aequispina, king crabs parasitized by the rhizocephalan, Briarosaccus callosus Boschma. Fishery Bulletin, 84, 327–332.

Hawkes, C. R., Meyers, T. R., & Shirley, T. C. (1986b). Prevalence of the Parasitic Barnacle Briarosaccus callosus on King Crabs of Southeastern Alaska. Transactions of the American Fisheries Society, 115, 252–257.

Hawkes, C. R., Meyers, T. R., & Shirley, T. C. (1987). Growth of Alaskan Blue King Crabs , Paralithodes platypus (Brandt), Parasitized by the Rhizocephalan Briarosaccus callosus Boschma. *Crustaceana*, *52*(1), 78–84.

He, P., Ennis, W., Walsh, P. (1994) Porcupine crab exploratory fishing off Labrador – report on the 1994 sea trials. Fish. Tech. Unit. Report No. 4/94, 22p. Available from Fisheries and Marine Institute, St. John’s, NL.

He, P. (1997). The porcupine crab (*Neolithodes grimaldii)* in the northwest Atlantic: biology, distribution, and behavior. Fish. Tech. Unit. Report No. 4/97, 46 p. + App. Available from Fisheries and Marine Institute, St. John’s, NL.

He, P. (2005). Characteristics of bycatch of porcupine crabs, *Neolithodes grimaldii* (Milne-Edwards and Bouvier 1894) from deepwater turbot gillnets in the northwest Atlantic. *Fisheries Research*, *74*, 35–43.

Hoggarth, D. D. (1990). The Effects of Parasitism by the Rhizocephalan *Briarosaccus callosus* Boschma on the Lithodid Crab, *Paralomis granulosa* (Jacquinot) in the Falkland Islands. *Crustaceana*, *59*(2), 156–170.

Isaeva, V. V., Dolganov, S. M., & Shukalyuk, a. I. (2005). Rhizocephalan Barnacles—Parasites of Commercially Important Crabs and Other Decapods. *Russian Journal of Marine Biology*, *31*(4), 215–220.

Jansen, P. A., Mackenzie, K., & Hemmingsen, W. (1998). Some parasites and commensals of Red king crabs, *Paralithodes camtschaticus* (tilesius), in the Barents Sea. *Bull. Eur. Ass. Fish Pathol.*, *18*(2).

Leland, J. C., Butcher, P. A., Broadhurst, M. K., Paterson, B. D., & Mayer, D. G. (2013). Damage and physiological stress to juvenile eastern rock lobster (*Sagmariasus verreauxi*) discarded after trapping and hand collection. *Fisheries Research*, *137*, 63–70. doi:10.1016/j.fishres.2012.09.001

Loher, T., Hill, P. S., Harrington, G. S., & Cassano, E. S. (1998). Management of Bristol Bay Red King Crab: A Critical Intersections Approach to Fisheries Management. *Reviews in Fisheries Science*, *6*(3), 169–251. doi:10.1080/10641269891314285

Lörz AN, Glenner H, Lützen J. (2008). First records of rhizocephalans from New Zealand, including first rhizocephalan records from hot vents and cold seeps. Crustaceana 81(8): 1013-1019.

Lovrich, G. A., Roccatagliata, D., & Peresan, L. (2004). Hyperparasitism of the cryptoniscid isopod Liriopsis pygmaea on the lithodid Paralomis granulosa from the. Diseases of Aquatic Organisms, 58, 71–77.

Lützen, J. (1985). Rhizocephala (Crustacea: Cirripedia) from the deep sea. In: Wolff T ed. Scientific results of the Danish deep-sea expedition around the world Galathea report vol. 16. Pp. 99-112.

Lützen, J., Glenner, H., & Lörz, A. (2009). Parasitic barnacles (Cirripedia: Rhizocephala) from New Zealand offshore waters. *New Zealand Journal of Marine and Freshwater Research*, *43*(2), 613–621. http://doi.org/10.1080/00288330909510027

Macpherson, E., (1988). Revision of the family Lithodidae Samouelle 1819 (Crustacean, Decapoda, Anomura) in the Atlantic Ocean. Monogr. Zool. Mar. 2, 153 pp.

McMullen, J.C., and H.T. Yoshihara. (1970). An incidence of parasitism of deep-waterking crab, *Lithodes aequispina*, by the barnacle *Briarosaccus callosus*. J. Fish.Res. Board Can. 27:818-821.

Otto, R.S., and R.A. MacIntosh. (1996). Observations on the biology of lithodid crab *Paralomis spinosissima* from the southern ocean near South Georgia. In: High latitude crabs: Biology, management, and economics. University of Alaska SeaGrant, AK-SG-96-02, Fairbanks, pp. 627-647.

Peresan, L., & Roccatagliata, D. (2005). First record of the hyperparasite *Liriopsis pygmaea* (Cryptoniscidae, Isopoda) from a rhizocephalan parasite of the false king crab *Paralomis granulosa* from the Beagle Channel (Argentina), with a redescription. *Journal of Natural History*, *39*(4), 311–324.

Pohle, G. W. (1992a). First Canadian record of *Paralomis bouvieri* Hansen, 1908 (Decapoda: Anomura : Lithodidae), infected by the rhizocephalan *Briarosaccus callosus* (Cirripedia: Peltogastridae) and carrying a hyperparasitic cryptoniscinid isopod (Epicaridea). *Canadian Journal of Zoology*, *70*.

Pohle, G. W. (1992b). First Record of the Rhizocephalan *Briarosaccus callosus* (Cirripedia, Peltogastridae) Infecting the Atlantic Porcupine Stone Crab *Neolithodes grimaldii* (Decapoda, Anomura, Lithodidae). *Crustaceana*, *62*(2), 133–136.

Poltev, Y. N. (2008). Parasitism of the rhizocephalan *Briarosaccus callosus* on the crab *Paralomis verrilli* (Crustacea: Lithodidae) in the waters of southeast Sakhalin. Russian Journal of Marine Biology, 34(3), 159-165.

Rose, C. S., Hammond, C. F., Stoner, A. W., Munk, J. E., & Gauvin, J. R. (2013). Quantification and reduction of unobserved mortality rates for snow, southern Tanner, and red king crabs (*Chionoecetes opilio, C. bairdi, and Paralithodes camtschaticus*) after encounters with trawls on the seafloor. *Fishery Bulletin*, *111*(1), 42–53. doi:10.7755/FB.111.1.4

Shirley, S.M., T.C. Shirley, and T.R. Meyers. (1986). Hemolymph responses of Alaskan king crabs to rhizocephalan parasitism. Can. J. Zool. 64:1774-1781.

Shukalyuk, A. I., Isaeva, V. V., Pushchin, I. I., & Dolganov, S. M. (2005). Effects of the *Briarosaccus callosus* infestation on the commercial Golden King Crab *Lithodes aequispina*. *The Journal of Parasitology*, *91*(6), 1502–1504.

Sloan, N. A. (1984). Incidence and effects of parasitism by the Rhizocephalan barnacle, *Briarosaccus callosus* Boschma, in the golden king crab, *Lithodes aequispina* Benedict, from deep fjords in Northern British Columbia. *Journal of Experimental Marine Biology and Ecology*, *84*, 111–131.

Sloan, N. A. (1985). Life history characteristics of fjord-dwelling golden king crabs *Lithodes aequispina. Marine Ecology Progress Series*, *22*, 219–228.

Somerton, D.A. (1981). Contribution to the life history of the deep sea king crab *Lithodes couesi*, in the Gulf of Alaska. Fish. Bull. U.S., 79: 259-269.

Sparks, A., & Morado, J. (1986). Histopathology and host response in lithodid crabs parasitized by *Briarosaccus callosus*. Diseases of Aquatic Organisms, 2, 31–38. doi:10.3354/dao002031

Squires, H.J., (1990). Decapod Crustacean of the Atlantic coast of Canada. Can. Bull. Fish. Aquat. Sci. 221, 532.

Stoner, A. W., Rose, C. S., Munk, J. E., Hammond, C. F., & Davis, M. W. (2008). An assessment of discard mortality for two Alaskan crab species, Tanner crab (*Chionoecetes bairdi*) and snow crab (*C .opilio*), based on reflex impairment. *Fishery Bulletin*, (106), 337–348.

Stoner, A. W. (2009). Prediction of discard mortality for Alaskan crabs after exposure to freezing temperatures, based on a reflex impairment index. *Fishery Bulletin*, (107), 451–464.

Winger, P. (2013). Workshop to Evaluate Porcupine Crab Resource Potential. Fisheries and Marine Institute Technical Report, CSAR P-408, 31p.

Zaklan, S. D. (2002). Review of the family Lithodidae (Crustacea: Anomura: Paguroidea): Distribution, biology, and fisheries. *Crabs in Cold Water Regions: Biology, Management, and Economics*, (Table 2), 751–845. doi:10.4027/ccwrbme.2002.53

1.3 Tables and Figures

Table 1.1: Classification of porcupine crab

Order	Decapoda
Family	Lithodidae
Genus	*Neolithodes*
Species	*grimaldii*

Table 1.2: Current known hosts and locations of rhizocephalan parasite *B. callosus*

Species	Location	Reference
Neolithodes agassizii (Smith 1882)	Atlantic; SE USA; Colombia	Boschma 1930, 1970
Neolithodes grimaldii (A. Milne-Edwards & Bouvier, 1894)	Atlantic; NE Canada	Pohle 1992a,b
Lithodes aequispina (Benedict, 1895)	NE Pacific; Bering Sea	Boschma 1970; Sloan 1984; Bower and Sloan 1985; Shukalyuk et al. 2005; McMullen & Yoshihara 1970; Hawkes et al. 1985a, 1986a; Shirley 1986; Sparks and Morado 1986;
Lithodes santolla (Molina, 1782)(= L. Antarctica Jacquinot 1844)	Antarctic; Subantarctic	Boschma 1970; Lovrich et al. 2004; Cañete et al. 2008
Lithodes couesi (Benedict, 1895)	NE Pacific; Bering Sea	Boschma 1970, Somerton 1981
Lithodes ferox (Filhol, 1885)	S Atlantic Ocean	Abello and Macpherson 1992
Lithodes murrayi (Henderson, 1888)	SW Indian Ocean	Arnaud and Do-Chi 1977

Paralithodes camtschatica (Tilesius, 1815)	NE Pacific, Bering Sea	Faxon 1895; Boschma & Haynes 1969; Boschma 1970; McMullen & Yoshihara 1970; Hawkes et al. 1986b; Shirley et al. 1986; Sparks and Morado 1986; Jansen et al. 1998
Paralithodes platypus (Brandt, 1850)	NE Pacific, Bering Sea	Hawkes et al. 1985a,b, 1986a, 1987; Shirley 1986
Paralithodes rathbuni	NE Pacific	Zaklan 2002
Paralithodes californiensis	NE Pacific	Zaklan 2002
Paralomis granulosa (Jacquinot, 1847)	Antarctic, Subantarctic	Hoggarth 1990; Lovrich 2004; Peresan 2005
Paralomis bouvieri (Hansen, 1908)	NE and NW Atlantic	Pohle 1992b
Paralmois aspera (Faxon, 1893)	Tropical E Pacific	Faxon 1895; Lützen 1985
Paralomis verrilli (Benedict, 1895)	SE Sakhalin island	Poltev 2008
Paralomis cubensis (Chace, 1939)	Northwestern Cuba	Chace 1939
Paralomis spinosissima (Birstein & Vinogradov, 1972)	Southern Ocean, Antarctic Ocean	Otto and Macintosh 1996
Paralomis birsteini (Macpherson, 1988)	Ross Sea, Antarctica, NZ	Ahyong and Dawson 2006; Lörz et al. 2008
Paralomis hirtella (de Saint Laurent & Macpherson, 1997)	New Zealand	Lörz et al. 2008; Lützen et al. 2009

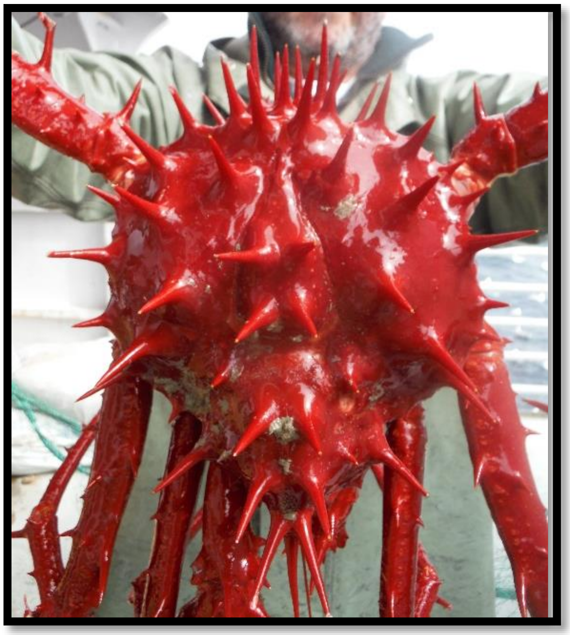

Figure 1.1: Dorsal view of porcupine crab carapace

Figure 1.2: Dorsal view of porcupine crab male

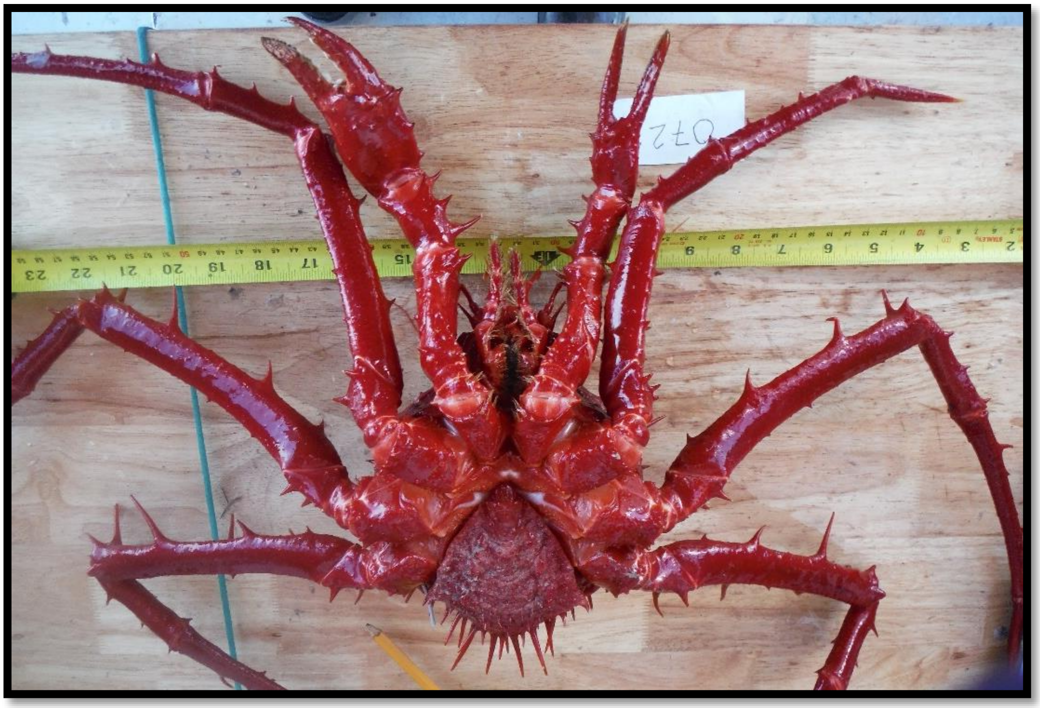

Figure 1.3: Ventral view of porcupine crab male displaying v-shaped abdominal flap.

Figure 1.4: Ventral view of porcupine crab female displaying large lobate abdominal flap.

Figure 1.5: Schematic diagram of *Peltogaster reticulatus* a close relative to *Briarosaccus callosus.* The kidney shaped externa is shown here connected to the interna (branching system of roots that occupy body cavity of host) (Isaeva et al. 2005).

Figure 1.6: Ventral view of a porcupine crab female with externa of *B. callosus* (brown) being exposed by pulling down on abdominal flap.

Chapter 2: Prevalence and effects of rhizocephalan (*Briarosaccus callosus)* parasitism on the deep water porcupine crab (*Neolithodes grimaldii*) captured on the Labrador Shelf.

Abstract

Porcupine crab (*Neolithodes grimaldii*) captured on the Labrador Shelf in NAFO Divison 0B were found to be infested with the rhizocephalan barnacle *Briarosaccus callosus.* Samples were collected over two consecutive years to determine prevalence and morphological effects on porcupine crab. The results have shown that porcupine crab in the two sites studied have a mean prevalence of 21% which is high compared 1-5% found in most open water king crabs. The prevalence was higher in females (34-46%) than males (18-19%) in both years of sample collection. Infestation by *B. callosus* was observed to have profound effects on the morphology of porcupine crab. For infested males the effects were reduction of chelae height, carapace length, and first and second merus length. It was also observed that the ventral flap of infested males was beginning to show segmentation similar to the larger segmented flaps of females. For females the effects appeared to be an increase in growth with a higher carapace length and lengthening of first and second merus of infested individuals. Negative reproductive effects of *B. callosus* on the porcupine crab were also observed. All infested females captured were in the non-ovigerous condition, and of those dissected 78% had empty ovary cavities with no eggs or oocytes internally. Of the infested males dissected 56% had atrophied testes or the testes were absent. The parasite externa was found to increase in size with host body length indicating initial infestation in the juvenile stages of host porcupine crab. The negative effects that *B. callosus* causes on morphology and reproduction as well as the high prevalence found in the porcupine crab highlight the potentially detrimental effects on local populations. Steps that could be taken to mitigate or reduce prevalence of *B. callosus* prevalence on porcupine crab are discussed.

2.1 Introduction

Rhizocephalan barnacles (Order Cirripedia) are common parasites of commercially important king crab species around the world (Meyers 1990; Walker 2001; Zaklan 2002). The rhizocephalan *Briarosaccus callosus* was first described by Boschma (1930) from a museum specimen of *Neolithodes agassizii* (Smith 1882) caught off the coast of North Carolina in 1886. It has since been recorded on 19 king crab species including the commercially important genera *Paralithodes, Lithodes,* and *Paralomis* (Table 1.1) (Meyers 1990; Pohle 1992b; Zaklan 2002). The parasite-host relationship of *B. callosus* has been most extensively studied in the North Pacific-Bering Sea region in red (*P. camtschatica*), blue (*Paralithodes platypus*) and golden king crabs (*Lithodes aequispina*), as well as in the Southern Atlantic-Sub-Antarctic region in the Falkland Islands (*Paralomis granulosa*) and Chilean (*Lithodes santolla*) king crab (Sloan 1984, 1985; Hawkes et al. 1986a,b, 1987; Hoggarth 1990; Lovrich et al. 2004; Cañete et al. 2008). The first recorded incidence of *B. callosus* on the porcupine crab (*N. grimaldii*) was from a single male crab captured in the Davis Strait in 1986 (Pohle 1992b). Although the occurance of *B. callosus* infestation on porcupine crab in the northeast Atlantic had been previously recorded, this sample only consisted of a single individual therefore little information about the morphological and reproductive effects or prevalence of *B. callosus* could be gleaned from this study (Pohle 1992b).

The host-parasite relationships of *B. callosus* are important to study due to their negative effects on reproduction of several commercially important king crab species (Meyers 1990; Walker 2001). The parasite *B. callosus* is comprised of two main parts: the interna which is a branching structure of roots that absorb nutrients from the host within the body cavity, and the reproductive portion, the externa, which protrudes outside the host and releases nauplii larvae into the water column through muscular contraction (Sparks & Morado 1986; Hoeg 1995). The interna is known to show a preference for connective tissue, therefore it usually penetrates such organs as the thoracic ganglion, brain, major nerves, and gastrointestinal tract (Sparks & Morado 1986). The hepatopancrease appears to always be penetrated and may provide the main source of nutrient absorption for the parasite (Sparks & Morado 1986). In male hosts *B. callosus* has been observed to destroy the androgenic gland shortly after infestation which is the gland responsible for producing the male hormones that trigger maturity (Veillet & Graf 1958).

The destruction of the androgenic gland by *B. callosus* causes remarkable morphological changes to the male host over time. This process has been termed feminization, as usually the male host will be prevented from attaining sexual or morphometric maturity and exhibit behaviours typical of egg bearing females (Sloan 1984, 1985; Hoeg 1995). Most king crab species including porcupine crab are sexually dimorphic with the males being larger than females. Morphological changes induced by *B. callosus* cause the males to undergo “feminization,” which includes reductions in growth such as significantly smaller right chela height, carapace length or width, and body weight compared with non-infested individuals (Sloan 1984; Hawkes et al. 1986a, 1987; Hoggarth 1990; Abello & Macpherson 1992; Hoeg 1995; Donaldson et al. 2005; Shukalyuk et al. 2005; Isaeva et al. 2005). Significantly smaller mean carapace lengths have been observed in infested *L. aequispina*, and *L. ferox* and significantly smaller carapace width has also been observed in *L. aequispina* (Sloan 1984; Abello and Macpherson 1992; Shukalyuk et al. 2005). In addition, mean height of the right chela was significantly smaller in infested *L. aequispina* males (Sloan 1984; Shukalyuk et al. 2005).

The allometric relationship of right chela height and carapace length is a common metric used to model growth at the morphometric molt to maturity in male decapod crustaceans (Jewett et al. 1985). This allometric relationship is usually presented as a linear regression with chela height on the y-axis and carapace length on the x-axis (Jewett et al. 1985; Hoggarth 1990). The differences in infested and non-infested organisms can be detected through the relationship between right chela height versus carapace length (Hoggarth 1990). A multiple regression comparison for infested and non-infested *P. granulosa* revealed that the slope of the right chela height-carapace length relationship for non-infested adult male crabs was significantly higher than 1) non-infested juveniles, 2) infested males with externa, and 3) infested males with scars from lost externa (Hoggarth 1990). Hoggarth (1990) also showed how maturity is affected by the parasite. Size at the molt to morphometric maturity was estimated by Somerton and Macintosh's (1983) method which provided a means of separating juvenile males from adult males with adult males exhibiting a larger right chelae relative to their body length than juveniles (Hoggarth 1990). Hoggarth (1990) demonstrated that *B. callosus* causes smaller right chela in relation to body size for male crabs by preventing the molt to morphometric maturity in *P. granulosa*. This morphometric change is likely to reduce productivity and therefore warrants investigation into whether similar effects are found in other commercial king crab species.

Studies of the growth of host crabs infested with *B. callosus* have been conducted based on field measurements and laboratory experiments (Hawkes et al. 1986a; Hawkes et al. 1987). Field measurements of *P. platypus* and *L. aequispina* were used to compare length-weight relationships between non-infested and infested male crabs (Hawkes et al. 1986a). This investigation revealed that weight at length was significantly smaller for infested males (Hawkes et al. 1986a). A laboratory study conducted to assess the growth of captive infested male *P. platypus* revealed that infested individuals have significantly smaller carapace growth increments between molts and also significantly smaller post-molt weight gains (Hawkes et al. 1987). The host's growth reduction was suggested to be caused by nutrient absorption from the parasite resulting in less energy available for growth (Hawkes et al. 1987). It was also observed that parasitized *P. platypus* were lethargic and did not eat as much or as often as uninfested hosts (Hawkes et al. 1987). Therefore the lethargy observed in *P. platypus* may be caused by nutrient absorption of *B. callosus* which may be a key factor in growth reductions in other infested king crab.

Negative reproductive effects caused by the interna of *B. callosus* have been recorded in many species (Sloan 1984; O'Brien and Van Wyk 1985; Hoggarth 1990; Isaeva et al. 2005; Shukalyuk 2005). The typical effects are sterilization of the male and female host caused by *B. callosus* which has been termed "parasitic castration" by Baudoin (1975) and Somerton (1981). For female hosts the reproductive effects are commonly expressed externally as most authors have observed all infested females to be in the non-ovigerous condition (Somerton 1981; Sloan 1985; Hawkes et al. 1985b; Campodonico et al. 1983; Hoggarth 1990). It has been observed by histological sections of internal organs that sterilization or degradation of female reproductive system may occur independent of actual penetration of the gonad by the roots (i.e. interna) of the parasite (Sparks and Morado 1986). Therefore, similar to the effects observed for growth, perhaps not enough energy is available to the host after nutrient absorption to allocate energy to reproduction. Infested females are likely permanently sterilized and therefore reduce the reproductive potential of the population with each additional infestation (Hoggarth 1990).

In male hosts, *B. callosus* has been recorded to destroy the androgenic gland and penetrate connective tissues but the reproductive effects of *B. callosus* are not as easily observable in males as they are for females. Presence of degraded testes with normal spermatozoa in the ducts have been observed and also conversely the complete absence of testis (Hawkes et al. 1985b; Sparks and Morado 1986). However, the spermatozoa observed may be remnants left over prior to infestation (Hoggarth 1990; Hoeg and Lutzen 1995). Histological investigations have noted that the testes of male *P. platypus* were atrophied by the root system (interna) of *B. callosus (*Hawkes et al. 1985b). The effects of *B. callosus* interna are different for female hosts as the cavity the ovary occupies is usually empty in infested individuals independent of actual penetration of the gonad by the interna. It is clear that *B. callosus* has negative effects on male hosts similar to female hosts but the extent of which may be dependent on the time of infestation and the extent of interna growth in the body cavity.

Several attempts have been made to quantify the extent of rhizocephalan parasite infestation. The most common method used is prevalence, which is characterized by the percentage of the total number of individuals sampled that are infested (Margolis et al. 1982). The prevalence of *B. callosus* varies depending on several factors. Size of the host has been suggested as a main factor influencing prevalence rates as usually larger externae are found on larger hosts (Bower and Sloan 1985). This effect has been observed and quantified on the golden king crab which suggests the larger externae will produce more larvae than smaller ones thus increasing prevalence (Bower and Sloan 1985). An infested porcupine crab collected by Pohle (1992a, b) had a large externa with a curve length (as per Bower and Sloan 1985) that exceeded the carapace length of the host and was substantially larger (4-5x) than externa found infesting *Paralomis bouvieri* in the northwest Atlantic.

In most cases, host crabs are believed to be parasitized early in life with prevalence inceasing with an increase in body size (Watters 1998). Prevalence usually follows a normal distribution illustrating an initial increase with an increase in body size followed by a reduction at the largest sizes (Sloan 1984; Watters 1998). For the host *Paralomis granulosa* it was observed that females had significantly higher prevalence of externae in smaller size classes, and higher prevalence of scarred individuals in larger size classes (Hoggarth 1990). One explanation

Hoggarth (1990) suggested for observed decreases in prevalence at the largest size classes is loss of externa, because the longer a host has a parasite attached to its ventral surface the greater the likelihood for it to be torn off. Another explanation for this decrease in prevalence in the larger size classes is that infestation is known to decrease growth rates such that one is more likely to sample infested crabs in smaller size classes than larger ones (Hawkes et al. 1987; Hoggarth 1990). Despite the evidence that prevalence is lower in larger size classes in some cases it is unclear whether infestation leads to an increase in mortality in larger size classes.

Habitat has been suggested as another main factor affecting prevalence of *B. callosus* in the host population. It has been suggested that more easily disturbed substrates such as silty bottom habitats may reduce gill cleaning efficiency and increase the likelihood of being infested by a *B. callosus* cyprid larva (Ritchie and Hoeg 1981; Hawkes et al. 1986a,b; Pohle 1989; Fleischer et al. 1992). In addition, Hawkes et al. (1986b) found that prevalence of parasites tends to be greater in turbid rather than clear water for red, blue, and golden king crab.

Oceanographic isolation is another habitat feature that may affect prevalence. For example, prevalence has been observed to be higher in enclosed areas such as bays and fjords compared to open water areas. Prevalence in the golden king crab (*L. aequispina)* was found to vary from 3% in open water to 41% in fjords in northern British Columbia (Sloan 1984) and 20% in fjords near Alaska (Hawkes et al. 1986b). In *P*. platypus, prevalence of up to 76% has been recorded in Alaskan fjords while prevalence was zero for large areas across the open ocean in the Pacific (Hawkes et al. 1985b, 1986b; Sparks and Morado 1986) (Table 2.1). This observation is similar to most other king crab species occupying open ocean habitats where 1-5% prevalence has been observed (Arnaud and Do-Chi 1977; Somerton 1981; Hawkes et al. 1986b; Hoggarth 1990; Abello and Macpherson 1992). Prevalence is believed to be higher in enclosed areas because of confinement of larval parasites resulting from weaker currents relative to open waters (Sloan 1984).

Some authors believe that the depth and population density of a species can cause local differences in prevalence (Sloan 1984; Hawkes et al. 1986b). Slizkin and Safronov (2000) has suggested that the highest prevalence is likely to occur in the deepest king crab species such as *Paralomis multispina, P. verrilli, Lithodes couesi, L. aequispina and Paralithodes platypus*. In a field study by Sloan (1984) prevalence was compared with catch per unit effort (CPUE) for golden king crab captured in traps and it was found that low CPUE (3 crabs per trap) had low prevalence (3%) and high CPUE (24 crabs per trap) had high prevalence (44%).

When *B. callosus* prevalence is compared by host sex ratio some species exhibit differences in prevalence between male and females, while others the prevalences are relatively equal for both sexes. For example, *B. callosus* prevalence was higher in male *P. spinosissima* and *L. aequispina* (Otto & Macintosh 1996; Hawkes et al. 1986b; Watters 1998), higher in females in *P. platypus* (Hawkes et al. 1985b, 1986b), and equal in *P. camtschatica, P. granulosa, and L. ferox* (Hawkes et al. 1986b; Hoggarth 1990; Abello & Macpherson 1992). Sloan (1984) noticed that the golden king crab had a 1:1 sex ratio prevalence overall when all years of data were combined, however, when the years are viewed separately, the first year of sampling had higher parasite prevalence in females and the second year higher prevalence in males. There may be behavioural aspects that affect sex ratio differences in different hosts, but the current evidence shows that the number of samples collected, when they are collected (season), and where they are collected can be crucial factors.

Reproductive sterility caused by *B. callosus* highlights the importance of preventing the spread of this parasite. Several important king crab fisheries worldwide are of great economic value and are hosts to *B. callosus* such as *P. camtschatica*, *P. granulosa,* and *L. aequispina*. To prevent increases in levels of infestation, several authors have suggested destroying all infested individuals regardless of sex or size (Sloan 1984, 1985; Isaeva et al. 2005; Shukalyuk et al. 2005). These preventative measures have been suggested based on the biology of the parasite. For example, a single externa is known to release 310-390 thousand larvae (Meyers 1990). These larvae are planktonic and will remain in the water column for weeks to months making extirpation or reduction of *B. callosus* very difficult (Hoeg & Lutzen 1995).

Prevalence of *B. callosus* infesting *L. aequispina* in the East Russian Sea of Okhosk has been observed to increase from 3% to 5% in males and from 5.7% to 11.4% in females in two years. This increase is due to discarding of infested individuals back into the ocean and retention of healthy individuals during the commercial fishery for *L. aequispina* (Selin 1998; Isaeva et al. 2005).

It is clear from this evidence that retention or destruction of infested individuals is important in areas where commercially exploitable species are found. With regards to human consumption of infested crabs, Meyers (1990) suggests that they should be marketed the same as non-infested crabs as the parasite does not negatively affect meat quality.

Histological analysis has shown that removal of the externa leads to the degradation of the interna (Sparks and Morado 1986). Degradation of the interna could be caused by a triggering of the normal autoimmune response as hosts with degraded interna were observed to be melanized and encapsulated (Sparks and Morado 1986). Isaeva et al. (2005) has suggested that *B.callosus* could have the potential of budding asexually similar to other rhizocephalan species (*P. reticulatus)* in the same family (Peltrogastridae) after externa removal. This asexual budding contradicts the findings of Sparks and Morado (1986) who suggest degradation of interna after externa removal. However, unless several hosts with removed externa were held in captivity for an extended period of time and a careful histological investigation carried out it cannot be ruled out as a possibility (Isaeva et al. 2005). The observation of multiple parasitism of *B. callosus* externa on a single host has been found in several king crab species (Sloan 1984). Several authors have suggested that the multiple parasitism results from several larvae infesting the same host as opposed to asexual budding of the interna (Sloan 1984).

Kuris (1974) has suggested the overall effect on the host of a single or multiple infestation is the same, reproductive sterility. Although the effect on a population within a stock may be the same for a single or multiple externa (one lost individual), the effect on prevalence may be higher for multiple externae as they may lead to more larvae being released compared to a single externa. The effect of multiple externa producing more larvae cumulatively than a single externa has yet to be studied in a laboratory setting. Due to externae's potential to spread larvae, and the observed increases in prevalence caused by discarding infested individuals (Selin 1998; Isaeva et al. 2005), the complete destruction or prevented discard of the infested host is suggested to be the best solution to reduce infestation levels in commercial stocks of king crab (Isaeva et al. 2005; Shukalyuk et al. 2005; Sloan 1984). The current study aims to secure an adequate sample size and sampling distribution to elucidate details about the effects on the host caused by *B. callosus*.

2.2 Materials and Methods

2.2.1 Sample locations

Samples were collected during the commercial fishery for Greenland halibut (*Reinhardtius hippoglossoides)* on the Labrador Shelf at 62'-63' N and 60'-61' W from 15 June to 6 July 2014 and 62'-63' N and 58'-59' W from 2-21 June 2015 (Figure 2.1). The porcupine crab were captured by means of commercial monofilament nylon gillnet (length = 91.4m, height = 25 meshes, mesh size = 191mm, twine diameter = 0.6mm, hang ratio = 0.5). Gillnet sets consisted of 50 nets in a string that soaked for 5-8 days before retrieval. Crabs were captured at depths 1,002-1,216 m in 2014 and 907-1,220 m in 2015.

2.2.2 Porcupine crab removal from gillnet, measurement, and preservation

Porcupine crab were removed from gillnets by rolling and spreading the monofilament across a sorting table to reduce the amount of damage to the crab and to prevent loss or removal of the externa of *B. callosus*. Morphological measurements of porcupine crab were then taken by means of Vernier calipers (±1.0 mm). The morphological measurements recorded were as follows: carapace length, carapace width, right chela height, right first and second merus length. Carapace length was measured from the right orbit to the greatest point on the right posterior lobe of the carapace (Figure 2.2). Carapace width was measured from the widest point on the left and right side of the carapace (Figure 2.2). Chelae height was measured vertically on the largest section of the chela (Figure 2.3). First and second merus were measured from the joint at the distal end to the base of the coxa at the proximal end (Figure 2.2). Weight (± 10 g) was also taken for fully intact crab. Each crab was examined for sex, presence of eggs or spent egg cases (furniculi) on pleopods, and presence of rhizocephalan parasite externa. Anteroposterior length (AP length) and height (Bower and Sloan 1985) of parasite externa was also recorded with Digital calipers (±1.0 mm) before and after preservation in 10% buffered formalin solution.

Once measurements were recorded, porcupine crab were either dissected onboard to determine presence of gonads, or preserved in 10% buffered formalin to be dissected in the laboratory on land. When preserved crabs arrived at the laboratory the males were dissected to examine for presence of atrophied or degraded testis and the females were dissected to check for the presence of ovaries with un-extruded eggs.

Parasite externa were also dissected and examined for presence of eggs in the mantle, hyperparasites, and presence of a frill at the mantle opening as shown in Figure 2.4. The recorded physical data from all measurements was digitized onto Microsoft excel and analyzed using SPSS (version 22).

2.2.3 Statistical procedures

The response of chela height (Y) of male porcupine crabs to infestation by the rhizocephalan barnacle (*Briarosaccus callosus*) was analyzed by analysis of covariance (ANCOVA) where carapace length was used as a covariate (X) since chela height varied with the length of the carapace. The response variable (Y) and the covariate were log(10) transformed for the analysis and years 2014 and 2015 were examined separately. ANCOVA was also used to examine the influence of *B. callosus* infestation on the first (M1) and second (M2) merus length separately in male and female crabs again with carapace length as a covariate. The log transformed values used for the model calculations were back transformed to display the relationship graphically. Because sample sizes were different and had different variances I used the unequal variance Welch's t-test to compare mean values of morphometric parameters measured (Welch 1947). To compare prevalence of *B. callosus* infestation between males and females a chi-square test was performed. All statistical procedures were executed in IBM SPSS Statistics (version 22).

2.3 Results

2.3.1 Size class distributions

In total 2889 porcupine crab were collected and analyzed, 1307 of these were captured in 2014, and 1582 in 2015. Of all the crabs collected males dominated (79%) the samples from both years (Table 2.2). There was a sex ratio was 3.7:1 with males outnumbering females. The male crabs captured exhibited larger mean carapace length (CL) than females in both 2014 (t_{1271} = 12.208, p <0.001) and 2015 (t_{1564} = 11.327, p <0.001). Mean sizes of carapace length for both years and sexes in addition to maximum/minimum carapace lengths can be found in table 2.2. Size distributions of male and female porcupine crab are displayed for 2014 (Figure 2.5) and 2015 (Figure 2.6). Size distributions of infested males and females are displayed for 2014 (Figure 2.7) and 2015 (Figure 2.8).

2.3.2 Prevalence of rhizocephlan parasite *B. callosus* on porcupine crab

The overall mean prevalence of crabs of both sexes infested with *B. callosus* for the sample over both years was 21%. The prevalence was similar for male porcupine crab in both 2014 (17.5%) and 2015 (18.5%). The prevalence was higher for females than males in both years with values of 34.1% for 2014 and 46.6% for 2015. The chi-squared test revealed significant values when testing for an association between prevalence and sex in 2014 (λ_1 = 33.617, p<0.001) and 2015 (λ_1 = 108.829, p<0.001). Therefore the null hypothesis that there is no association is rejected and we accept the alternative hypothesis that prevalence is dependent on sex. Since female prevalence was higher than male in both years we can conclude that female prevalence is significantly higher than males for both years. The largest infested female captured in year one was 142 mm carapace length while the largest non-infested female was 132mm (Table 2.2). Similarly in year two the largest infested female captured was 159 mm carapace length while the largest non-infested female captured was 137 mm (Table 2.2). The opposite trend was observed for male crabs as the largest infested male captured in year one was 141 mm carapace length while the largest non-infested male was 173mm (Table 2.2). Similarly, in year two the largest infested male captured was 148 mm carapace length while the largest non-infested male captured was 167 mm (Table 2.2).

The size class with the highest prevalence was between 91 and 100 mm in males (Figure 2.9). For females the size classes past 121 mm were exceptionally high with the highest being 141-150 mm and 151-160 mm with 100% prevalence (Figure 2.9). Porcupine crab were sometimes found with multiple externa attached to one host. Over both years 34 individuals were found with multiple externa on one host with up to 4 externa per host in females and up to 3 externa per host in males (Table 2.3).

2.3.3 Morphology

Infested males were significantly smaller than non-infested individuals for mean values of carapace length, chela height, merus 1, and merus 2 (Table 2.4).

Infested females were significantly smaller than non-infested individuals for mean values of carapace length, chela height, merus 1, and merus 2 (Table 2.5).

Scatterplots of the right chela height-carapace length relationships for parasitized and non-parasitized male porcupine crab indicate different curvilinear relationships for 2014 and 2015 (Figure 2.10). Results from the ANCOVA on log transformed variables indicated that slopes of the infected and non-infected relationships in 2014 were significantly different ($F = 79.25$, $P < 0.0001$) with growth of the right chela being greater in non-infected male crabs. Similar results were found in male crabs in 2015 ($F = 205.88$, $P < 0.0001$).

Scatterplots of merus length of the first (M1) and second (M2) walking leg versus carapace length also illustrate different relationships of infected and non-infected crabs both for males and females (Figure 2.11 and 2.12). For males, the slopes of the regression lines differed between infected and non-infected crabs both for M1 ($F = 122.04$, $P < 0.0001$) and M2 ($F = 94.16$, $P < 0.0001$). Similar to chela height, merus length of non-infected crabs increased faster than infected organisms.

In female crabs, merus length of the first and second walking leg also differed between infected and non-infected crabs both for M1 ($F = 14.6$, $P = 0.0002$) and M2 ($F = 15.9$, $P < 0.0001$). However, the situation for female crabs differed from that of male crabs. For both M1 and M2, growth of infected crabs was faster than that of non-infected organisms.

It was observed that the ventral flap of the infested males were in various stages of segmentation with some more pronounced than others. A large segmented ventral flap used to house developing eggs is the normal condition of females but not non-infested males. Non-infested males have small ventral flaps with no segmentation as they do not house developing eggs. A qualitative display of ventral flaps of infested males in various stages of increasing segmentation is provided alongside the normal female condition (Figure 2.13).

2.3.4 Internal dissections and externa

It was observed during removal of the externa that younger externa had a brown cuticular shield with no visible growth rings and a frill around the mantle opening of the externa. The young externa are shown in pane 1 of Figure 2.4 with the mantle opening indicated by a black arrow and the cuticular shield indicated by a white arrow. Conversely, older externa were observed to have many growth rings on the cuticular shield and the mantle opening had become invaginated due to successive molts of the mantle. The older externa are shown in pane 2 of

Figure 2.4 with the mantle opening indicated by a black arrow and the cuticular shield indicated with a white arrow.

Of the 73 male crabs that were found to have completely atrophied testes, 70 possessed older externa with invaginations, and 3 were young with frilled openings (Table 2.6). For the 88 male crabs with older externa 80% had atrophied testes. For the 42 male crabs with younger externa 7% had atrophied testes. Porcupine crab with invaginated externa have a higher proportion of atrophied testes which indicates that externa with frilled openings are not as well developed internally.

During both years of field collection it was observed that the parasite *B. callosus* had negative effects on reproduction for female porcupine crab. This effect was the absence of eggs or empty egg cases (furniculi) on the pleopods of females of adequate size to be sexually mature and mating. These observations were followed up with internal dissections of the reproductive organs in 2015 (Table 2.6).

Porcupine crab were found in all shell conditions (soft, old hard shell, new hard shell) with externa most importantly found on post-molt soft shelled condition hosts. This indicates that externa are not lost when the host molts. The cuticular shield on the externa accretes rings with each successive molt of the externa mantle as they are not lost during host molting. Under microscope it was clear that many externa of *B. callosus* on porcupine crab had many rings on the cuticular shield indicating the externa stay attached to the host for multiple molt cycles.

A simple linear regression was performed on the externa measured to observe if there was a significant relationship between externa AP length and host carapace length. Externa was found to positively correlate with the host carapace length producing a regression line with a slope of 0.6164 and a correlation coefficient of 0.4679 (Figure 2.14). The t-statistic for the slope was significant at the .05 critical alpha level (t_{441} = 19.692, p <0.001). Therefore the null hypothesis is rejected and I conclude that there was a positive significant relationship between externa AP length and host carapace length.

2.4 Discussion

This study found high prevalence of *B. callosus* infestation in the porcupine crab sampled on the Northern Labrador Shelf. This study also revealed the negative effects of *B. callosus* on the reproductive organs of male and female porcupine crab, and negative effects on the growth of male porcupine crab. There appeared to be opposite effects on the growth of male and female porcupine crab, with parasitized males exhibiting a reduction in maximum size while parasitized females appeared to grow to a larger maximum size than non-parasitized individuals.

There was a larger proportion of males captured during the course of the study compared to females. The dominance of male crabs in both years (4:1 male: female) suggests that behavioural or biological factors may affect the composition of porcupine crab catches in bottom gillnets. Porcupine crab exhibited sexual dimorphism with males attaining a greater carapace length; their larger size may lead to increased activity and greater likelihood of being captured in bottom gillnets than females as suggested by He (2005). A behavioral aspect known as podding may also have led to the sexual bias of porcupine crab in bottom gillnet captures (Dew 1990). Podding consists of large numbers of crab individuals coming together in larger swarms either daily or seasonally for feeding, protection, or reproduction. Podding aggregations can be largely or entirely comprised of female individuals (Dew 1990). Porcupine crab captured in this study were not captured in large groups but the sex ratios found may indicate this behaviour prior to capture.

The prevalence of *B. callosus* observed in the porcupine crab samples in this study were high for an open water king crab. Most open water king crab species found in the literature parasitized by *B. callosus* have prevalences that range from 1-5% compared to the prevalence observed in this study of 21%. Some proposed reasons behind the high level of infestation observed are: 1) interactions with the gillnet fishery for Greenland halibut, 2) unobserved aspects of the porcupine crabs behaviour that facilitate infestation, 3) deep water residency with potentially low current strength, or 4) parasite larval release matching reproduction of host when most likely to be aggregated.

With regard to effects of interaction with the Greenland halibut gillnet fishery, since there is no directed fishery for porcupine crab the possibility of selective retention or mortality of non-infested individuals leading to an increase in prevalence appears to be low. This scenario is different than the results found for *L. aequispina* in the East Russian Sea of Okhosk where prevalence increased from 3% to 5% in males and from 5.7% to 11.4% in females in two years apparently due to discarding of infested individuals and retention of non-infested individuals in the commercial fishery (Selin 1998; Isaeva et al. 2005). Porcupine crab have historically been captured as bycatch in the gillnet fishery for Greenland halibut but whether this may have had an effect on the parasite prevalence observed today is unknown. One possible scenario for a local increase in prevalence may be related to difference in survival rate of small and large crab during the removal process. Larger adult crab are more difficult to remove and are more likely to be destroyed. The release of small or juvenile infested porcupine crab may be gradual on a gillnet set basis. However, over the course of the fishery the incremental increase in small or juvenile infested individuals and the destruction of larger non-infested individuals may result in reduced competition for resources and contribute to the high prevalence rates observed in NAFO 0B porcupine crab.

The results presented in this study suggest that porcupine crab are infested early in the life cycle as Hoggarth (1990) suggested for *Paralomis granulosa*. This conclusion is based on the relationship between *B. callosus* externa length and host porcupine crab carapace length (Figure 2.14). Poltev (2008) found the same result for *Paralomis verilli* when carapace width of the host crab was compared to externa AP length of *B. callosus*. The size of the externa was also observed to be influenced by presence or absence of a frill around the mantle opening and also the presence or absence of hyperparasites on the externa (Figure 2.4).

The prevalences of *B. callous* were higher in females than males for both sample years which suggests a difference in the chance of infestation or survival between sexes. This result is similar to Sloan (1984) who found that females had higher prevalence and it was proposed that their migration to deeper enclosed areas such as fjords to spawn may contribute to high prevalence. Gregarious behaviour like podding in females could explain the high rates of infestation found in porcupine crab and the differences in prevalence between sexes. Given that infestation occurs in the juvenile stage of the porcupine crab life cycle then it would appear females begin to segregate from males in the juvenile stage. In addition, if ocean current strengths are lower in the deep water areas that porcupine crab are captured (>800m) an oceanographic isolation effect may exist similar to the fjord confinement of larvae observed by Sloan (1984).

Morphological differences between sexes could also play a role in prevalence as large crabs are potentially more mobile than smaller crabs and males are on average larger than females. If larger males are more mobile and smaller females are less mobile this could explain observing small pockets of increased infestation due to the reduction in mobility of females and also gregariousness for protection during feeding or mating periods. The result of observing multiple externa on a single host with up to four externa on a female host suggests there may be some confinement of parasite larvae present possibly caused by reduced mobility or aggregation of female hosts.

It has been observed for red king crab that juveniles routinely pod together for protection daily during feeding periods (Dew 1990). If podding behaviour is present in porcupine crab it would explain an increase in prevalence of infestation at smaller size classes, and this behaviour could make them more vulnerable to parasite confinement or cross infestation between individuals. This would also coincide with the trend observed in externa morphology as individuals parasitized early would have time to grow and lead to large externa being found on larger hosts.

The size reduction, or feminization, observed in male porcupine crab is consistent with the previous studies carried out on other king crabs infested with *B. callosus* (Sloan 1984; Hawkes et al. 1986a, 1987; Hoggarth 1990; Abello & Macpherson 1992; Shukalyuk et al. 2005; Isaeva et al. 2005). Reductions in body size of male porcupine crab are important to note but the most significant morphological reduction observed is that of the right chela. The absence of an increase in the size of the right chela is an important as it reduce a male porcupine crab's ability to grasp and compete for females to reproduce. A typical allometric growth trajectory for a non-infested male king crab reaching morphometric maturity would be an increase in size of the right chela relative to carapace length. The absence of this allometric growth in infested crabs suggests that crabs infested early in the life cycle may prevent the crabs from reaching sexual maturity, preventing growth to morphometric maturity. For crabs infested later in life the infestation of *B.*

callosus may cause a reduction in post-molt size gains and prevent any further increases in right chela height although it is most likely that hosts are infested early in life (Hoggarth 1990).

An additional morphological change that was observed in infested male porcupine crab was the changes to the ventral flap or abdomen. Infested male porcupine crab exhibited a larger ventral flap than non-infested male crabs. The ventral flap on infested male crabs was often segmented which is a feature typical of female crabs (Figure 2.13). The externae occurred under the abdomen which made quantitative measurements very different from host to host and thus comparisons of the abdomen were not carried out during this study. In a study conducted on green crab (*Carcinus maenas)* infested with the rhizocephalan *Sacculina carcini* it was observed and quantified that infested males had broader, larger, and sometimes segmented abdomens when compared to non-infested males (Kristensen et al. 2012). Although quantitative comparisons were not conducted in this study the morphologic changes discussed were observed qualitatively ie. Figure 2.13.

The effects that *B. callosus* had on the growth of female porcupine crab were interesting because instead of size reduction as seen in male crabs, the infestation by *B. callosus* appeared to result in an increase in the maximum size of female crabs. This result is different than the effects of *B.callosus* on *L. aequispina* as recorded by Sloan (1984) who found no influence on female body size. Not only were mean values of morphometric parameters (carapace length, right chela height, merus 1 and merus 2 length) significantly greater for infested female porcupine crab, but the largest females collected in 2014 and 2015 were infested.

A suggested cause for this increase in growth for infested females may be due to the sterilization effects of *B. callosus*. Since the production of ovaries with yolk filled eggs is inherently costly an absence of this energetic expenditure would leave more energy available for somatic growth. Although the parasite may absorb a portion of the energy from the host for its own growth, the wealth of energy available to infested females in lieu of prevented reproduction may cause an increase in somatic growth relative to non-infested females.

The effects that *B.* callosus have on the porcupine crab are significant in the context of developing an emerging porcupine crab fishery. A potential fishery would set size limits of harvestable crabs. This would leave fisheries management with the decision of what to do with infested crabs. One scenario could be the harvest of all infested individuals regardless of sex and size ignoring the set size limits for harvest as suggested by Meyers (1990) and Hawkes et al. (1986). Provided that infestation does not negatively affect the meat quality of porcupine crab this could be a viable option to increase catches while decreasing local prevalence of *B. callosus*. This scenario makes intuitive sense especially in the case of infested females as those found in this study were of larger size. A second scenario could be the destruction or retention of all infested individuals upon capture (Sloan 1984, 1985; Isaeva et al. 2005; Shukalyuk et al. 2005). If infested individuals are unmarketable this may be the best decision as it would reduce competition for resources with uninfested crabs. The destruction and discarding of infested individuals may prove to be much easier than retention from an industry point of view. However, any attempts to mitigate *B. callosus* infestation in these local stocks may provide an increase in productivity of the porcupine crab resource and therefore potential economic opportunities in the future.

From the dissections of infested porcupine crab it became clear that there are negative effects on reproduction in males and females. For males the effects appeared to vary possibly depending on the duration of time since infestation began or the extent of interna growth. The observations that the cuticular shield is brown and the mantle opening is frilled in early externa supports the theory that the duration of infestation was an indicator of whether the testis would be degraded but present, or completely atrophied. As was previously discussed it was also observed that larger externae were found on larger hosts similar to the findings of Bower and Sloan (1985) and Poltev (2008) (Figure 2.14). This supports the theory that infestations generally occur early in the life cycle (i.e. juvenile stage) and that the growth of the interna increases with time causing increasing spread throughout the tissues of the body cavity.

The absence of eggs on the pleopods of females of adequate size to be sexually mature (i.e. 80mm or greater as per Hurley et al. 2016) in 2014 and 2015 was indicative of a negative reproductive effect (Hurley et al. 2016). The external indicators of sterilization in female porcupine crab were reinforced by the internal dissections completed later in the laboratory. The dissection of females with no eggs on the pleopods revealing an absence of ripe ovarian tissue where it should reside in the majority of those dissected (80%) indicates they were undoubtedly sterilized by the infestation of *B. callosus*. Since the prevalence of infestation was high for females for both years sampled, these results indicate that *B. callosus* reduces the reproductive potential of the local NAFO 0B stock of porcupine crab.

The main objective of this study was to investigate the prevalence and effects of *B. callosus* on porcupine crab. High prevalence in porcupine crab and the observation of clear effects on reproduction and morphology have satisfied this objective. This study was limited to depths of ~1200 m and porcupine crab are known to occur to depths of 1500 m and are possibly more abundant at these depths. The limitations of collection depth and the size of the area sampled may result in a recorded prevalence that may not be representative of the entire population. The potential negative effects on the productivity of the local porcupine crab population sampled in this study highlights the need for continued research of porcupine crab in other areas. Adoption of some of the potential strategies to control parasite prevalence and continued monitoring will lead to the best conservation of the porcupine crab populations in NAFO 0B for the future.

2.5 References

Abello, P., & Macpherson, E. (1992). Epibiosis and Rhizocephalan infestation patterns in relation to the reproductive biology of *Lithodes ferox* (Filhol, 1885) (Anomura: Lithodidae). *Journal of Crustacean Biology*, *12*(4), 561–570.

Arnaud P.M., Do-Chi, T. (1977) Données biologiques et biométriques sur les lithodes *Lithodes murrayi* (Crustacea: Decapoda: Anomura) des îles Crozet (SW océan Indien). Mar Biol 39:147–159.

Baudoin, M. (1975). Host Castration as a Parasitic Strategy. *Evolution*, *29*(2), 335–352.

Boschma, H. (1930). *Briarosaccus callosus*, a new genus and new species of rhizocephalan parasite of *Lithodes agassizii* Smith. Proc. U.S. Natl. Mus. 76:1-8.

Boschma, H., and E. Haynes. (1969). Occurrence of the rhizocephalan *Briarosaccus callosus* Boschma in the king crab *Paralithodes camtschatica* (Tilesius) in the Northeast Pacific Ocean. Crustaceana 16:97-98.

Bower, S. M., & Sloan, N. A. (1985). Morphology of the externa of *Briarosaccus callosus* Boschma (Rhizocephala) and the relationship with its host *Lithodes aequispina* Benedict (Anomura). *The Journal of Parasitology*, *71*(4), 455–463.

Bungay, T., Grant, S.M., Winger, P., and Moret, K. (2014). Mapping catch statistics for porcupine crab (*Neolithodes grimaldii*) in the Northwest Atlantic. Centre for Sustainable Aquatic Resources, Marine Institute of Memorial University of Newfoundland. Technical Report P-431: 25 p.

Campodonico, I., M. B. Hernandez & E. Riveros. (1983). Investigacion, Manejo y Control de las pesquerias de centolla y centollon de la XII Region. Informe Consolidado: Recurso centollon. Informe Instituto Patagonia, 25: 1-97

Cañete, J. I., Cárdenas, C. A., Oyarzún, S., Plana, J., Palacios, M., & Santana, M. (2008). *Pseudione tuberculata* Richardson, 1904 (Isopoda: Bopyridae): a parasite of juveniles of the king crab *Lithodes santolla* (Molina, 1782) (Anomura: Lithodidae) in the Magellan Strait, Chile. *Revista de Biologia Marina Y Oceanografia*, *43*(2), 265–274.

Dew, C. B. (1990). Behavioral Ecology of Podding Red King Crab, *Paralithodes camtschatica*. *Can. J. Fish. Aquat. Sci.*, *47*, 1944–1958.

Donaldson, W. E., Byersdorfer, S. C., National Sea Grant Office (U.S.), & Alaska Sea Grant College Program. (2005). *Biological field techniques for lithodid crabs*. Fairbanks, Alaska: University of Alaska Fairbanks, Alaska Sea Grant College Program.

Fleischer, J., Grell, M., Hoeg, J. T., & Olesen, J. (1992). Morphology of grooming limbs in species of Petrolis and Pachycheles (Crustacea: Decapoda: Anomura: Porcellanidae): a scanning electron microscopy study. *Marine Biology*, *435*, 425–435.

Hawkes, C. R., Meyers, T. R., & Shirley, T. C. (1985a). Parasitism of the Blue King crab, *Paralithodes platypus*, by the Rhizocephalan, *Briarosaccus callosus*. *Journal of Invertebrate Pathology*, *45*, 252–253.

Hawkes, C.R., Meyers, T., & Shirley, T. C. (1985b). Larval biology of *Briarosaccus callosus* Boschma (Cirripedia: Rhizocephala). *Proceedings of the Biological Society of Washington*, *98*(4), 935–944.

Hawkes, C. R., Meyers, T. R., & Shirley, T. C. (1986a). Length-weight relationships of blue, *Paralithodes platypus*, and golden, *Lithodes aequispina*, king crabs parasitized by the rhizocephalan, *Briarosaccus callosus* Boschma. *Fishery Bulletin*, *84*, 327–332.

Hawkes, C. R., Meyers, T. R., & Shirley, T. C. (1986b). Prevalence of the Parasitic Barnacle *Briarosaccus callosus* on King Crabs of Southeastern Alaska. *Transactions of the American Fisheries Society*, *115*, 252–257.

Hawkes, C. R., Meyers, T. R., & Shirley, T. C. (1987). Growth of Alaskan Blue King Crabs, *Paralithodes platypus* (Brandt), Parasitized by the Rhizocephalan *Briarosaccus callosus* Boschma. *Crustaceana*, *52*(1), 78–84.

He, P. (2005). Characteristics of bycatch of porcupine crabs, *Neolithodes grimaldii* (Milne-Edwards and Bouvier, 1894) from deepwater turbot gillnets in the northwest Atlantic. Fisheries Research, 74, 35–43.

Hoeg, J. T. (1995). The biology and life cycle of Rhizocephala (Cirripedia). *Journal of the Marine Biological Association of the United Kingdom*, *75*, 517–550.

Hoeg, J. T., & Lützen, J. (1995). Life cycle and reproduction in the Cirripedia Rhizocephala. *Oceanography and Marine Biology: An Annual Review*, *33*, 427–485.

Hoggarth, D. D. (1990). The Effects of Parasitism by the Rhizocephalan, *Briarosaccus callosus* Boschma on the Lithodid Crab, *Paralomis granulosa* (Jacquinot) in the Falkland Islands. *Crustaceana*, *59*(2), 156–170.

Hurley, M., Furey, G., and Grant, S. (2016) Masters Thesis. Memorial University of Newfoundland.

Isaeva, V. V., Dolganov, S. M., & Shukalyuk, a. I. (2005). Rhizocephalan Barnacles—Parasites of Commercially Important Crabs and Other Decapods. *Russian Journal of Marine Biology*, *31*(4), 215–220.

Jewett, S. C., Sloan, N. A., & Somerton, D. A. (1985). Size at Sexual Maturity and Fecundity of the Fjord-Dwelling Golden King Crab *Lithodes aequispina* Benedict from Northern British Columbia. *Journal of Crustacean Biology, 5*(3), 377–385.

Kristensen, T., Nielsen, A. I., Jørgensen, A. I., Mouritsen, K. N., Glenner, H., Christensen, J. T., Høeg, J. T. (2012). The selective advantage of host feminization: A case study of the green crab *Carcinus maenas* and the parasitic barnacle *Sacculina carcini*. *Marine Biology*, *159*(9), 2015–2023.

Kuris, A. M. (1974). Trophic interactions: similarity of parasitic castrators to parasitoids. Q. Rev. Biol. 49:129-148.

Lovrich, G. A., Roccatagliata, D., & Peresan, L. (2004). Hyperparasitism of the cryptoniscid isopod *Liriopsis pygmaea* on the lithodid *Paralomis granulosa* from the. *Diseases of Aquatic Organisms*, *58*, 71–77.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M., & Schad, G. (1982). The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). The Journal of Parasitology, 68(1), 131-133.

McMullen, J.C., and H.T. Yoshihara. (1970). An incidence of parasitism of deep-waterking crab, Lithodes aequispina, by the barnacle Briarosaccus callosus. J. Fish.Res. Board Can. 27:818-821.

Meyers, T. R. (1990). Diseases of Crustacea. Diseases caused by protistans and metazoans. *Diseases of Marine Animals*, *3*, 350–389.

O'Brien, J. and Van Wyk, P. (1985). Effects of crustacean parasitic castrators (Epicaridean isopods and Rhizocephalan barnacles) on growth of crustacean hosts. p. 191-218. In: A.M. Wenner (ed) Factors in adult growth. Crustacean Issues, Vol. 3. Rotterdam, A.A. Balkema

Otto, R.S., and R.A. MacIntosh. (1996). Observations on the biology of lithodid crab *Paralomis spinosissima* from the southern ocean near South Georgia. In: High latitude crabs: Biology, management, and economics. University of Alaska Sea Grant, AK-SG-96-02, Fairbanks, pp. 627-647.

Pohle, G. (1989). Structure, function, and development of setae on gill-grooming appendages and associated mouthparts of pinnotherid crabs (Decapoda: Brachyura). *Canadian Journal of Zoology, 67*(7), 1690–1707. doi:10.1139/z89-243

Pohle, G. W. (1992a). First Canadian record of *Paralomis bouvieri* Hansen, 1908 (Decapoda: Anomura: Lithodidae), infected by the rhizocephalan *Briarosaccus callosus* (Cirripedia: Peltogastridae) and carrying a hyperparasitic cryptoniscinid isopod (Epicaridea). *Canadian Journal of Zoology*, *70*.

Pohle, G. W. (1992b). First Record of the Rhizocephalan *Briarosaccus callosus* (Cirripedia, Peltogastridae) Infecting the Atlantic Porcupine Stone Crab *Neolithodes grimaldii* (Decapoda, Anomura, Lithodidae). *Crustaceana*, *62*(2), 133–136.

Poltev, Y. N. (2008). Parasitism of the rhizocephalan *Briarosaccus callosus* on the crab *Paralomis verrilli* (Crustacea: Lithodidae) in the waters of southeast Sakhalin. Russian Journal of Marine Biology, 34(3), 159-165.

Ritchie, L. E., & Høeg, J. T. (1981). The Life History of *Lernaeodiscus porcellanae* (Cirripedia: Rhizocephala) and co-evolution with its porcellanid host. *Journal of Crustacean Biology*, *1*(3), 334–347.

Selin, N. I. (1998). Expedition report on investigation of underutilized and poorly studied species and regions with commercial significance in the seas of the Far East in 1998. TINRO Publishing Company, scientific archive N22940, Vladivostok, Russia. [In Russian.]

Shukalyuk, A. I., Isaeva, V. V., Pushchin, I. I., & Dolganov, S. M. (2005). Effects of the *Briarosaccus callosus* infestation on the commercial Golden King Crab *Lithodes aequispina*. *The Journal of Parasitology, 91*(6), 1502–1504.

Slizkin, A. G., and A. G. Safronov. (2000). Commercial crabs of Kamchatkan waters. North Pacifica Publishing Company, Petropavlovsk-Kamchatski, Russia, 180 p. [In Russian.]

Sloan, N. A. (1984). Incidence and effects of parasitism by the Rhizocephalan barnacle, *Briarosaccus callosus* Boschma, in the golden king crab, *Lithodes aequispina* Benedict, from deep fjords in Northern British Columbia. *Journal of Experimental Marine Biology and Ecology*, *84*, 111–131.

Sloan, N. A. (1985). Life history characteristics of fjord-dwelling golden king crabs *Lithodes aequispina*. *Marine Ecology Progress Series*, *22*, 219–228.

Somerton, D.A. (1981). Contribution to the life history of the deep sea king crab *Lithodes couesi*, in the Gulf of Alaska. Fish. Bull. U.S., 79: 259-269.

Somerton, D. A., & MacIntosh, R. A. (1983). The size at sexual maturity of Blue king crab, *Paralithodes platypus*, in Alaska. *Fishery Bulletin, 81*(3), 621–628.

Sparks, A., & Morado, J. (1986). Histopathology and host response in lithodid crabs parasitized by *Briarosaccus callosus*. *Diseases of Aquatic Organisms*, *2*, 31–38. doi:10.3354/dao002031

Veillet, A. & F. Graf (1958). Degenerescence de la glande androgene des crustaces decapodes parsites par les rhizocephales. Bull. Soc. Scient. Nancy 18:123-127

Walker, G. (2001). Introduction to the Rhizocephala (Crustacea: Cirripedia). *Journal of Morphology*, *249*, 1–8.

Watters, G. (1998). Prevalences of parasitized and hyperparasitized crabs near South Georgia. *Marine Ecology Progress Series*, *170*, 215–229.

Welch, B. L. (1947). The generalization of "Student's" problem when several different population variances are involved. Biometrika 34 (1–2): 28–35.

Zaklan, S. D. (2002). Review of the family Lithodidae (Crustacea: Anomura: Paguroidea): Distribution, biology, and fisheries. *Crabs in Cold Water Regions: Biology, Management, and Economics*, (Table 2), 751–845. doi:10.4027/ccwrbme.2002.53

2.6 Tables and Figures

Table 2.1: Recorded prevalence of *B. callosus* on king crabs (Lithodidae).

Species	Area	Reference	Latitude	Longitude	Depth (m)	Infested	Total	Prevalence (%)
Paralithodes platypus (Brandt, 1850)	Alaskan fjord population	Hawkes et al. 1985b, 1986b	58 50' N	135 50' W				76
Lithodes aequispina (Benedict, 1895)	NE Pacific; Bering Sea	McMullen & Yoshihara 1970	57 58' N	153 45' W	210	6	11	55
Lithodes aequispina (Benedict, 1895)	Northern BC Fjords	Sloan 1984				1233	3045	40.5
Neolithodes grimaldii (A. Milne-Edwards & Bouvier, 1894)	Davis Strait, NAFO 0B	This study	62-63 N	58-60 W	1000-1300	574	2822	20.3
Paralomis spinosissima	Southern Ocean, Antarctic Ocean	Otto and Macintosh 1996						15
Lithodes aequispina (Benedict, 1895)	NE Pacific; Bering Sea	Shukalyuk 2005	54 40' N	154 10' E	570	43	769	5.6
Paralomis verrilli	SE Sakhalin island	Poltev 2008	47˚27'-47˚34' N	144˚59' - 144˚29' E				4.36
Lithodes ferox (Filhol, 1885)	S Atlantic Ocean	Abello and Macpherson 1992	23-30 S		50-500	68	2257	3
Lithodes murrayi (Henderson, 1888)	SW Indian Ocean, Crozet Islands	Arnaud and Do-Chi 1977						3
Paralithodes camtschatica (Tilesius, 1815)	NE Pacific, Bering Sea	Sparks and Morado 1986	55 00'N	169 00' W		2		2

Paralithodes camtschatica (Tilesius, 1815)	NE Pacific, Bering Sea	Boschma & Haynes 1969	58 02' N	133 59' W	0-40	2	99	2
Paralithodes camtschatica (Tilesius, 1815)	NE Pacific, Bering Sea	Hawkes 1985a	58 20' N	134 30' W		3		2
Paralithodes camtschatica (Tilesius, 1815)	Northern BC Fjords	Sloan 1984	BC Fjords					2
Lithodes couesi (Benedict, 1895)	Around deep sea mounts	Somerton 1981						1
Paralithodes platypus (Brandt, 1850)	Pacific Ocean	Sparks and Morado 1986						1
Paralomis granulosa (Jacquinot, 1847)	Beagle Channel, Tieraa del Fuego, Argentina	Lovrich 2004	54˚51' S	68˚12' W	10-50	85	29570	0.3

Table 2.2: Summary of porcupine crab captured in 2014 and 2015

Year	2014						2015					
Sex	Male			Female			Male			Female		
Category	Total	Non-infested	Infested	Total	Non-infested	Infested	Total	Non-infested	Infested	Total	Non-infested	Infested
Number Captured	1024	845	179	274	181	93	1244	1014	230	332	167	155
Minimum Length	22	22	60	37	37	74	34	34	61	35	35	55
Maximum Length	173	173	141	142	142	142	167	167	155	159	159	159
Mean Length ± sd	121.3 ±23.1	124.3 ±23.6	106.3 ±13.7	101.8 ± 23.1	97.5 ±25.2	110.4 ±14.5	113.1 ±23.3	115.4 ±24.0	104.0 ±17.6	96.5 ±19.1	90.9 ±19.2	103.2 ±17.0

Table 2.3: Summary of *B. callosus* externae found attached to porcupine crab in 2014 and 2015

Year	Sex	1 Externa	2 Externa	3 Externa	4 Externa
2014	Male	154	8		
	Female	80	4		
2015	Male	182	12	1	
	Female	127	7	1	1

Table 2.4: Summary of morphometric features of *B. callosus* infested and non-infested male porcupine crab.

	Non-infested			Infested			Welch's Two sample t-test		
Parameter	n	Mean	± sd	n	mean	± sd	t	Df	p
Carapace length	1815	119	± 24	431	105	± 16	14.64	858	< 0.001
Chela height	1669	30	± 10	364	19	± 4	35.68	1606	< 0.001
Merus 1 length	910	109	± 23	194	88	± 12	17.49	531	< 0.001
Merus 2 length	826	121	± 27	175	99	± 15	14.88	448	< 0.001

Table 2.5: Summary of morphometric features of *B. callosus* infested and non-infested female porcupine crab.

	Non-infested			Infested			Welch's Two sample t-test		
Parameter	n	Mean	± sd	n	mean	± sd	t	df	p
Carapace length	348	94	± 23	239	106	± 17	-7.286	583	< 0.001
Chela height	313	16	± 4	213	18	± 4	-6.279	498	< 0.001
Merus 1 length	159	72	± 11	125	84	± 13	-8.294	247	< 0.001
Merus 2 length	142	81	± 13	124	94	± 15	-8.035	247	< 0.001

Table 2.6: Internal reproductive effects of *B. callosus* infestation

Sex	Total dissected	Testis(atrophied or translucent) OR Ovary(absent)	Testis(degraded) OR Ovary(degenerated)
Male	130	73 (56%)	57
Female	102	80 (78%)	22

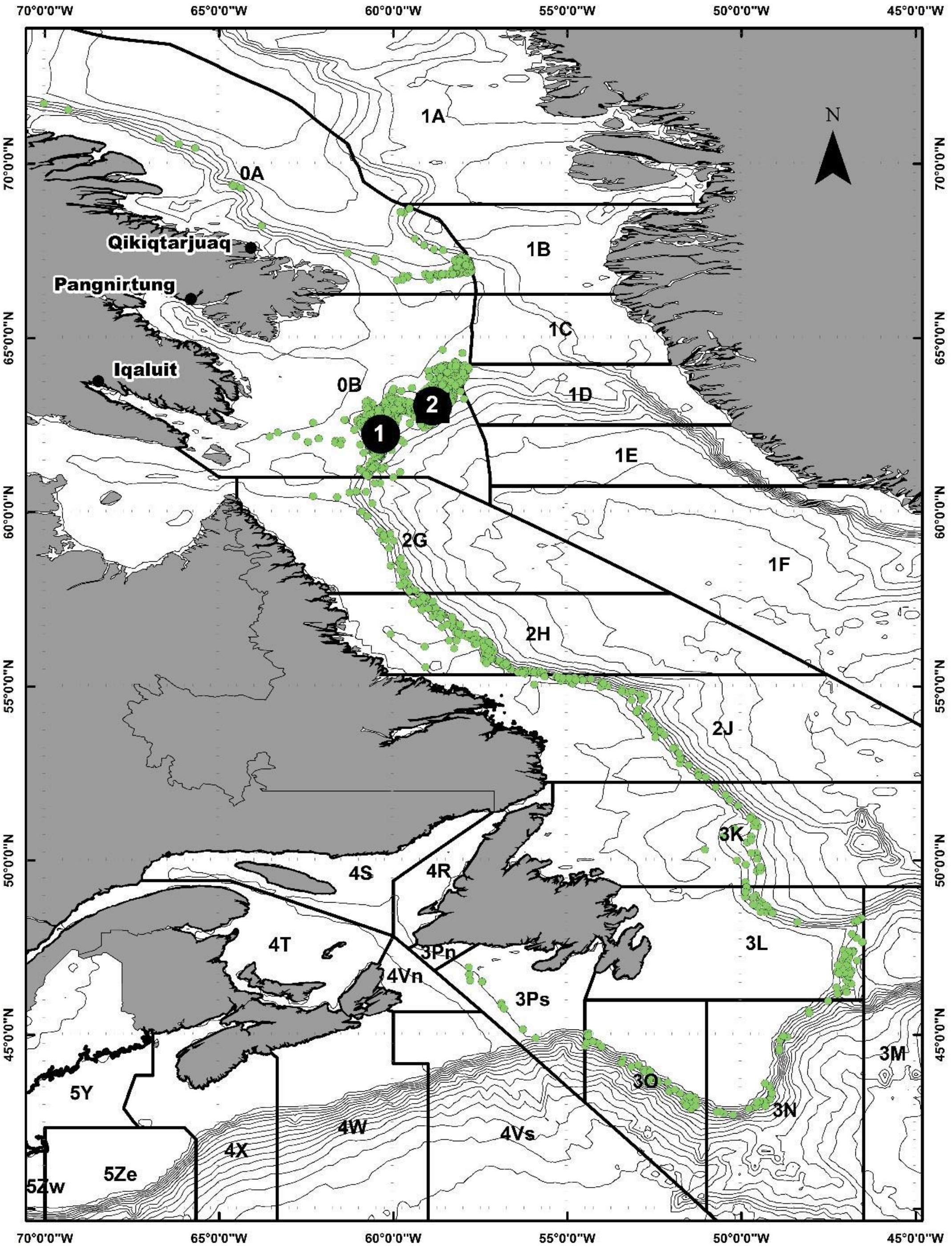

Figure 2.1: Map of the northwest Atlantic illustrating the 1995-2012 research survey and observer catch records for porcupine crab (green dots) (Bungay et al. 2014) and fishing locations in 2014(1) and 2015(2).

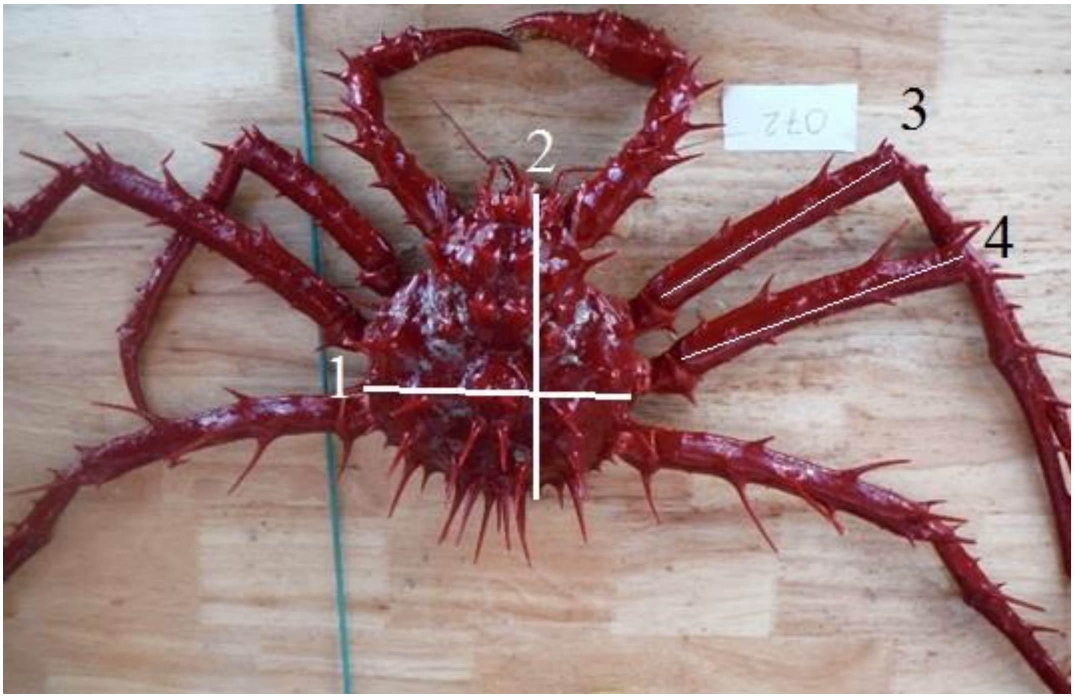

Figure 2.2: Dorsal view of porcupine crab displaying measurements taken: 1) carapace width, 2) carapace length, 3) first merus, 4) second merus.

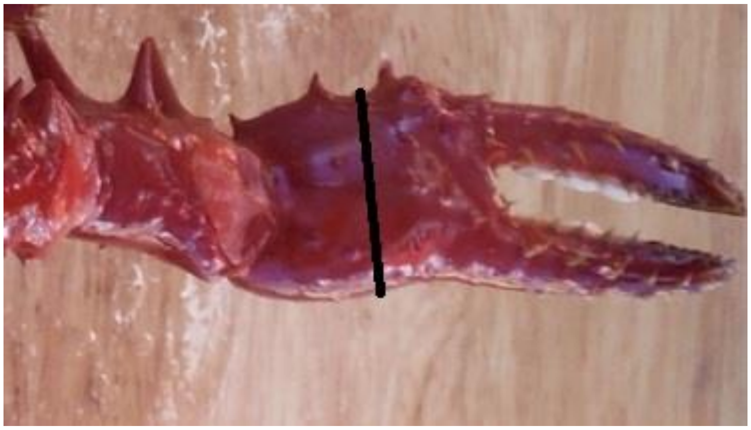

Figure 2.3: Chela of porcupine crab displaying measurements taken: black line indicates chela height measurement.

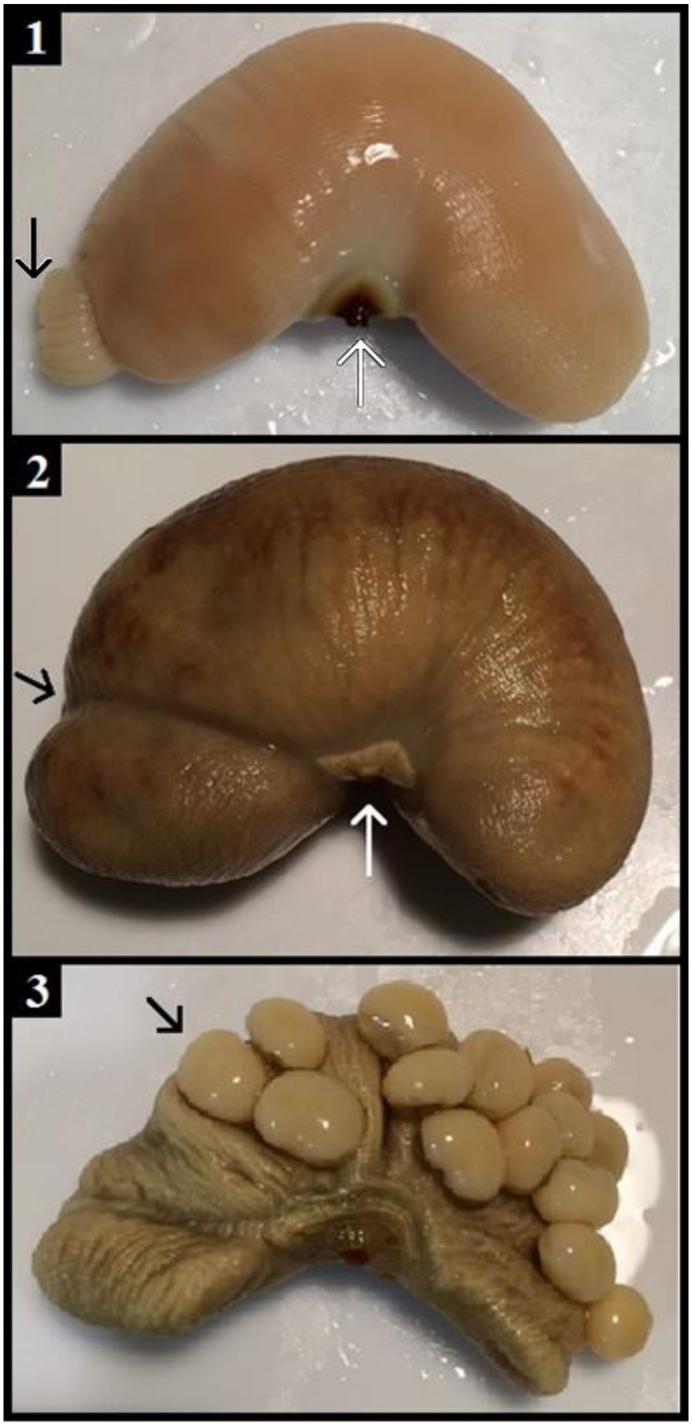

Figure 2.4: Externa of *B. callous* removed from porcupine crab: (1) young externa, black arrow indicates externa opening, white arrow indicates cuticular shield, (2) old externa, black arrow indicates externa opening, white arrow indicates cuticular shield, (3) black arrow indicates one female reproductive body of the hyperparasitic isopods which are infesting this externa.

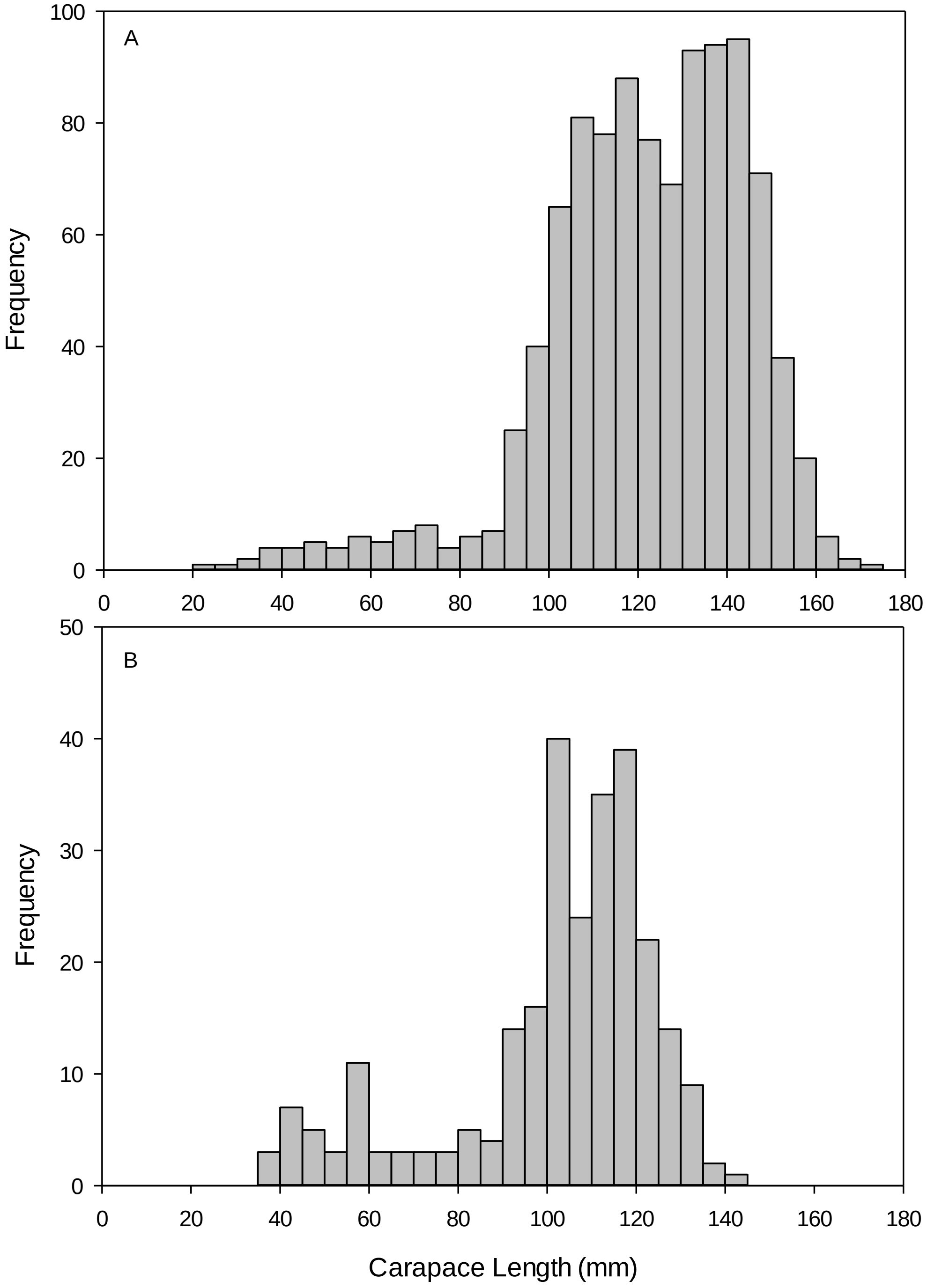

Figure 2.5: Size distribution of male (A) and female (B) porcupine crab captured during 2014.

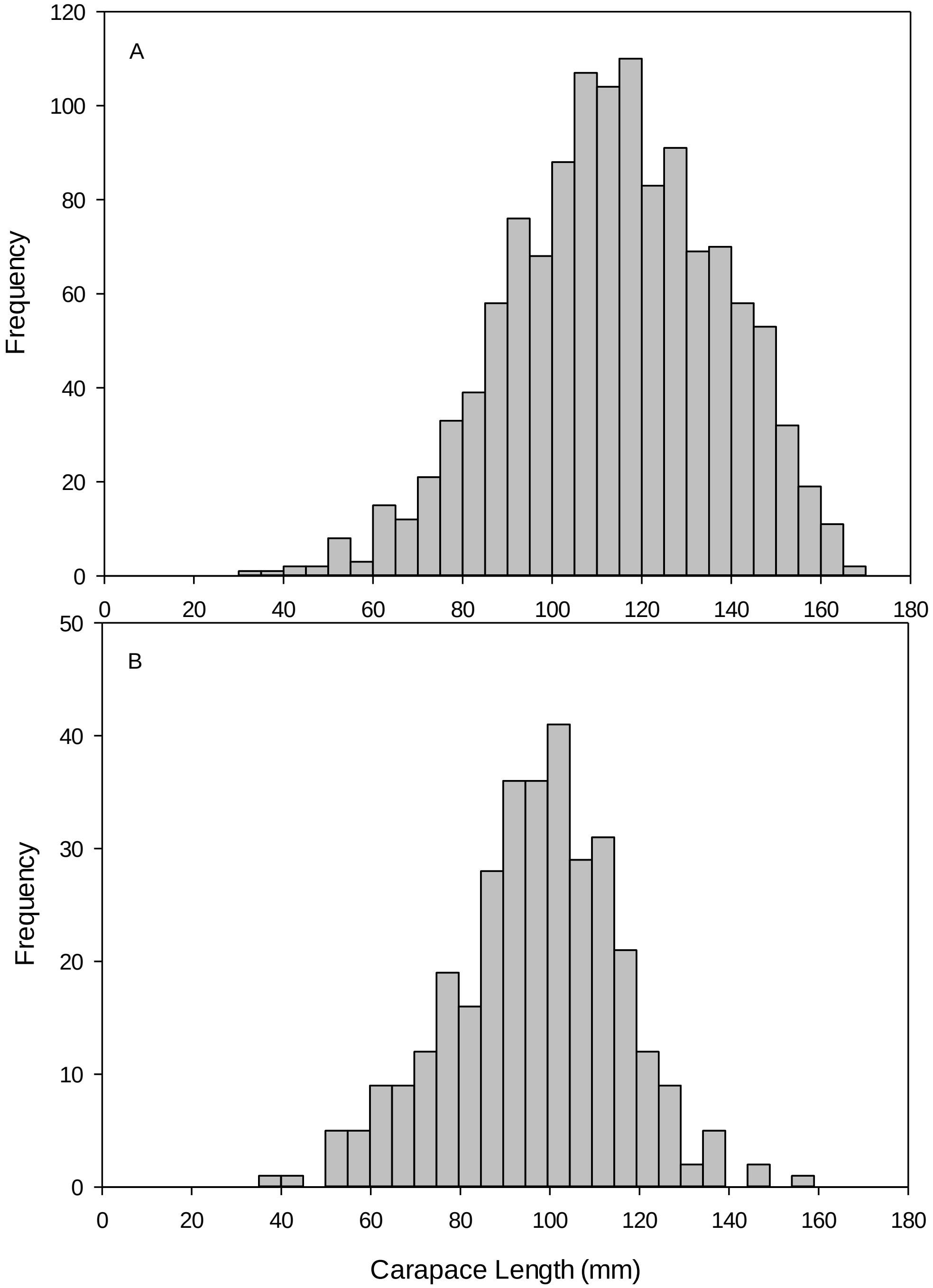

Figure 2.6: Size distribution of male (A) and female (B) porcupine crab captured during 2015.

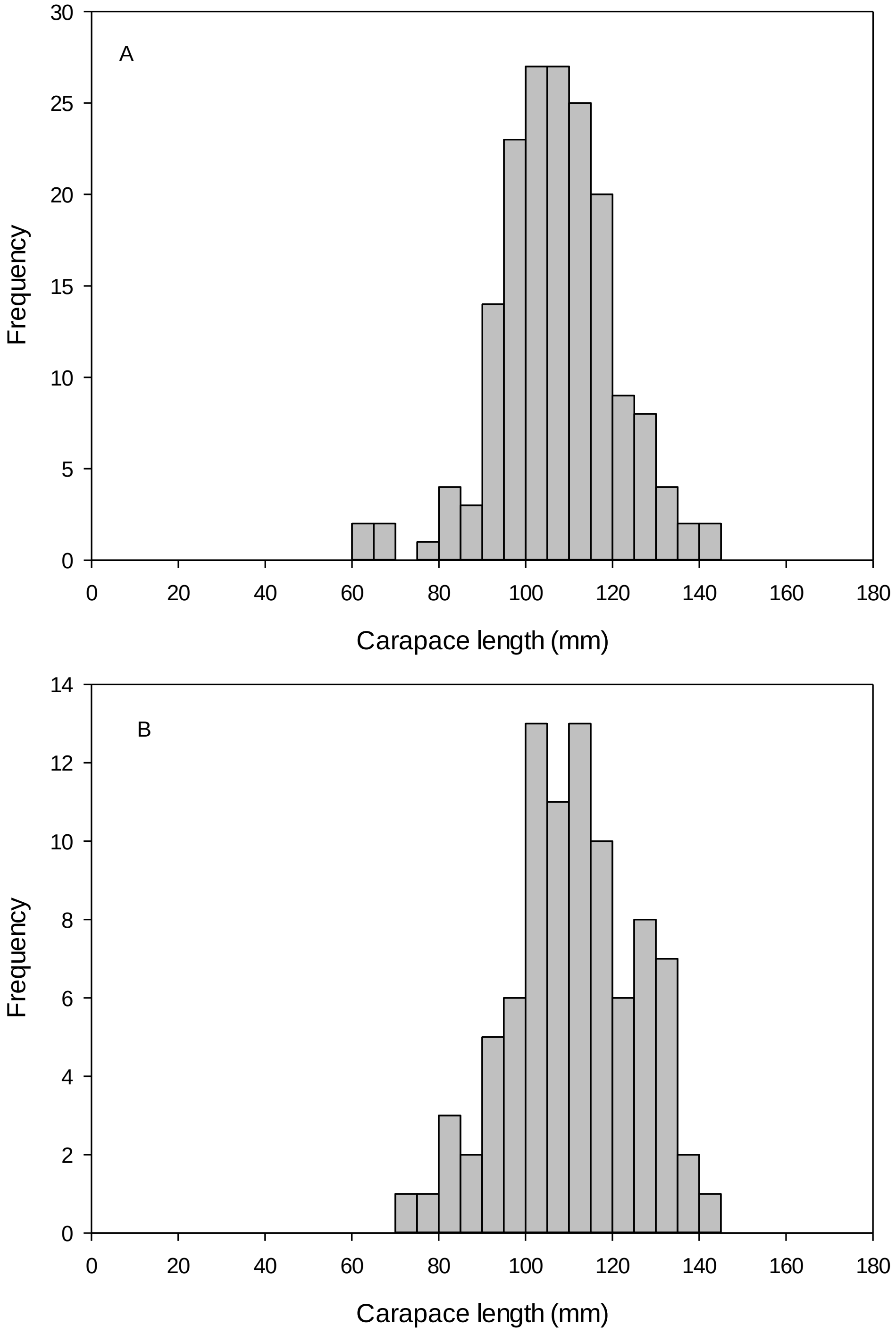

Figure 2.7: Size distribution of male (A) and female (B) porcupine crab infested with *B. callosus* captured during 2014.

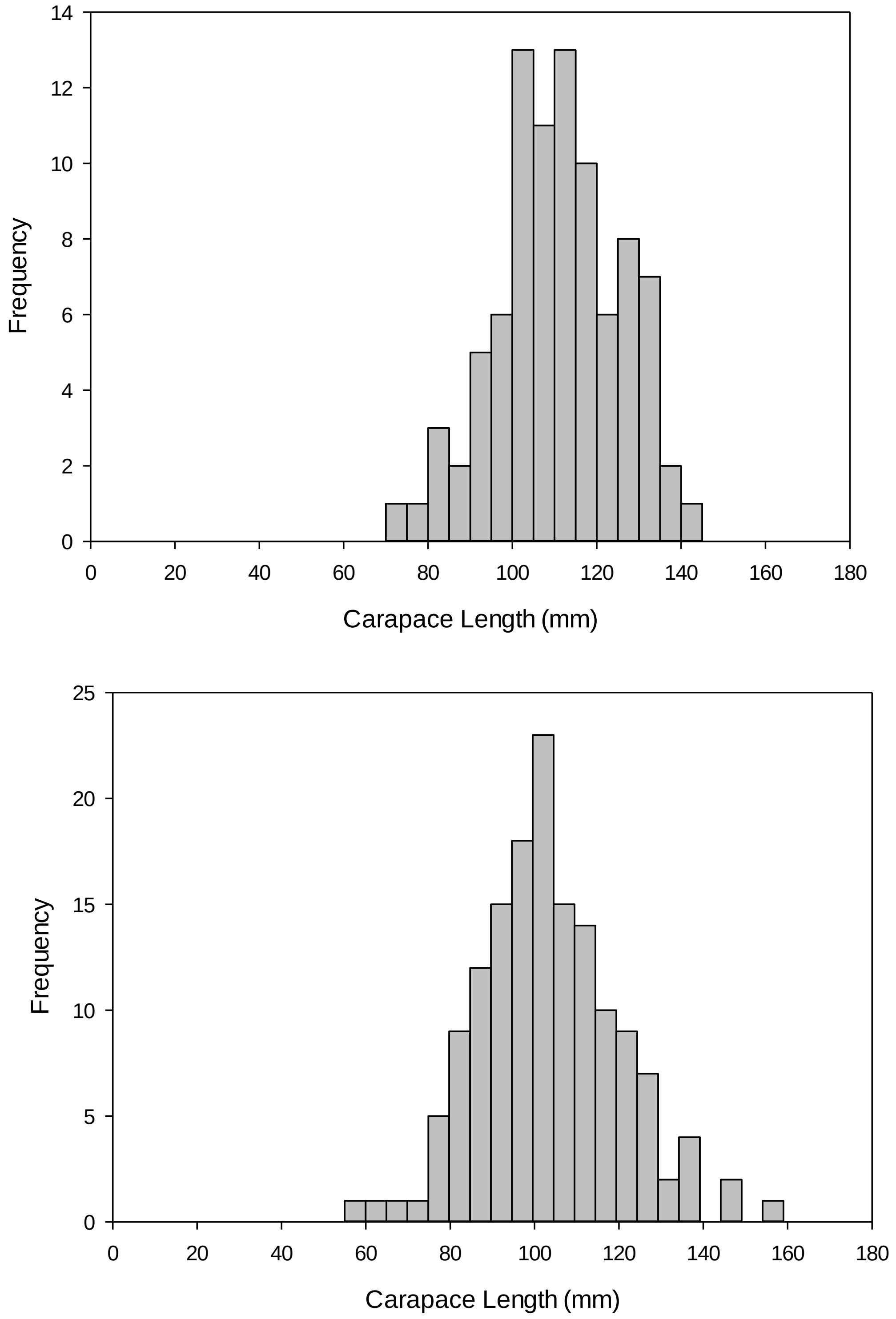

Figure 2.8: Size distribution of male (A) and female (B) porcupine crab infested with *B. callosus* captured during 2015.

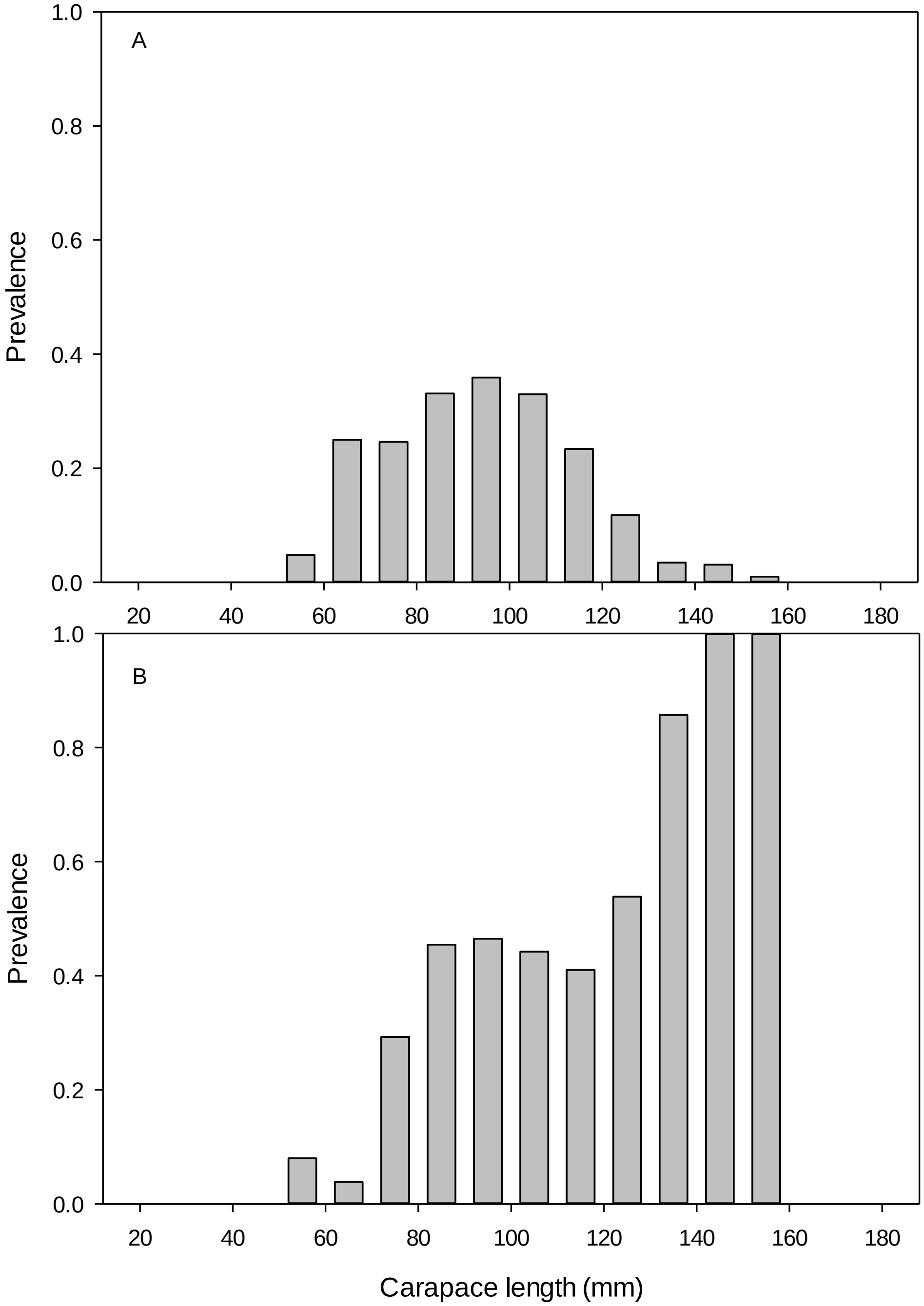

Figure 2.9: Size distribution of prevalence of *B. callosus* infestation on NAFO 0B male (A) and female (B) porcupine crab for both years.

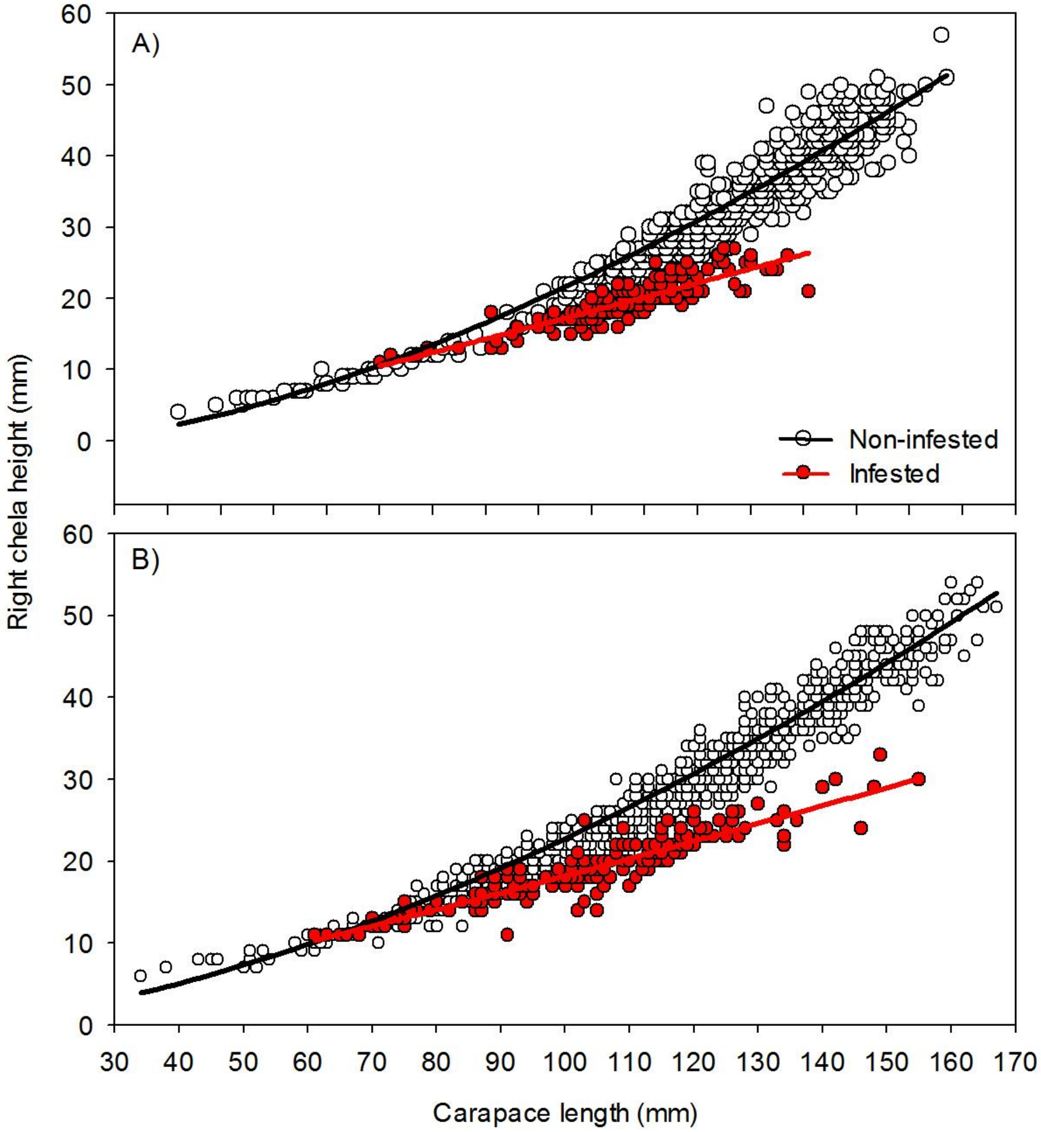

Figure 2.10: Relationships between right chela height and carapace length of *B. callosus* infested and non-infested male porcupine crab in 2014 (A) and 2015 (B).

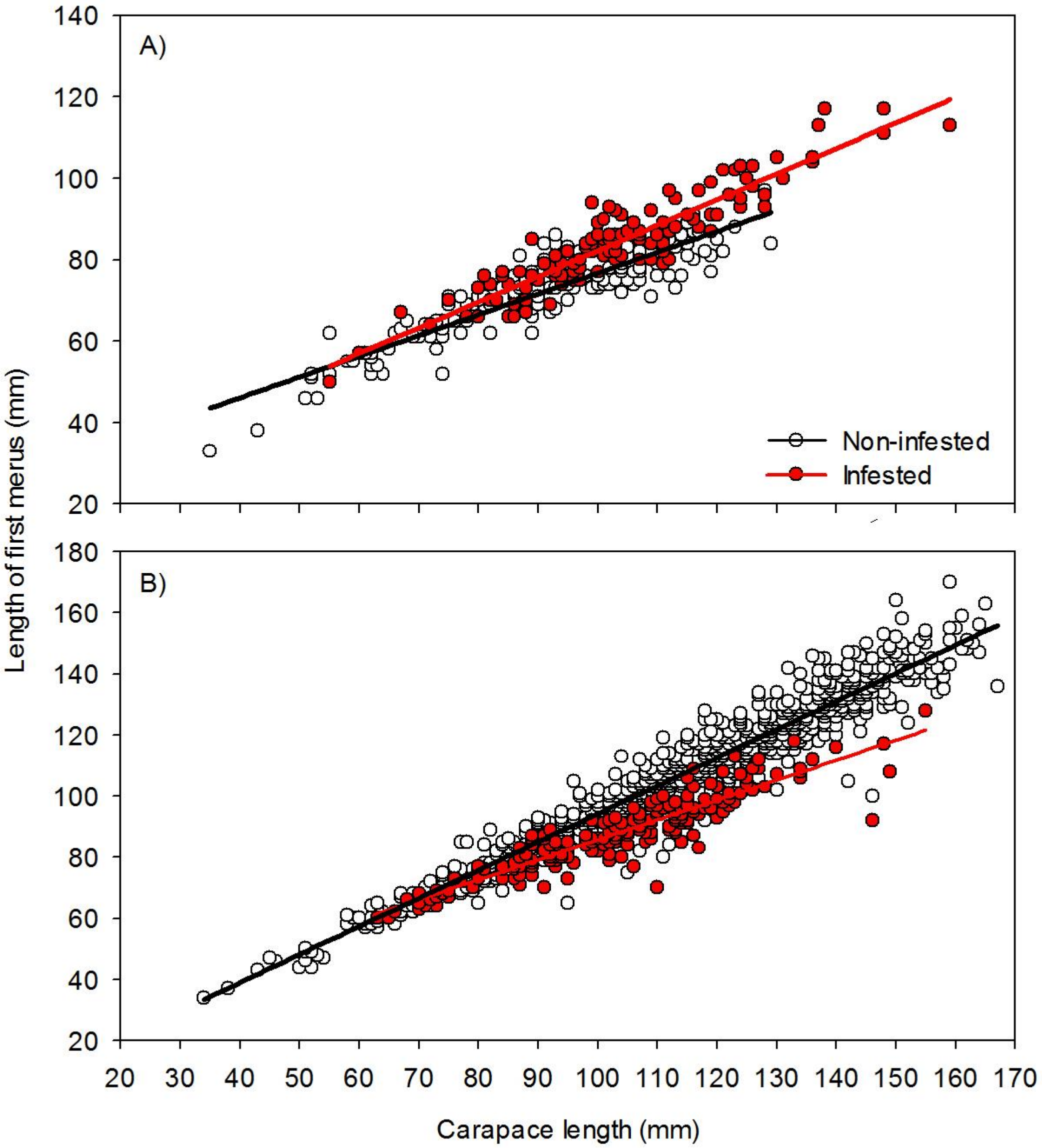

Figure 2.11: Relationships between first merus length and carapace length of *B. callosus* infested and non-infested female (A) and male (B) porcupine crab in 2015.

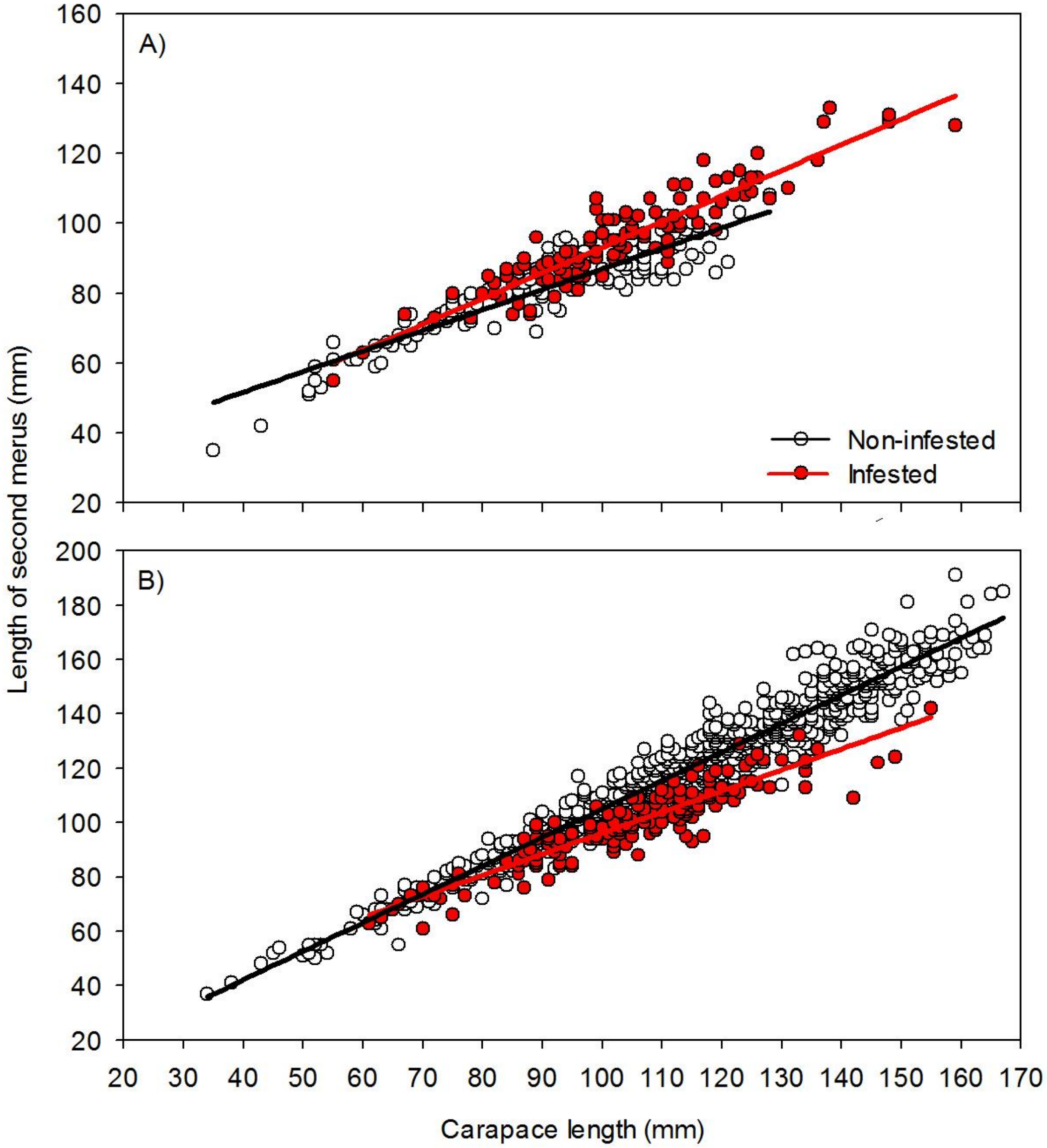

Figure 2.12: Relationships between second merus length and carapace length of *B. callosus* infested and non-infested female (A) and male (B) porcupine crab in 2015.

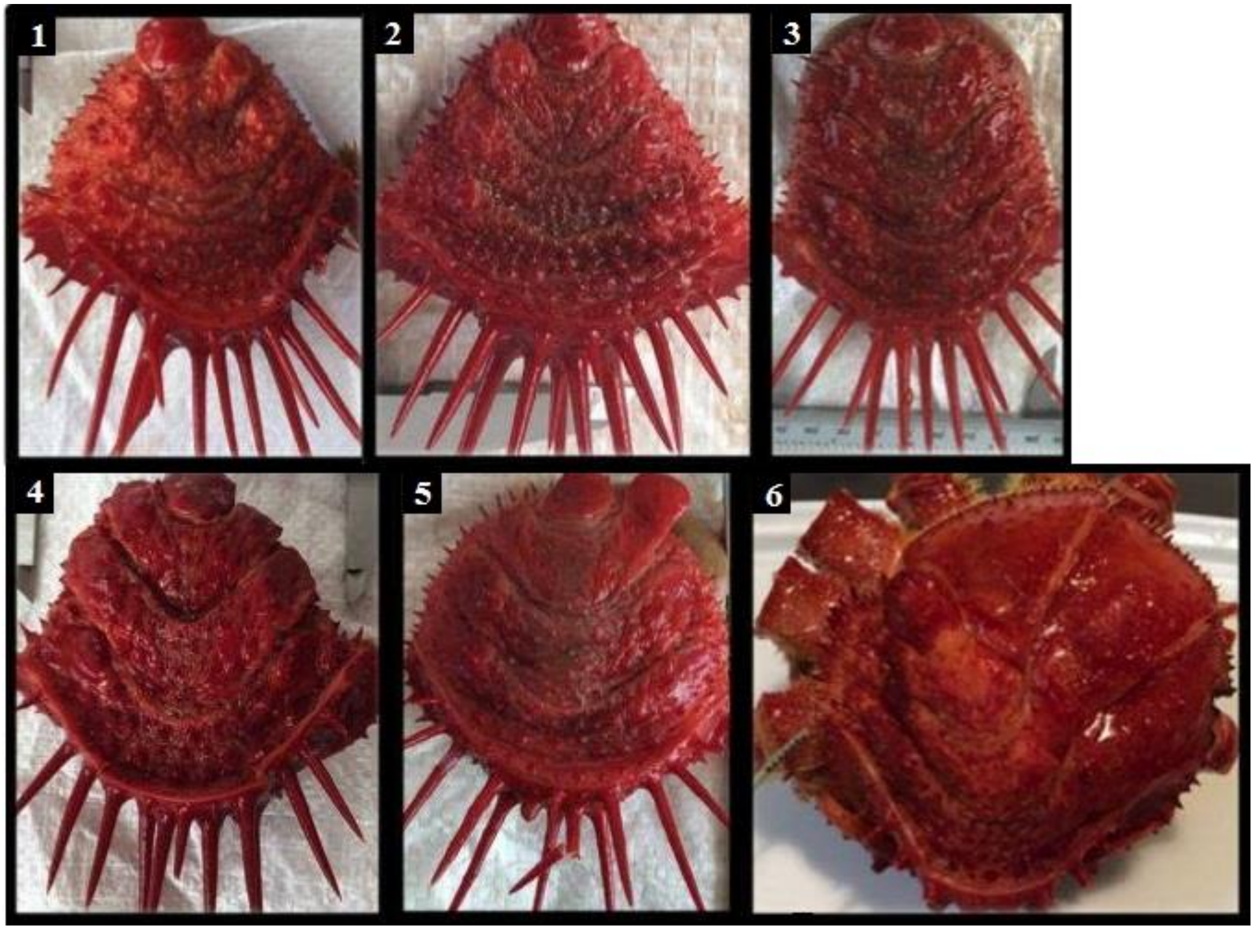

Figure 2.13: Feminization of the abdomen of infested male porcupine crab (1-5) displayed with increasing segmentation from 1-5 and compared to a non-infested female abdomen (6).

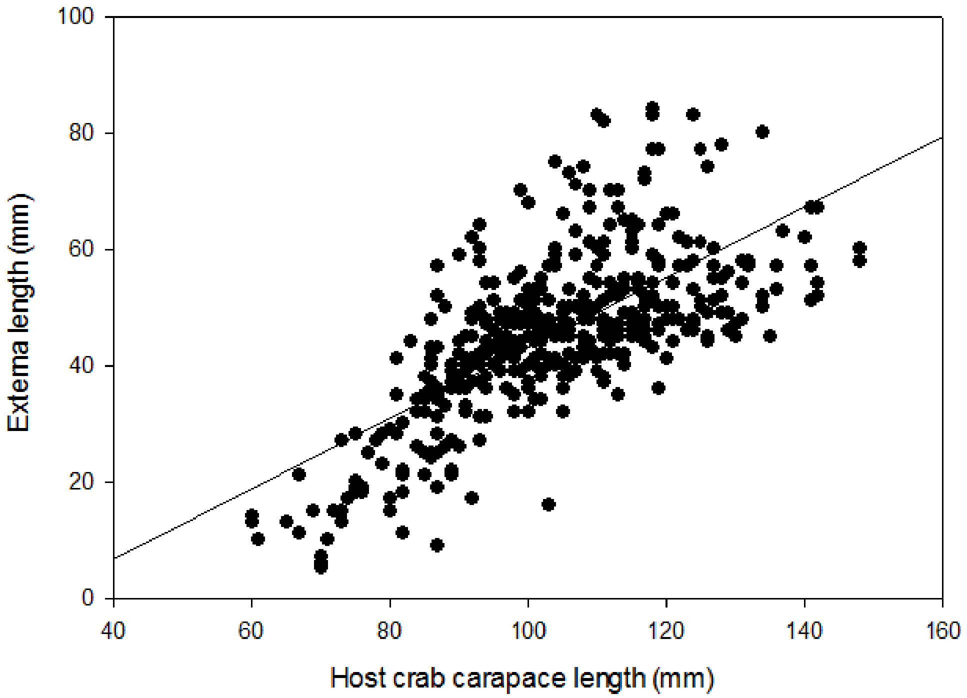

Figure 2.14: Relationship between *B. callosus* externa length and carapace length of host porcupine crab.

Chapter 3: Development of a Reflex action mortality predictor (RAMP) matrix for assessing post-capture survival of gillnet captured Porcupine crab (*Neolithodes grimaldii)*

Abstract

Investigations of post-capture survival of non-targeted species captured incidentally in commercial fisheries are important for conservation and sustainability of the affected species and ecosystems. Porcupine crab (*Neolithodes grimaldii)* are frequently captured in bottom gillnet fisheries for Greenland halibut (*Reinhardtius hippoglossoides)* yet there is no information on the survival of discarded animals. This study utilized the reflex action mortality predictor (RAMP) method to test for post-capture survival through means of reflex impairment. The results showed that porcupine crab were highly responsive to the eight reflex actions tested. The crabs tested were highly responsive to reflex actions and many exhibited no reflex impairment (loss of reflexes). Crab mortalities were observed that did not fail any reflex actions. Analyzing these results revealed that mortality to 5-9 days post-capture could not be predicted with a high amount of confidence as a low amount of variability was explained by the models used (5-15%). These results show that differences exist between porcupine crab captured in gillnets and previous studies conducting the RAMP method on bottom trawl captured king crab. The RAMP method did not successfully predict discard mortality but observations were made of effects on mortality from handling related injury. Between the two years of sample collection mortality was much higher when crabs were selected that had undergone rough handling and exhibited external injuries irrespective of RAMP score. It was observed that porcupine crab that were handled gently had high rates of survival (~74%) compared to the roughly handled crab (~28%), although neither had lost many reflexes. This result suggested that regardless of the RAMP score crabs that are handled gently without excessive trauma do have a greater chance of survival.

3.1 Introduction

The capture of non-targeted species in commercial fishing operations is of concern with regard to the long term sustainability of a resource and effects on the ecosystem. Alverson et al. (1994) found that the number of non-targeted species discarded can exceed the target species retained. Based on this knowledge, mortality of non-targeted species remains a large source of uncertainty in commercial fisheries (Alverson et al. 1994; Witherell and Pautzke 1997; Broadhurst et al. 2006). The effect that capture has on non-targeted species with regards to survival can be sub-divided into two categories: species captured that are immediately killed during the capture process (observed mortality), and species that are damaged or stressed and die subsequent to capture and discard (unobserved mortality) (Stevens 1990; Davis 2002; Suuronen 2005).

The species being captured and the method of capture may be a large factor influencing observed and unobserved mortality. Different methods of capture and handling such as hand collection, traps or pots, bottom gillnets, and bottom trawls produce varying degrees of damage and stress to organisms. For example, Leland et al. (2013) found for juvenile rock lobster that hand collection can cause increased antennae and pereopod loss when compared to trapping although the physiological response appeared to be benign compared to control animals over a recovery period. With regard to capturing crustaceans using baited pots, fixed rates of discard mortality have been set for snow crab (*Chionocetes opilio)*, tanner crab (*Chionocetes bairdi)*, and red king crab (*Paralithodes camtschatica)* captured in pots and have been found to be higher in bottom trawling operations for red king crab and snow crab (Siddeek 2003). Several studies have aimed to quantify the damage caused to crustaceans from bottom trawls because trawling operations usually disregard the unobserved mortality of non-targeted species. These studies have utilized recapture nets and modified trawl gears to observe the effects of trawls on crustaceans captured by different regions of conventional bottom trawl gear (Rose 1999; Hammond et al. 2013; Rose et al. 2013).

In NAFO Division 0B, a commercial fishery for Greenland halibut (*Reinhardtius hippoglossoides)* has been in operation since the early 1980's. The main capture gear used in this fishery is monofilament bottom gillnets consisting of strings of 50 nets connected together in sequence with a weighted line to keep the net on the bottom, and a float line to hold the net vertically off the seabed. These strings are usually left to soak for five days before being hauled. Historically this fishery has encountered bycatch of many species including the porcupine crab (*Neolithodes grimaldii)* (He 2005). The retrieval of monofilament gillnet using hydraulic or gas powered haulers and associate handling methods can lead to damage and stress to porcupine crab. These damages can be in the form of crushing or compressing of the carapace within the hauler, loss of limbs, and damage to the body during disentanglement, and air exposure or wind chill. In addition, the duration of gill net entanglement while on the seabed, air exposure, and wind chill may also influence stress and behaviour during handling (Romero et al. 2007; Urbina et al. 2013).

Physical and physiological stressors have been studied in crustaceans captured incidentally in fishing gears around the world to test for post-capture survival. Exposure to air is a common physiological stressor and although the effect may vary between habitats and temperatures it remains a consequence of retrieving crustaceans from the ocean. Air exposure has been studied in a large variety of crustaceans including: snow crab *Chionocetes opilio* (Grant 2003), brown crab *Cancer pagurus* (Webster 1996; Danford et al. 2002), king crabs *Lithodes santolla* (Urbina et al. 2013), *Paralomis granulosa* (Romero et al. 2007), and *Paralithodes* *camtschatica* (Carls and O'Clair 1990) and lobsters *Homarus americanus* (Chang et al. 1998), and *Panulirus Cygnus* (Crear and Forteath 2001). For all of these studies the species in question was exposed at standard time intervals and observed or tested for immediate or delayed effects. When testing the components of haemolymph such as production of proteins and enzymes, as well as oxygen absorption it has been observed that the critical amount of time a species can be exposed to air without incurring irreversible damage differs from species to species (Romero et al. 2007; Urbina et al. 2013). Tests analyzing the haemolymph components have revealed that reversible air exposure can even differ between species in the same family such as the king crabs with *L. santolla* and *P. granulosa* which have critical exposure limits of 30 hours and 6 hours respectively (Romero et al. 2007; Urbina et al. 2013). Different methods of holding crustacean species have been used depending on the focus of the study. For studies testing haemolymph components holding tanks are often used, while other studies that focused on the long term mortality caused by air exposure have utilized field enclosures (Grant 2003; Romero et al. 2007; Urbina et al. 2013).

Another main component of measuring mortality and stress in crustaceans is the physical damage that occurs during gear retrieval and handling processes. The damage to crustaceans can range from limb or segment loss to small or large cracks or pieces missing from critical areas of the body such as the carapace and thorax (Rose 1999; Stoner et al. 2008). Recording limb loss for crustaceans can be difficult and misleading as crustaceans have the ability to autotomize or intentionally detach limbs that have incurred injury. Scientists in the field of crustacean mortality have gone through a long progression of testing injury in various forms (Rose 1999; Stoner et al. 2008, Hammond et al. 2013). The earliest qualitative notes described damage in terms of what could be seen externally. This then led to counting lost limbs or segments, and finally to developing injury indices where injuries are ranked in categories based on specific criterion (Rose 1999; Stoner et al. 2008).

There are a variety of methods in which physical and physiological stressors are quantified in crustaceans with mixed results and success. External observations of damage and testing of haemolymph components have proven to be limiting when testing the cause of mortality and the ability to predict mortality based on stress or injury. Some authors have focused on quantifying behaviour or physical reflexes of crustaceans which may lead to valuable predictions of unobserved mortality (Stevens 1990; Warrenchuk and Shirley 2002; Stoner et al. 2008; Hammond et al. 2013). The first of such behaviours that was tested in several crustaceans was the ability of an animal to right itself once placed upside down following injury or retrieval from fishing gear (Stevens 1990; Carls and O'Clair 1995; Zhou and Shirley 1995; Warrenchuk and Shirley 2002). Righting behaviour has been found to be variable and may not be a valuable predictor of mortality. Similar to the measurements of righting behaviour, observations of spontaneous movement in crustaceans have been quantified but also found to be variable and have questionable validity in predicting mortality (Stevens 1990; Purves et al. 2003). Studies conducted on the post-capture behavior of fish using a set of reflexes or reflex actions was found to be a good predictor of mortality and was coined the reflex action mortality predictor (RAMP) method (Davis and Ottomar 2006; Davis 2007). A detailed explanation of the RAMP is covered in Table 3.1. The RAMP method has since been tested for crustaceans such as red king crab, snow crab, and tanner crab which routinely encounter bottom trawl gear (Stoner et al. 2008, 2009; Hammond et al. 2013; Rose et al. 2013). Using the RAMP method Stoner et al. (2008) and Hammond et al. (2013) have found that the mortality curves produced from the data are a good predictor of unobserved mortality in several crustaceans that encounter bottom trawls.

The current study aims to 1) determine post-capture survival of porcupine crab captured in gillnets and 2) use the RAMP method to test its accuracy of predicting unobserved mortality in gillnet captured porcupine crab. As the field study was conducted over two consecutive years, the second year focused on improving upon methods used in the first year and indirectly assessing if an apparent heightened response in crabs may alter RAMP outcomes. The results of this study will be important as the gillnet fishery for Greenland halibut occurs annually with no current knowledge on the discard survival of porcupine crab. In the current fishery practice, the large spines of porcupine crab result in fishermen grasping them by the soft abdomen when being disentangled from gillnets which can result in damage to or complete removal of the abdomen. If fishermen are to change their handling practices it will be important to determine whether porcupine crab have an increased likelihood of survival when greater care is taken during removal from gillnets.

3.2 Materials and Methods

3.2.1 Sample locations

Samples were collected during the commercial fishery for Greenland halibut (*R. hippoglossoides)* in the Davis Strait at 62’-63’ N and 60’-61’ W from June 15th to July 6th 2014 and 62’-63’ N and 58’-59’ W from June 2nd to June 21st 2015 (Figure 2.1). The porcupine crab were captured by means of commercial monofilament nylon Greenland halibut gillnet which was 91.4m long with a 191mm mesh size, a twine thickness of 0.6mm, a 0.5 hang ratio, and standing 25 meshes high. Gillnet sets consisted of 50 nets in a string that soaked for 5-8 days before retrieval. Crabs were captured at depths 1,002-1,216 m in 2014 and 907-1220 m in 2015.

3.2.2 Measurements and reflex action identification

Once removed from a gillnet porcupine crab were held in seawater for ~5-10 mins before being assessed to produce a controlled state from which to take reflex actions. Morphological measurements were then recorded in a timely manner (< 5 mins) to avoid excess handling (as per chapter 2 methods) and notes were taken on the sex, shell condition, limb loss, spine loss, and visible damage. Reflex actions were then performed. In the first year reflex actions were performed with seven reflexes (Table 3.1) which included eye retraction. However, after initial tests of this reflex action it was apparent that eye retraction was not a reliable action for porcupine crab and it was substituted with chelae retraction. In the second year an additional reflex action of secondary antennae was added bringing the total number of reflexes to eight. The eight reflex actions were recorded as 0 if the reflex was a positive response (i.e. noticeable movement) and 1 if the action was a negative response (i.e. failed reflex) (Table 3.1). After all information was collected the crabs were then either returned to the ocean, or retained for post-capture survival experiments. A T-bar tag was attached to crabs used in the survival experiments. The tag was attached to the rearmost right coxa joint of the leg and the crab were placed in a tank noting the time and date.

3.2.3 Survival Assessments

To assess post-capture survival, crabs were held in one of four onboard tanks with flowing seawater for 4-9 days in Year I and 2-11 days in Year II. The tanks had dimensions of 0.9m x 0.9m x 0.7m (L x W x H) receiving seawater at a flow rate of 24 litres/min. Temperatures in the tanks ranged from 1.28-1.67 Celsius. Measurements of temperature were recorded in each tank over the course of the experiment with temperature data loggers. Effort was made to ensure equal flow of seawater to each tank, and inflow hoses were placed into the bottom of the tank to provide circulation as water exited the tank from overflow holes at the top. Before being placed in the tanks reflex actions were taken initially after measurements. Care was taken when submersing crabs into the tank so that the anterior region was facing upward to allow air bubbles trapped in the gill chambers to escape. The tanks each contained 9-12 crabs depending on carapace length or overall size to maintain stocking densities and maximum number of crabs assessed. In the first year, after being tagged and placed into a tank the crabs were monitored once daily for mortalities. Daily survival assessments consisted of a visual observation of secondary antennae movement which avoided excess handling or removing crabs from the water. Mortalities were determined by failure of all eight reflex actions. Mortalities were immediately removed and notes of visible damage were taken as well as photographs. Live crabs were removed after a holding period of four to nine days and the reflex actions were recorded once more before preservation or returning to the ocean, all crabs returned to the ocean were euthanized. When crabs died or were removed for their second and final reflex assessment, new crabs were placed into those tanks to replace them and maintain stocking densities.

In the second year of the experiment crabs were checked for reflex impairments twice a day to narrow the time interval when mortalities occurred or observed changes in reflex impairments. Also in the second year as many additional details as possible were recorded with regard to the external observable injuries. These observations included, cracks to the thorax and carapace, damage to or removal of abdomen, leg and segment loss, and spine breaks. Similar to other authors these observations were combined into an injury index which could be used to assess if it accounted for any of the variability in the RAMP model. Injury scores were as follows: 0 – no injuries, 1 – new leg or cheliped loss, 2- minor carapace or thorax damage (a crack of ~3cm that did not expose internal tissue), 3-major carapace or thorax damage (a large crack traversing the entire body that exposed internal tissue) , 4- damages to multiple parts(legs, carapace, thorax).

3.2.4 Reflex impairments measured

Reflex action scores were combined into an impairment index for statistical procedures. The impairment index is calculated as the number of one scores (failed reflexes) that an individual exhibits. This index which ranges from 0-8 where 0 is assumed to indicate a crab in healthy condition with a low risk of mortality, and an 8 indicates a dead crab.

3.2.5 Statistical procedures

Logistic regression was executed to see if sex, carapace length, injury score or parasite infestation were significant predictors in the RAMP model. A generalized linear model with binomial error was used to analyze the reflex impairment data. All the crabs that were contained in the model were held within onboard tanks and were scored with a 0 if they survived the holding period or 1 if they did not. The cumulative reflex impairment score was added as a parameter in the model with values of 0-8. Comparisons of Akaike information criterion (AIC) were used for the models in Year I and II to observe any changes in the amount of variability explained.

3.3 Results

3.3.1 Reflex actions

In both years the initial seven reflex actions used were highly repeatable and exhibited rapid responses for the crabs tested (Table 3.1). The eighth reflex action of secondary antennae movement added in the second year was also highly responsive. In both years the crabs exhibited a high level of activity immediately following capture and most did not show any reflex impairment at all in Year I (68%) or Year II (76%) (Table 3.3). The most common reflex actions that were lost immediately following capture in both years were leg flare and kick (Table 3.2). When only one reflex was lost leg flare constituted a high proportion in year 1 (70%) and year 2 (49%) (Table 3.2). The kick reflex also constituted a high proportion when only one reflex was lost in year 1 (24%) and year 2 (48%) (Table 3.2).

3.3.2 Tank experiment Year I

In the first year for the 156 animals placed in onboard tanks there were 41 deaths leading to an overall mortality rate of 26%. Most (94%) of the animals placed in survival tanks had no reflex impairment or only 1 or 2 reflexes were impaired. Although the mortality rates were higher at higher reflex impairment scores it is based on a low number of individuals (Table 3.5). For the 115 crabs that survived to the end of the holding period the reflex impairment scores of 73 crabs (63%) remained unchanged from the initial ramp score to the final ramp score. Forty crabs (35%) that initially showed reflex impairment appeared to improve and showed a lower score in the final test. Two crabs (2%) showed an increase in impairment and had higher reflex action score in the final test before being removed from the tanks. Logistic regression revealed that the changes in AIC from adding or removing sex, carapace length and infestation were small (3.05 from highest to lowest). The model with the lowest AIC (model 2) accounted for 4.48% of the variability (Table 3.6). The generalized linear model showed that mortality appeared to be positively correlated with increasing reflex impairment score although sample sizes were low at high reflex scores as only 5.8% of crabs tested had initial reflex impairment scores above two (Table 3.5). The binomial GLM model 1 (Table 3.6) of alive or dead (0 or 1) used as a response with reflex score as a categorical variable was used to fit the model values to compare with the raw data values at each given reflex score (Figure 3.1). Summary for model 1 is shown in Table 3.4.

3.3.3 Tank experiment Year II

There was a high rate of mortality (72%) in Year II. The crabs from Year II were checked for reflex impairment scores twice daily which differed from Year I. For the 29 crabs that survived the holding period, 16 (55%) had the same impairment score before and after, four (14%) had lower scores after, and nine (31%) had higher scores after. Logistic regression revealed that the changes in AIC from adding or removing sex, carapace length, injury and infestation were small (8.45 from highest to lowest). The model with the lowest AIC (model 3) accounted for 15.22% of the variability (Table 3.10). When reflex score was used in a generalized linear model to predict mortality the mortality rate did correlate with increasing reflex impairment score. Similar to Year I, however, very few crabs exhibited high reflex impairment scores and only 13.5% of those tested had initial reflex scores above two (Table 3.5). Although mortality did correlate positively with increasing reflex impairment score it should be noted that crabs with no reflex impairments at all (score of 0) or those with only one failed reflex showed a high amount of mortality (63% and 80%). The binomial GLM model 1 (Table 3.8) of alive or dead (0 or 1) used as a response with reflex score as a categorical variable was used to fit the model values to compare with the raw data values at each given reflex score (Figure 3.1). Summary for model 1 is shown in Table 3.7.

3.3.4 Holding times

The period of time that crabs were held appeared to be appropriately long to observe mortalities. The majority of mortalities occurred within the first 72 hours in Year I (83%) and in

Year II (81%) (Figures 3.2). Despite low reflex impairment score there were crabs in both years that survived longer time periods before dying indicating there may be other causes of mortality such as a heightened stress response that prolongs death. In year one two crabs with an initial reflex impairment of zero died on the seventh day of holding and one with an initial reflex impairment score of one died on the eighth day of holding. In year two, two crabs died on the ninth day of holding despite having zero reflex impairment score and no observable external injuries. In year one the range of time to mortality for crabs with zero reflex impairment score ranged from 7-178 hours and in year two from 9-236 hours.

3.4 Discussion

This study has shown that the quantification of post capture survival for porcupine crab captured in bottom gillnets using the RAMP method did not lead to a high amount of confidence in predicting mortality. Observations of external injuries quantified in Year I and Year II of the study found a similar result to Rose (1999) in that the injuries themselves cannot provide a direct estimate of mortality. This result was solidified in Year II as porcupine crab with severe injuries were selected to study but despite the damage no loss in reflexes was found. The injury results correspond with the literature in that initial attempts to quantify the discard survival in crustaceans utilized the observations of external injuries. However, while important, these measures highlight the need for more comprehensive tests to predict mortality (Rose 1999; Rose et al. 2013).

Through the two year field study on porcupine crab specific behaviours termed reflex actions were identified similar to the early work by Davis and Ottomar (2006). These reflex actions were comprised into a reflex impairment index in attempts to assess the probability of survival of animals captured in commercial fishing. The specific reflex actions identified by

Stoner et al. (2008) for tanner and snow crab were tested on porcupine crab and some additional reflex actions were added. All of the reflex actions tested during the current study were highly responsive and satisfied the condition set for a positive response in the methods (Table 3.1).

Stoner et al. (2008) found that the RAMP method for crustaceans captured in commercial bottom trawls accurately predicted mortality for snow crab and tanner crab. Additional research by Hammond et al. (2009; 2013) and Rose et al. (2013) increased the confidence of the established RAMP curves and presented the viability of this method for use in other crustaceans. The first main objective of the current study was to determine whether porcupine crab survive capture and handling**.** The objective was met when this study compared the survival of animals captured between the two sample years. It was found that when crabs are handled gently, and do not have large cracks in the body that expose the body cavity they tend to survive (>70% alive) removal from the gillnet. Conversely, when crabs are handled roughly such as large cracks in the body exist exposing the body cavity, or limbs are removed by force the survival is much lower (~30% alive).

The second goal was to assess if the RAMP method is as accurate at predicting the mortality in porcupine crab as it is for other species tested in previous research. The results of this study were not as clear as those conducted using the RAMP method for crustaceans captured using bottom trawls. The explained variability for Year I and Year II for porcupine crab using the reflex actions and injury index were small (2.7-15.78%) compared to 70-80% in the studies by Stoner et al. (2008) and Hammond et al. (2013). Due to this result it appears that the RAMP method used in this way did not provide a good prediction of mortality for porcupine crab captured in nets that are left to soak for 5-8 days. However, there may have been unobserved effects on the animals due to being held in onboard tanks that may have affected results such as differences in water parameters compared to their native environment. Without control tanks containing the ideal bottom temperature and water parameters typical of porcupine crab habitat a direct comparison cannot be made.

This study is unique in that the gear used for capture is different than that used in prior post-capture survival research and that the porcupine crab inhabits much deeper waters than previously studied king crab species. The main gear differences that arise between the current study and previous studies are the nature of passive versus active capture gear and the handling procedures that result. In the capture phase for bottom trawling the gear is actively pulled over the seabed which results in collisions with crabs (Nguyen et al. 2014) and collecting animals which are entrained and towed for a period of time before being hauled to the surface. The removal phase then consists of the codend of the net being opened on deck where a brief sorting process occurs by sex and species. For gillnets, the capture phase consists of the gear being left to soak for a number of days, and crabs are caught when they incidentally encounter the gear and become entangled. After this entanglement phase they are hauled to the surface through a column of water. The removal phase involves vigorous handling to twist and rotate the animals out of the meshes of the gillnet which is made more difficult by the long spines characteristic of the porcupine crab. The results of this study showed that porcupine crab appear to be highly responsive to reflex actions although few crabs in both years exhibited any lost reflex actions at all even when severely injured.

A proposed reasoning behind the low number of lost reflexes may be an adaptive response to the stress of the removal phase following capture. The main objective of the second year of this study was to indirectly test whether the heightened response of the crabs makes it difficult to assess survival with RAMP alone. This objective was tested by selecting critically injured crabs and assessing RAMP twice daily for changes in reflex actions. This objective was met as there was a stark increase in the mortality of crabs that were heavily injured despite RAMP scores being low and showing little change over time. This result reinforced the conclusion that RAMP score does not allow a confident prediction of mortality for gillnet captured porcupine crab.

Removal times of porcupine crab from gillnets may vary depending on the method used to disentangle the crabs and the skill of the individual to execute it. However, regardless of removal duration, there is still a dramatic change in environment from the crabs being entangled in their native environment, remaining entangled for 1-8 days and then being briefly pulled to the surface and vigorously handled. This change may induce a heightened stress response in the animals which could account for the responsiveness when reflex actions are performed upon them, even those that are critically injured. A potential way this could be tested further is by watching for rises in physiological stress parameters such as stress hormones. Webster (1996) found that changes in the stress hormone circulating hyperglycaemic hormone (CHH) was shown to increase in *Cancer pagurus* when subjected to increased air exposure. Another way to test if differences in stress response occur would be to use alternative fishing gear on the same species to see if the same results are observed.

The increase in activity, possibly as a result of stress may account for the differences in survival observed between Year I (~70%) and Year II (~30%) of the experiment. This is because damage of vigorously handled crabs and repeated handling for the RAMP assessments in the second year led to significantly higher mortality yet reflex scores remained low for 86.5% of the crabs tested.

Both models for Year I and Year II appeared to correlate increasing mortality with the increase in the reflex impairment score although the higher reflex impairment score values were based on low numbers (5.8% crabs scored above 2 in Year I, and 13.5% of crabs scored above 2 in Year II). Statistically the models did not explain a high amount of variability with either reflex action score alone or with added variables (2.7 – 15.78 %). The low amount of accounted variability was observed in both years which shows that although there was an increase in mortality with increasing RAMP score it is highly variable and not actually a very good predictor for this species and capture gear.

When observing the differences in mortality that occurred at reflex impairment scores of zero (no reflex impairments) it is clear that there are potential unaccounted sources of mortality such as the amount of time porcupine were captured in gillnets before retrieval and also differences in water parameters between the onboard tanks and the porcupine crab's native environment. Based on the results of the second objective (stark differences in mortality between rough and gently handled crab) it appears that a heightened stress response in damaged crabs may be a factor affecting predictability of mortality and reliability of reflex impairments to gauge vitality. Another main factor that may affect mortality but not reflex impairment scores is that the water parameters in the onboard tanks are probably different than the habitat of porcupine crab (deeper than 800 m). The combination of stressors from porcupine crab being removed from their native environment and placed in potentially non-optimal one may induce changes in natural mortality that are not consequence of handling and can only be tested by future studies utilizing control tanks to rule out these factors.

In addition to reflex impairment, our best attempts were taken to note any external observable injuries, although this did not cause any major changes in modelling the predicted mortality. The critical injuries to these animals that lead to mortality must be comprehensive involving shock or trauma to the internal organs. Future studies could examine internal injuries to porcupine crab that may result from shock or pressure in laboratory or field conditions. This type of a study may reveal similar results to Grant (2003) who observed that increasing the drop height of snow crab caused increases in mortality, attributed to concussive damage to internal tissues. Observing mortalities of crabs with zero reflex impairments and injuries from less than 12 hours to greater than 7 days indicates that there must be other factors that lead to the eventual death of the animal. The stress in handling is proposed as a main source of this eventual mortality which was clearly demonstrated by the increase in mortality in the second year by selecting for more damaged animals. Additional supporting evidence for this point is that a general lack of increasing RAMP scores among crabs that died was observed in Year II despite daily handling and reassessment of RAMP score.

3.5 References

Alverson, D. L., M. H. Freeberg, S. A. Murawski, and J. G.Pope. (1994). A global assessment of fisheries bycatch and discards. FAO (United Nations Food and Agriculture Organization) Fish. Tech. Pap. 339, 235 p.

Broadhurst, M. K., Suuronen, P., & Hulme, A. (2006). Estimating collateral mortality from towed fishing gear. *Fish and Fisheries* (Vol. 7).

Carls, M. G., and C. E. O'Clair (1990). Influence of cold air exposure on ovigerous red king crabs (*Paralithodes camtschatica*) and Tanner crabs (*Chionoecetes bairdi*) and their offspring. *In* Proceedings of the international symposium on king and Tanner crabs, p. 329–343.

Chang, E. S., Keller, R., & Chang, S. a. (1998). Quantification of crustacean hyperglycemic hormone by ELISA in hemolymph of the lobster, *Homarus americanus*, following various stresses. *General and Comparative Endocrinology*, *111*(3), 359–66. doi:10.1006/gcen.1998.7120

Crear, B. J., & Forteath, G. N. R. (2001). Recovery of western rock lobster, *Panulirus cygnus*, from emersion and handling stress: the effect of oxygen concentration during re-immersion. *Journal of Shellfish Research*, *20*(3), 921–929.

Danford, a. R., Hagerman, L., & Uglow, R. F. (2002). Effects of emersion and elevated haemolymph ammonia on haemocyanin-oxygen affinity of *Cancer pagurus*. *Marine Biology*, *141*(6), 1019–1027. doi:10.1007/s00227-002-0906-6

Davis, M. W. (2002). Key principles for understanding fish bycatch discard mortality. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*, 1834–1843. doi:10.1139/f02-139

Davis, M. W. (2007). Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish. *ICES Journal of Marine Science*, *64*(8), 1535–1542. doi:10.1093/icesjms/fsm087

Davis, M. W., & Ottmar, M. L. (2006). Wounding and reflex impairment may be predictors for mortality in discarded or escaped fish. *Fisheries Research*, *82*(1-3), 1–6. doi:10.1016/j.fishres.2006.09.004

Elwood, R. W., Barr, S., & Patterson, L. (2009). Pain and stress in crustaceans? *Applied Animal Behaviour Science*, *118*(3-4), 128–136. doi:10.1016/j.applanim.2009.02.018

Grant, S. M. (2003). Mortality of snow crab discarded in Newfoundland and Labrador's trap fishery: at-sea experiments on the effect of drop height and air exposure duration. *Canadian Technical Report of Fisheries and Aquatic Sciences*. 2481: vi + 28 p.

Hammond, C. F., Conquest, L. L., & Rose, C. S. (2009). Using Reflex Action Mortality Predictor (RAMP) to investigate if trawl gear modifications reduce unobserved mortality of Chionoecetes sp. M.S. Thesis, 52 p. Univ. Washington, Seattle, WA.

Hammond, C. F., Conquest, L. L., & Rose, C. S. (2013). Using reflex action mortality predictors (RAMP) to evaluate if trawl gear modifications reduce the unobserved mortality of Tanner carb (*Chionocetes bairdi*) and snow crab (*C. opilio*). *ICES Journal of Marine Science*, *70*(7), 1308–1318.

Leland, J. C., Butcher, P. A., Broadhurst, M. K., Paterson, B. D., & Mayer, D. G. (2013). Damage and physiological stress to juvenile eastern rock lobster (*Sagmariasus verreauxi*) discarded after trapping and hand collection. *Fisheries Research*, *137,* 63–70. doi:10.1016/j.fishres.2012.09.001

Nguyen, T.X., Winger, P.D., Legge, G., Dawe, E.G., and Mullowney, D.R. (2014). Underwater observations of the behaviour of snow crab (*Chionoecetes opilio*) encountering a shrimp trawl off northeast Newfoundland. Fisheries Research, 156: 9-13.

Parsons, D. M., & Eggleston, D. B. (2005). Indirect effects of recreational fishing on behavior of the spiny lobster *Panulirus argus*. *Marine Ecology-Progress Series*, *303*(1), 235–244. doi:10.3354/meps303235

Purves, M. G., Agnew, D. J., Moreno, G., Daw, T., Yau, C., & Pilling, G. (2003). Distribution, demography, and discard mortality of crabs caught as bycatch in an experimental pot fishery for toothfish (*Dissostichus eleginoides*) in the South Atlantic. *Fishery Bulletin*, *101*(4), 874–888.

Romero, M. C., Ansaldo, M., & Lovrich, G. A. (2007). Effect of aerial exposure on the antioxidant status in the subantarctic stone crab *Paralomis granulosa* (Decapoda: Anomura). *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *146*(1-2 SPEC. ISS.), 54–59. doi:10.1016/j.cbpc.2006.06.009

Rose, C. S. (1999). Injury rates of Red king crab, *Paralithodes camtschaticus*, Passing Under Bottom-trawl Footropes. *Marine Fisheries Review*, *61*(2), 72–76.

Rose, C. S., Hammond, C. F., Stoner, A. W., Munk, J. E., & Gauvin, J. R. (2013). Quantification and reduction of unobserved mortality rates for snow, southern Tanner, and red king crabs (*Chionoecetes opilio, C. bairdi, and Paralithodes camtschaticus*) after encounters with trawls on the seafloor. *Fishery Bulletin*, *111*(1), 42–53. doi:10.7755/FB.111.1.4

Siddeek, M. S. M. (2003). Determination of biological reference points for Bristol Bay red king crab. *Fisheries Research*, *65*(1-3), 427–451. doi:10.1016/j.fishres.2003.09.030

Stevens, B. G. (1990). Survival of King and Tanner Crabs Captured by Commercial Sole Trawls. *Fishery Bulletin*, *88*, 731–744.

Stoner, A. W., Rose, C. S., Munk, J. E., Hammond, C. F., & Davis, M. W. (2008). An assessment of discard mortality for two Alaskan crab species, Tanner crab (*Chionoecetes bairdi*) and snow crab (*C .opilio*), based on reflex impairment. *Fishery Bulletin*, (106), 337–348.

Stoner, A. W. (2009). Prediction of discard mortality for Alaskan crabs after exposure to freezing temperatures, based on a reflex impairment index. *Fishery Bulletin*, (107), 451–464.

Stoner, A. W. (2012). Assessing Stress and Predicting Mortality in Economically Significant Crustaceans. *Reviews in Fisheries Science*, *20*(3), 111–135. doi:10.1080/10641262.2012.689025

Suuronen, P. (2005). *Mortality of fish escaping trawl gears*. *FAO Fisheries Technical Paper* (Vol. 478). Retrieved from http://www.fao.org/docrep/field/003/ab825f/AB825F00.htm#TOC

Uhlmann, S. S., Broadhurst, M. K., Paterson, B. D., Mayer, D. G., Butcher, P., & Brand, C. P. (2009). Mortality and blood loss by blue swimmer crabs (*Portunus pelagicus*) after simulated capture and discarding from gillnets. *ICES Journal of Marine Science*, *66*(3), 455–461. doi:10.1093/icesjms/fsn222

Urbina, M. A., Paschke, K., Gebauer, P., Cumillaf, J. P., & Rosas, C. (2013). Physiological responses of the southern king crab, *Lithodes santolla* (Decapoda: Lithodidae), to aerial exposure. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *166*(4), 538–545. doi:10.1016/j.cbpa.2013.08.006

Warrenchuk, J.J., & Shirley T. C. (2002) Effects of wind chill on the snow crab (*Chionoecetes opilio),* pp 81-96. In: *Crabs in Cold Water Regions: Biology, Management, and Economics* (Paul, A. J., E. G. Dawe, R. Elner, G. S. Jamieson, G. H. Kruse, R. S. Otto, B. Sainte-Marie, T. C. Shirley, and D. Woodby, Eds.). Fairbanks, AK: Alaska Sea Grant, University Alaska Fairbanks, AK-SG-02-01 (2002).

Webster, S. (1996). Measurement of crustacean hyperglycaemic hormone levels in the edible crab *Cancer pagurus* during emersion stress. *The Journal of Experimental Biology*, *199*(Pt 7), 1579–85. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9319482

Witherell, D., & Pautzke, C. (1997). A Brief History of Bycatch Management Measures for Eastern Bering Sea Groundfish Fisheries. *Marine Fisheries Review*, *59*(4), 15–22.

Zhou, S. J., & Shirley, T. C. (1995). Effects of handling on feeding, activity and survival of red king crabs, *Paralithodes camtschaticus* (Tilesius, 1815). *Journal of Shellfish Research*, *14*(1), 173–177.

3.6 Tables and Figures

Table 3.1: Specific reflex actions identified as useful to test stress in porcupine crab for RAMP tank experiment

Reflex Action	Test	Positive response	Negative response
Leg Flare	Lift crab by carapace, dorsum up.	One or more legs notably flare upwards or are held high in near horizontal orientation.	Legs droop down or are not held in the near horizontal orientation.
Leg Retraction	Grasp leg gently and pull.	Manipulated leg retracts proximally.	Legs does not retract.
Chela closure	Run metal probe along teeth of chela.	Chela closes down hard when manipulated, or opens and closes with little manipulation.	Chela does not close down when manipulated, and does not move in the absence of manipulation.
Cheliped retraction	Grasp cheliped and gently pull anteriorally.	Cheliped retracts posteriorally toward mouth region.	Cheliped does not retract.
Mouth retraction	Depress the maxillae down with a metal probe	Depressed maxillae move upward and inward protecting mouthparts.	Maxillae remain depressed after manipulation.
Kick	Placed in the ventral side up position, pull back gently on the abdominal flap	Immediate and strong movement of one or more chelipeds or walking legs towards the abdominal flap region.	Displays no movement of chelipeds or walking legs.
Primary antennae movement	Hold antennae in the dorsal position with a probe, or run probe along length of antennae	Atennae slightly moves at tip, or moves with little manipulation	Antennae exhibit no movement.
Secondary antennae movement	Touch the tip of the antennae with a probe	Antennae reacts with the typical jerking movement seen in tank-held crab	Antennae exhibit no movement.

Table 3.2: Portions of reflex actions failed in *Neolithodes grimaldii* (Porcupine crab) in 2014 and 2015. The left column represents the portion of each type of reflex that failed when only one

reflex failed. The right column represents the portion of each type of reflex that failed among all reflexes that failed.

	Crabs used in tank experiment				Crabs not used in tank experiment			
	Only failed reflex		Portion of all reflexes failed		Only failed reflex		Portion of all reflexes failed	
	Year I	Year II	Year I	Year II	Year I	Year II	Year I	Year II
Reflexes	(n=29)	(n=15)	(n=58)	(n=73)	(n=219)	(n=193)	(n=432)	(n=457)
Leg flare	0.72	0.47	0.55	0.33	0.70	0.50	0.51	0.36
Kick	0.28	0.53	0.33	0.34	0.24	0.47	0.26	0.33
Leg retraction	0	0	0.07	0.1	0.04	0.01	0.10	0.09
Chelae retraction	0	0	0.03	0.14	0.01	0	0.09	0.07
Mouth closure	0	0	0.02	0.03	0	0	0.01	0.04
Chelae closure	0	0	0	0.07	0.005	0.01	0.03	0.05
Antenna 1	0	0	0	0	0.005	0.02	0.01	0.03
Antenna 2	0	0	0	0	0	0	0	0.02

Table 3.3: Summary of reflex impairment scores for Year I and II (NA = not applicable)

	Year I	Year II

Total # of individuals examined	1122	1273
Number that failed reflex actions	357	303
Number that exhibited no lost reflexes	765	970
Number that exhibited 1 lost reflex	259	208
Number that exhibited 2 lost reflexes	61	45
Number that exhibited 3 lost reflexes	20	15
Number that exhibited 4 lost reflexes	9	11
Number that exhibited 5 lost reflexes	5	7
Number that exhibited 6 lost reflexes	3	7
Number that exhibited 7 lost reflexes	N/A	2
Number that exhibited 8 lost reflexes	0	8

Table 3.4: Summary of model 1 used to fit values seen in figure 3.1(A).

	Coefficients	Std. Error	Z value	P value
(Intercept)	-1.288	0.224	-5.764	8.235e-9
Reflex score	0.373	0.169	2.207	2.274e-2

Null deviance: 179.71 on 155 degrees of freedom
Residual deviance: 174.85 on 154 degrees of freedom

Table 3.5: Summary of observed mortality from tank experiment Year I and II.

	Year I	Year II

Reflex	Total	Dead	Mortality	Total	Dead	Mortality
0	97	23	0.24	67	42	0.63
1	38	9	0.24	15	12	0.80
2	12	3	0.25	8	7	0.88
3	3	2	0.67	7	7	1
4	5	3	0.60	3	3	1
5	1	1	1	3	3	1
6	0	0	0	1	1	1

Table 3.6: Year I Comparison of values of AIC and % variability explained for Reflex action mortality predictor logistic regression for porcupine crab.

Model	Explanatory Variables	df	AIC	% Variability explained
1	Reflex Score (RS)	2	178.85	2.70 %
2	RS + Sex	3	177.65	4.48 %
3	RS + Sex + CL	4	179.00	4.84 %
4	RS + Sex + CL + Inf	5	180.70	5.01 %

Variables CL and Inf, represent Carapace length (mm) and presence of rhizocephalan parasite infestation

Table 3.7: Summary of model 1 used to fit values seen in figure 3.1(B).

	Coefficients	Std. Error	Z value	P value
(Intercept)	0.501	0.247	2.031	0.042
Reflex score	0.968	0.389	2.490	0.013

Null deviance: 123.11 on 103 degrees of freedom

Residual deviance: 110.33 on 102 degrees of freedom

Table 3.8: Year II Comparison of values of AIC and % variability explained for Reflex action mortality predictor logistic regression for porcupine crab

Model	Explanatory Variables	df	AIC	% Variability explained
1	Reflex Score (RS)	2	114.33	10.37 %
2	Injury Score (IS)	2	120.60	5.28 %
3	RS + IS	3	110.35	15.22 %
4	RS + IS + (RS*IS)	4	112.32	15.25 %
5	RS + IS + Sex	4	112.15	15.40 %
6	RS + IS + Sex + CL	5	113.94	15.57 %
7	RS + IS + Sex + CL +	6	115.66	15.78 %

Variables CL and Inf, represent Carapace length (mm) and presence of rhizocephalan parasite infestation

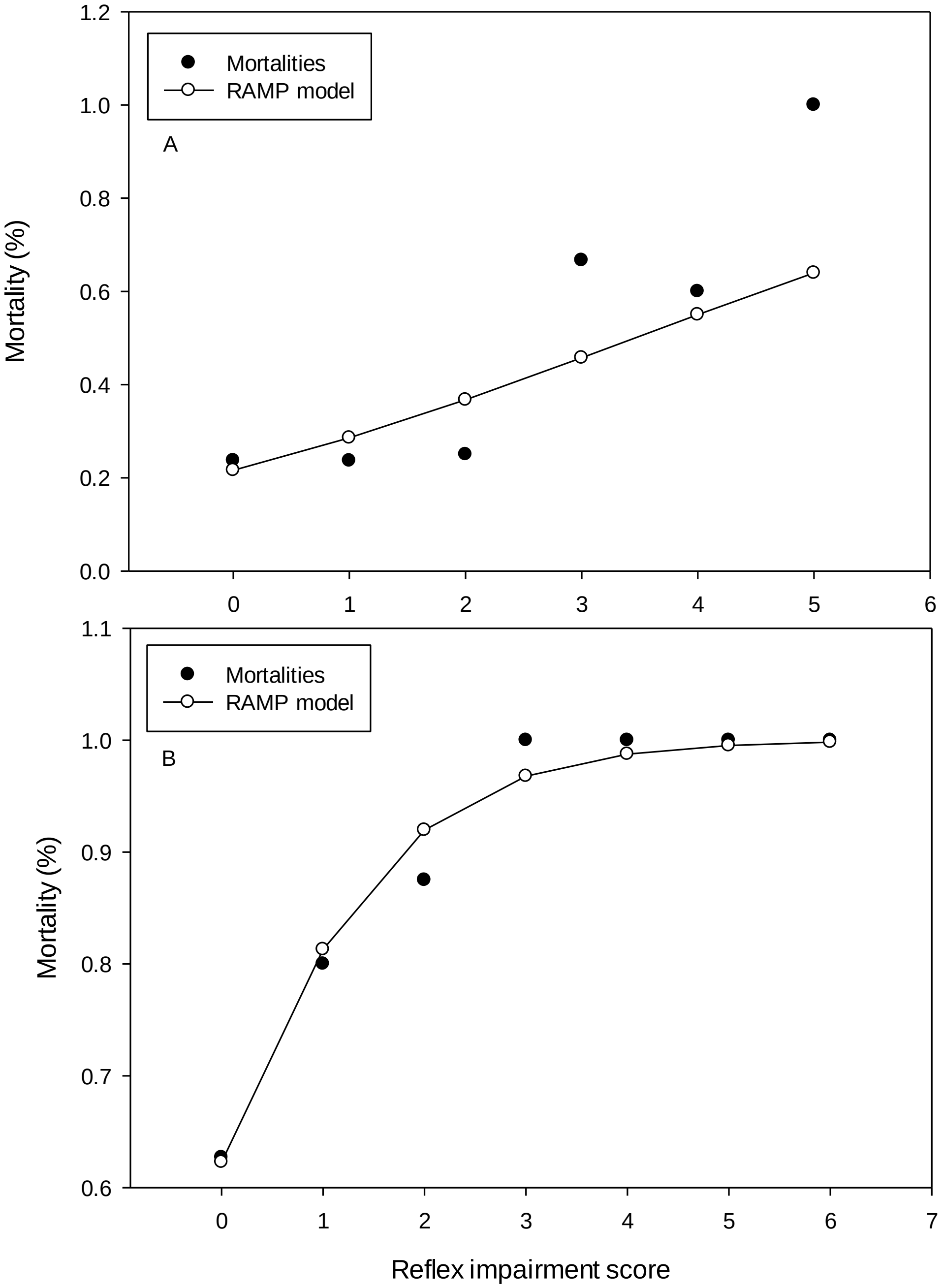

Figure 3.1: Comparison of observed mortalities and values fitted from GLM for Year I (A) and Year II (B) tank experiment.

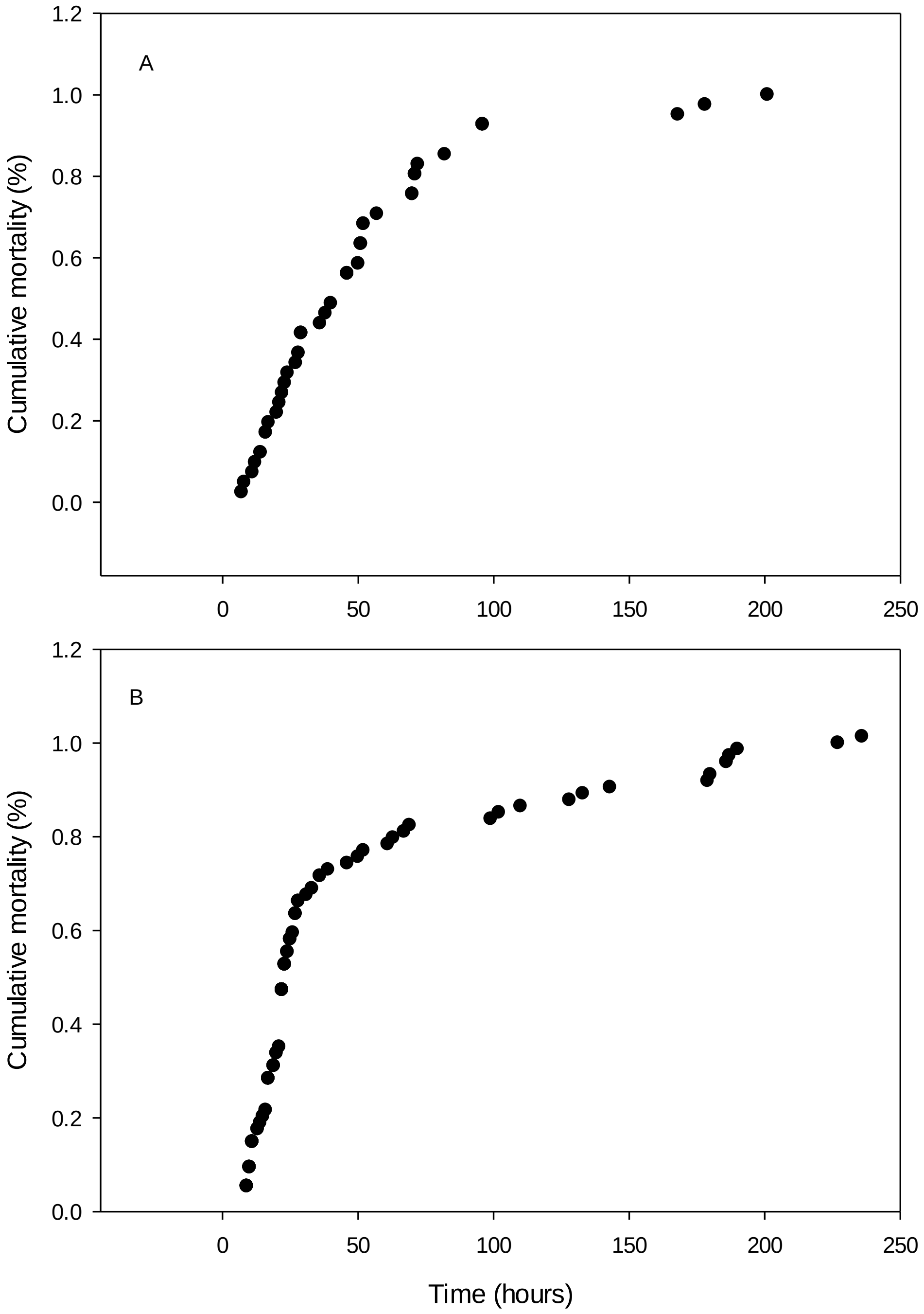

Figure 3.2: Cumulative mortality of crabs held in Year I (A) and Year II (B) survivorship experiment

4.1 Summary

The objectives of this study were to increase the baseline knowledge on the prevalence of parasite infestation and biological effects caused by *B. callosus*, and to assess post-capture survival of porcupine crab. Two years of successive sampling yielded a large amount of biological measurements taken in the field, as well as, subsequent dissection and analysis in the laboratory. Having two field seasons allowed for the knowledge gained on the anatomy and biology of the study species gained in the first year to be applied to the second year. This allowed for the methods utilized in the first year to be improved upon in the second year.

In chapter two the sampling conducted had limitations in the area and depths at which the samples were taken. These limitations are a consequence of being part of commercial fishing operations. Future studies may aim to increase the depths at which porcupine crab are captured to compare prevalence rates with those found at the depths sampled in this study. In addition, future studies could also sample different areas within NAFO Division 0B to see if the prevalence rates found are consistent across the Division or if small pockets of increased prevalence are present.

Time and space constraints of the study platform and a lack of transportation capability of live crabs prevented any long term observations of porcupine crab in captivity. Continued observations of infested porcupine crab held in captivity would help to answer some of the questions posed by this study. Hoggarth (1990) suggested that males are permanently sterilized due to larger scarred individuals having reduced right chela allometry parameters seen in those with attached externa. If porcupine crab could be held in captivity after removal of the externa it would potentially provide information on whether males and females are permanently sterilized, or if reproductive capability can return subsequent to externa removal. In addition, further observation would revealed whether the interna is covered in scar tissue as the body elicits an auto-immune response following an externa removal as suggested by Sparks and Morado (1986), or if a new externa can grow from an old interna as suggested by Isaeva (2005).

Continued monitoring of porcupine crab populations infested with *B. callosus* into the future is important for the conservation of this species. Continued monitoring could reveal if methods to control infestation such as destruction of infested individuals would affect the overall prevalence of local porcupine crab populations.

For chapter three the monitoring and holding times of porcupine crab used to test post-capture survival were limited by the commercial fishing practices. Future studies should aim to have increased holding times to observe if mortality results differ. Based on the results of this study the shock or concussive damage to porcupine crab internal organs has been suggested as one factor leading to eventual mortality; if a new RAMP model could be developed in the future to incorporate concussive damage possibility by means of drop height it could increase confidence in predicting mortality. Another method that could be adopted for future studies is to observe the process taken and time elapsed during the removal process to see if there is a correlation with post-capture survival.

The results found for testing the RAMP method on gillnet captured porcupine crab differed from king crab captured by bottom trawl. To provide a comparison to the methods in this study and previous RAMP research, future studies could capture porcupine crab by means of bottom trawl. This would allow a direct comparison to see if the results of testing the RAMP method differ based on capture gear used. While the focus of this study was on the post-capture survival of porcupine crab as bycatch in the Greenland halibut gillnet fishery a study conducted on bottom trawl captured porcupine crab could strengthen the result that this species exhibits heightened stress response when captured and subsequently removed from gillnet.

Through the information collected in this study the baseline knowledge on the biology of porcupine crab has been improved. The difficulties of using the RAMP approach on gillnet captured porcupine crab have been highlighted, which can be referenced for future studies. The negative effects and prevalence of *B. callosus* discovered showcase the importance of research conducted on non-targeted species captured as bycatch. The results of this study can be used to improve future research into understanding the biology of the porcupine crab and to aid in the conservation of this species into the future.