Laboratory study of reproduction and development of *Lopholithodes foraminatus* (brown box crab), with a discussion of reversed asymmetry

by

William Duguid
B.Sc, University of Victoria, 2002

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

© William Duguid, 2009
University of Victoria

All rights reserved. This thesis may not be reproduced in whole or in part, by photocopy or other means, without the permission of the author.

Supervisory Committee

Laboratory study of reproduction and development of *Lopholithodes foraminatus* (brown box crab), with a discussion of reversed asymmetry

by

William Duguid
B.Sc, University of Victoria, 2002

Supervisory Committee

Dr. Louise R. Page (Department of Biology)
Supervisor

Dr. Tom E. Reimchen (Department of Biology)
Departmental Member

Dr. Verena Tunnicliffe (Department of Biology & School of Earth and Ocean Sciences)
Departmental Member

Abstract

Supervisory Committee

Dr. Louise R. Page (Department of Biology)
Supervisor

Dr. Tom E. Reimchen (Department of Biology)
Departmental Member

Dr. Verena Tunnicliffe (Department of Biology & School of Earth and Ocean Sciences)
Departmental Member

Lithodid crabs present intriguing questions about evolution of reproductive strategies and developmental evolution of asymmetry. Lopholithodes foraminatus *(Decapoda: Anomura: Paguroidea) from British Columbia have a biennial reproductive cycle. Eighteen months of egg-brooding included an embryonic diapause of 12 months. Larvae were released over a long period of up to 3 months due to pronounced differential developmental rate that was apparently not due to differential oxygen availability among brooded eggs. I describe the behaviour, growth, and morphology of 4 zoeal stages, a non-feeding glaucothoe, and early juvenile instars. Approximately 25% of glaucothoe showed reversed asymmetry, which was surprising considering its rarity among field collected adults. Incidence of reversed asymmetry was not affected by rearing temperature or by cheliped removal and did not differ among offspring of reversed and normal females. Lability in the direction of asymmetry during development is enigmatic in light of long-term evolutionary stability of this character among lithodids.*

Table of Contents

List of Tables

List of Figures

Acknowledgments

I am grateful to many people who provided advice at various stages of this work including: Dr. Greg Jensen, Dr. Anna Epelbaum, Mr. Antan Phillips, Dr. Richard Palmer, Dr. Akash Sastri, and Dr. Edd Hammill. The staff of the Aquatics Unit of the Animal Care Facility at the University of Victoria, in particular Brian Ringwood, provided invaluable assistance with animal husbandry and data collection. In the field Mark Sloan, Rob Nugent, and Kyle Stubbs helped to pull traps (and eat prawns). Jen Tyler, Alison Page, Candice St. Germaine, Heidi Gartner, Jon Rose, Mathis Stoekle, Nicole Laforge, Ian Beverage, and Kim Davies provided moral support. This project would never have been initiated without Joe Watson of Campbell River B.C. who donated boat time and expertise to capture adult box crabs. I thank my committee members, Tom Reimchen and Verena Tunnicliffe, for their time and their valuable suggestions. Most importantly I thank Louise, Faron, and my parents, all of whom have stood by me, supported me and had faith in me over the long haul. This work was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) discovery grant to Dr. Louise Page.

Dedication

I dedicate this thesis to my parents, David and Patricia Duguid. They have always had faith in me and have supported my education in every way possible.
I will always be grateful.

Chapter 1: Introduction

OVERVIEW

Marine decapod crustaceans are morphologically and ecologically diverse; occupying benthic and pelagic habitats across a wide depth range in all oceans. Most decapod infraorders include species harvested for human consumption, some of which support economically important commercial fisheries. The large size and formidable armament of benthic decapods such as crabs and lobsters make them important top-down regulators of marine ecosystems (e.g. Behrens Yamada & Boulding, 1996; Lafferty, 2004; Jørgensen, 2005; Jones & Shulman, 2008). However, the dramatic increase in size experienced by most species during development also results in significant ontogenetic changes in ecological role (e.g. Richards, 1992; Sainte-Marie & Chabot, 2002).

Most marine decapods have complex life histories, normally involving pelagic larval forms. These larvae differ so dramatically from adults in morphology that they were originally identified as separate genera. The logistical challenges of rearing larvae, in particular the lack of a suitable food source, inhibited studies of decapod development until the second half of the 20th century (see historical review by Ingle, 1998). To date, the complete life history of many ecologically and economically important decapods remains unstudied. This lack of knowledge regarding the basic biology of many species limits our ability to understand ecological interactions and to develop conservation strategies; highlighting the continued need for traditional natural history research (Dayton, 2003; Fleischner, 2005; Greene, 2005).

DECAPOD LIFE HISTORY

Decapod crustaceans employ a diversity of reproductive strategies. Most taxa are gonochoristic, although true shrimps (infraorder Caridea) also exhibit protandric hermaphroditism (e.g. Gavio et al., 2006) or protandric simultaneous hermaphroditism (e.g. Baeza et al., 2009). Male decapods produce spermatophores. These may be attached to the external surface of the female or deposited into seminal receptacles that are either isolated from, or continuous with, the female reproductive tract. With the exception of the more derived Brachyura, the absence of sperm storage structures, or sperm storage in exoskeletal compartments, forces coordination of mating with the molt cycle (Subramoniam, 1993). Many decapods exhibit mate guarding, in which the male attends the female for a period prior to (and sometimes after) mating (e.g. Wada et al., 1997). Mate guarding is common for animals in which female sexual receptiveness is temporally limited (Grafen & Ridley, 1983; Jormalainen, 1998).

Decapod eggs are fertilized as they are extruded, either by passing through spermatheca containing stored spermatozoa (higher Brachyura, Warner, 1977), or by external fertilization with spermatozoa received in spermatophores from a simultaneously spawning male or stored in compartments of the female's exoskeleton. With the exception of penaeoid and sergestoid shrimp (suborder Dendrobranchiata), which release fertilized eggs into the water column, female decapods brood eggs attached to their pleopods. In species where the egg mass is tightly packed in the space between the thorax and abdomen (e.g. some brachyuran crabs), females may provide oxygen to their embryos by abdominal flapping (Naylor et al., 1999; Baeza & Fernández, 2002; Fernández & Brante, 2003). Female decapods may also groom their egg mass with

specialized appendages to remove dead embryos and other debris (e.g. Pohle, 1989; Forster & Baeza, 2001). The duration of embryogenesis depends on temperature, but may be extended by periods of diapause. The time spent in diapause may (Moriyaso & Lanteigne, 1998; Stevens et al., 2008) or may not (Wear, 1974) be dependent on temperature. All decapods in the suborder Pleocyemata (true crabs, anomuran crabs, lobsters, and shrimp) pass through the nauplius stage (a larval form which defines the Crustacea), during embryogenesis, and most hatch as zoea larvae (exceptions include freshwater crayfish and other groups which pass through the zoeal stages in the egg and hatch as fully formed juveniles).

Newly hatched zoeae are covered by a transparent prezoeal cuticle that is shed soon after hatching (Hong, 1988). Zoeae swim by vigorously beating the exopods of the maxillipeds, which bear long, plumose, natatory setae. The majority of decapods pass through several pelagic, planktotrophic (feeding) zoeal stages. However, lecithotrophic (non-feeding) development has evolved in some representatives of several infraorders. Lecithotrophy is often associated with a reduction in the number of zoeal instars (Anger, 2001). Upon completing zoeal development, most species of decapods pass through a stage defined by a combination of larval and juvenile characteristics. The maxillipeds are now mouthparts, as in adults, and pleopods (abdominal appendages) are used for swimming. The pereopods are functional and may be used to walk on the substrate. This stage has been variously termed a postlarva, megalopa, or decapodid (Anger, 2006). In pagurid and lithodid crabs, it may be referred to as a glaucothoe. In species with benthic, non-swimming adults (e.g. brachyuran crabs, hermit crabs, lithodid crabs), the ability to swim using the pleopods is lost in the molt from the megalopa to the first juvenile instar.

LITHODID BIOLOGY

Representatives of the anomuran family Lithodidae (king and stone crabs) resemble the true crabs of the infraorder Brachyura. Like brachyurans, they have a broad, calcified carapace and hold the abdomen against the ventral surface of the thorax. Unlike brachyurans, lithodids have 3 rather than 4 pairs of walking legs. The 5th pereopods (homologous to the 4th walking legs of brachyurans) are folded back on themselves and held within the branchial chamber. These appendages are used to clean the gills and can also be brought outside of the branchial chamber to groom the egg mass (Pohle, 1989). The Lithodidae consists of two subfamilies, the Lithodinae and the Hapalogastrinae. Subfamily Lithodinae has a global distribution and includes all of the large, commercially important lithodids including the red king crab (*Paralithodes camtschaticus*), blue king crab (*Paralithodes platypus*), golden king crab (*Paralithodes aequispinus*), southern king crab (*Lithodes santolla*), and false southern king crab (*Paralomis granulosa*). Subfamily Hapalogastrinae includes 5 genera of smaller, relatively inconspicuous crabs inhabiting intertidal and coastal subtidal waters of the North Pacific. While lithodines have a well calcified abdomen, not unlike that of brachyuran crabs, hapalogastrines have a soft abdomen lacking calcified exoskeletal plates. Lithodids exhibit conspicuous asymmetry: both sexes have a larger right chela, and females have a dextrally offset abdomen with pleopods generally present on both sides of the first abdominal segment but only on the left-hand side of segments 2-5. Cases of reversed asymmetry are rare enough to warrant published notes (Campodonico, 1978; Zaklan, 2000; Motoh & Toyota, 2006).

The directional asymmetry of the Lithodidae, along with other morphological characters, has led many authors to speculate that this family could be derived from an

ancestor in the right-handed hermit crab family Paguridae (reviewed in McLaughlin & Lemaitre, 1997). This hypothesis has recently been supported by morphological (Richter & Scholtz, 1994), developmental (Macdonald et al., 1957), and molecular evidence (Cunningham et al., 1992; Morrison et al., 2002; Zaklan, 2002; Tsang et al., 2008); but is still debated by some researchers (McLaughlin & Lemaitre, 1997; McLaughlin et al., 2004; McLaughlin et al., 2007). The soft abdomen of hapalogastrines has been interpreted as intermediate between the soft abdomen of pagurids and the calcified abdomen of lithodines (see review by McLaughlin & Lemaitre, 1997). The hypothesis that hapalogastrines are basal within the Lithodidae has been supported by developmental (Konishi, 1986) and molecular evidence (Zaklan, 2002). Some authors suggest that the Lithodidae likely arose in the North Pacific as the Hapalogastrinae are limited to this region and the diversity of both subfamilies is centered in the Northeast Pacific (Marakov, 1968; Zaklan, 2002)

Male lithodids lack intromittent organs and females have no sperm storage capacity. Egg extrusion and fertilization therefore occur soon after the female molts (e.g. Wada et al., 1997). Embryogenesis may be completed within a single year (e.g. *Paralithodes camtschaticus,* Stevens & Swiney, 2007) or may require almost 2 years (e.g. *Paralomis granulosa,* Lovrich & Vinuesa, 1993). Relative to other decapods, female lithodids release zoeae over an extended period, possibly as a result of differential development rates resulting from an oxygen gradient within the egg mass (Thatje et al., 2003). Lithodids pass through between 2 (e.g. lecithotrophic development of *Paralomis spinosissima*, Watts et al., 2006) and 4 (e.g. planktotrophic development of *Lopholithodes mandtii*, Crain & McLaughlin, 2000) zoeal stages. As all hapalogastrines so far

investigated pass through 4 feeding zoeal stages and one glaucothoe stage, this likely represents the ancestral developmental pattern in the Lithodidae (Konishi, 1986; Zaklan, 2002).

Lopholithodes foraminatus

The brown box crab, *Lopholithodes foraminatus* (Simpson, 1859), is a moderately large lithodine (males to 20cm carapace width) found on the West Coast of North America from California to Alaska. *L. foraminatus* inhabits soft substrates (Jensen, 1995) in relatively deep water (highest CPUE at 101-125 m in the Strait of Georgia, Zhang, 2001). Box crabs may be deposit feeders (Jensen, 1995); but beyond this, relatively little is known about their ecology. *L. foraminatus* is considered to have a high culinary value (McDaniel, 1985). Possibly due to this fact, box crabs have been harvested in a small scale commercial fishery in Oregon since 1982 and interest has been expressed in developing a commercial fishery in British Columbia (Zhang et al., 1999; Zhang, 2001). *L. foraminatus* is also caught as by-catch in groundfish and shrimp trawl fisheries (Kato, 1992; Zhang et al., 1999). No data have been published on the reproductive cycle or larval development of *Lopholithodes foraminatus*, and Zhang et al. (1999) identified this lack of information as an obstacle to the development of management strategies for the species.

OBJECTIVES

In this thesis I characterize the reproductive cycle of *Lopholithodes foraminatus*, describe the behaviour, growth and morphology of larvae and post-larvae, and investigate the phenomenon of reversed asymmetry in juveniles.

I determined the reproductive cycle of *L. foraminatus* by maintaining adults in the lab over almost a 3 year period and relating their reproductive status to that of individuals captured in the field at different times of year. I supplemented these observations with microscopic examination of embryos throughout development. I also collected data on the timing and duration of larval release. To test the hypothesis that extended hatching in lithodid crabs is a consequence of differential developmental rates resulting from an oxygen gradient within the egg mass, I assayed for a spatial gradient in developmental rate within the egg mass of one female.

I reared *Lopholithodes foraminatus* zoeae, glaucothoe, and juvenile stages and described their behaviour, growth, colour, and morphology. I also tested whether zoeae and glaucothoe were planktotrophic or lecithotrophic.

In the process of rearing glaucothoe and juvenile *L. foraminatus* I noticed a high incidence of offspring with a larger left claw (reversed asymmetry). I also captured a brooding female exhibiting reversed asymmetry of the chelae and abdomen. These circumstances provided an opportunity to address a number of questions regarding the phenomenon of reversed asymmetry in lithodid crabs:

Is the frequency of reversed asymmetry related to the direction of maternal asymmetry?

Do left-handed and right-handed glaucothoe represent distinct phenotypes?

Does reversed asymmetry of the chelae in glaucothoe predict the subsequent development of morphological asymmetry?

Is the frequency of reversed asymmetry affected by rearing temperature?

Can the direction of asymmetry be reversed by cheliped removal?

Do reversed glaucothoe show evidence of deleterious phenotypic characters?

REFERENCES

Anger K. (2001) Morphology: abbreviated development. In Vonk R. (ed.) *Biology of Decapod Crustacean Larvae*. Lisse: A. A. Balkema Publishers, pp. 32-36.

Anger K. (2006) Contributions of larval biology to crustacean research: a review, *Invertebrate Reproduction and Development*, 49, 175-205.

Baeza J.A. and Fernández M. (2002) Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding. *Functional Ecology*, 16, 241-251.

Baeza J.A., Schubart C.D., Zillner P., Fuentes S. and Bauer R.T. (2009) Molecular phylogeny of shrimps from the genus *Lysmata* (Caridea: Hippolytidae): the evolutionary origins of protandric simultaneous hermaphroditism and social monogamy. *Biological Journal of the Linnean Society*, 96, 415-424.

Behrens Yamada S. and Boulding E.G. (1996) The role of highly mobile crab predators in the intertidal zonation of their gastropod prey. *Journal of Experimental Marine Biology and Ecology*, 204, 59-83.

Campodonico G.I. (1978) Un caso de inversión en la asimetría abdominal de las hembras de *Paralomis granulosa* (Jacquinot) (Decapoda, Anomura, Lithodidae). *Anales del Instituto de la Patagonia*, 9, 231-232.

Crain J.A. and McLaughlin P.A. (2000) Larval and early juvenile development in the Lithodidae (Decapoda: Anomura: Paguroidea) reared under laboratory conditions. 1. subfamily Lithodinae: *Lopholithodes mandtii* Brandt, 1848. *Invertebrate Reproduction and Development*, 37, 43-59.

Cunningham C.W., Blackstone N.W. and Buss L.W. (1992) Evolution of king crabs from hermit crab ancestors. *Nature*, 335, 539-542.

Dayton P.K. (2003) The importance of the natural sciences to conservation (an American Society of Naturalists symposium paper). *The American Naturalist*, 162, 1-16.

Fernández M. and Brandte A. (2003) Brood care in brachyuran crabs: the effect of oxygen provision on reproductive costs. *Revista Chilena de Historia Natural*, 76, 157-168.

Fleischner T.L. (2005) Natural history and the deep roots of resource management. *Natural Resources Journal*, 45, 1-13.

Forster C. and Baeza J.A. (2001) Active brood care in the anomuran crab *Petrolisthes violaceus* (Decapoda: Anomura: Porceallanidae): Grooming of brooded embryos by the fifth pereiopods. *Journal of Crustacean Biology*, 21, 606-615.

Gavio M.A., Orensanz J.M. and Armstrong D. (2006) Evaluation of alternative life history hypotheses for the sand shrimp *Crangon franciscorum* (Decapoda: Caridea). *Journal of Crustacean Biology*, 26, 295-307.

Grafen A. and Ridley M. (1983) A model of mate guarding. *Journal of Theoretical Biology*, 102, 549-567.

Green H.W. (2005) Organisms in nature as a central focus for biology. *Trends in Ecology and Evolution*, 20, 23-27.

Hong S.Y. (1988) The prezoeal stage in various decapods crustaceans. *Journal of Natural History,* 22, 1041-1075.

Ingle R.W. (1998) Decapod larval taxonomic research in the North Eastern Atlantic and Mediterranean: past achievements and future prospects. *Invertebrate Reproduction and Development*, 33, 97-107.

Jensen G.C. (1995) *Pacific Coast crabs and shrimps*. Monterey, California: Sea Challengers.

Jones P.L. and Shulman M.J. (2008) Subtidal-intertidal trophic links: American lobsters [*Homarus americanus* (Milne-Edwards)] forage in the intertidal zone on nocturnal high tides. *Journal of Experimental Marine Biology and Ecology*, 361, 98-103.

Jørgensen L.L. (2005) Impact scenario for an introduced decapod on Arctic epibenthic communities. *Biological Invasions*, 7, 949-957.

Jormalainen V. (1998) Precopulatory mate guarding in crustaceans: male competitive strategy and intersexual conflict. *The Quarterly Review of Biology*, 73, 275-304.

Kato S. (1992) Box crab. In Leet W.S., Dewees C.M. and Haugen C.W. (eds) *California's living marine resources and their utilization*. Davis CA: Sea Grant Extension, pp. 192.

Konishi K. (1986) Larval development of the stone crab, *Hapalogaster dentata* (De Haan, 1844) (Crustacea: Anomura: Lithodidae) reared in the laboratory. *Journal of the Faculty of Science of Hokkaido University Series VI, Zoology*, 24, 155-172.

Laffery K.D. (2004) Fishing for lobsters indirectly increases epidemics in sea urchins. *Ecological Applications*, 14, 1566-1573.

Lovrich G.A. and Vinuesa J.H. (1993) Reproductive biology of the false southern king crab (*Paralomis granulosa*, Lithodidae) in the Beagle Channel, Argentina. *Fishery Bulletin*, 91, 664-675.

Marakov V.V. (1962) In A.A. Shtakelberg (ed.) *Fauna of U.S.S.R. (Crustacea).* Jerusalem: Israel Program for Scientific Translations, pp. 1-283.

McDaniel M. (1985) Sensory examination of meat from Dungeness and box crabs. Oregon State University. Dept. of Food Science and Technology. Corvalis, Or. 12pp.

MacDonald J.D., Pike R.B. and Williamson D. (1957) Larvae of the British species of *Diogenes*, *Pagurus*, *Anapagurus*, and *Lithodes* (Crustacea, Decapoda). *Proceedings of the Zoological Society of London*, 128, 209-257.

McLaughlin P.A. and Lemaitre R. (1997) Carcinization in the Anomura – fact or fiction? I. Evidence from adult morphology. *Contributions to Zoology*, 67, 79-123.

McLaughlin P.A., Lemaitre R. and Tudge C.C. (2004) Carcinization in the Anomura - fact or fiction? II. Evidence from larval, megalopal and early juvenile morphology. *Contributions to Zoology*, 73, 165-205.

McLaughlin P.A., Lemaitre R. and Sorhannus U. (2007) Hermit crab phylogeny: a reappraisal and its "fall-out". *Journal of Crustacean Biology*, 27, 97-115.

Morrison C.L., Harvey A.W., Lavery S., Tieu K., Huang Y. and Cunningham C.W. (2002) Mitochondrial gene rearrangements confirm the parallel evolution of the crab-like form. *Proceedings of the Royal Society Series B*, 269, 345-350.

Motoh H. and Toyota K. (2006) A case of reversed asymmetry in a lithodid crab, *Paralomis hystrix*. *Cancer*, 15, 25-27.

Moriyasu M. and Lanteigne C. (1998) Embryo development and reproductive cycle in the snow crab, *Chionoecetes opilio* (Crustacea: Majidae), in the southern Gulf of St. Lawrence, Canada. *Canadian Journal of Zoology*, 76, 2040-2048.

Naylor J.K., Taylor E.W. and Bennet D.B. (1999) Oxygen uptake of developing eggs of *Cancer pagurus* (Crustacea: Decapoda: Cancridae) and consequent behaviour of the ovigerous females. *Journal of the Marine Biological Association of the United Kingdom*, 79, 305-315.

Pohle G. (1989) Gill and embryo grooming in lithodid crabs: comparative functional morphology based on *Lithodes maja.* In Felgenhauer B.E., Watling L. and Thistle A.B. (eds) *Functional Morphology of Feeding and Grooming in Crustacea. Crustacean Issues*, 6. Rotterdam: A.A. Balkema, pp. 75-94.

Richards A.R. (1992) Habitat selection and predator avoidance: ontogenetic shifts in habitat use by the Jonah crab Cancer borealis (Stimpson). *Journal of Experimental Marine Biology and Ecology*, 156, 187-197.

Richter S. and Scholtz G. (1994) Morphological evidence for a hermit crab ancestry of lithodids (Crustacea, Decapoda, Anomala, Paguroidea). *Zoologischer Anzeiger*, 223, 187-219.

Sainte-Marie B. and Chabot D. (2002) Ontogenetic shifts in natural diet during benthic stages of American lobster (*Homarus americanus*) off the Magdalen Islands. *Fishery Bulletin*, 100, 106-116.

Stevens B.G. and Swiney K.M. (2007) Hatch timing, incubation period, and reproductive cycle of captive primiparous and multiparous red king crab, *Paralithodes camtschaticus*. *Crustacean Biology*, 27, 37-48.

Stevens B.G., Swiney K.M. and Buck L. (2008) Thermal effects on embryonic development and hatching for blue king crab *Paralithodes platypus* (Brandt, 1850) held in the laboratory, and a method for predicting dates of hatching. *Journal of Shellfish Research*, 27, 1255-1263.

Subramoniam T. (1993) Spermatophores and sperm transfer in marine crustaceans. *Advances in Marine Biology*, 29, 129-214.

Thatje S., Calcagno J.A., Lovrich G.A., Sartoris F.J. and Anger K. (2003) Extended hatching periods in the subantarctic lithodid crabs *Lithodes santolla* and *Paralomis granulosa* (Crustacea: Decapoda: Lithodidae*). Helgoland Marine Research*, 57, 110-113.

Tsang L.M., Ma K.Y., Ahyong S.T., Chan T.-Y. and Chu K.H. (2008) Phylogeny of Decapoda using two nuclear protein-coding genes: Origin and evolution of the Reptantia. *Molecular Phylogenetics and Evolution*, 48, 359-368.

Wada A., Ashidate M. and Goshima S. (1997) Observations on the reproductive behavior of the spiny king crab *Paralithodes brevipes* (Anomura: Lithodidae). *Custacean Research*, 26, 56-61.

Warner G.F. (1977) *The Biology of Crabs*. London: Paul Elek (Scientific Books).

Watts J., Thatje S., Clarke S. and Belchier M. (2006) A description of larval and early juvenile development in *Paralomis spinosissima* (Decapoda: Anomura: Paguroidea: Lithodidae) from South Georgia waters (Southern Ocean*). Polar Biology*, 29, 1028-1038.

Wear R.G. (1974) Incubation in British decapod Crustacea, and the effects of temperature on the rate and success of embryonic development. *Journal of the Marine Biological Association of the United Kingdom,* 54, 745-762.

Zaklan S.D. (2000) A case of reversed asymmetry in *Lithodes maja* (Linnaeus, 1758) (Decapoda, Anomura, Lithodidae). *Crustaceana*, 73, 1019-1022.

Zaklan S.D. (2002) Review of the family Lithodidae (Crustacea: Anomura: Paguroidea): Distribution, biology, and fisheries. In Paul A.J., Dawe E.G., Elner R., Jamieson G.S., Kruse G.H., Otto R.S., Sainte-Marie B., Shirley T.C. and Woodby D. (eds), *Crabs in Cold Water Regions: Biology, Management, and Economics*. Fairbanks: University of Alaska Sea Grant College Program, pp. 751-845.

Zhang Z.Y. (2001) Evaluation of a box crab (*Lopholithodes foraminatus*) trap test in British Columbia. *Canadian Science Advisory Secretariat Research Document* 2001/158. Ottawa: Fisheries and Oceans Canada, 41 pp.

Zhang Z.Y., Workman G. and Phillips A.C. (1999) A review of the biology and fisheries of the box crab (*Lopholithodes foraminatus* Stimpson) in British Columbia. *Canadian Stock Assessment Secretariat Research Document* 99/131. Ottawa: Fisheries and Oceans Canada, 38 pp.

Chapter 2: Reproductive timing and embryogenesis of *Lopholithodes foraminatus* (Stimpson, 1859) in British Columbia waters.

ABSTRACT

A paucity of data on the reproductive cycle of crabs in the family Lithodidae prevents both the development of management strategies and the formulation of hypotheses regarding the evolution of lithodid life history strategies. Life history parameters of Lopholithodes foraminatus *from British Columbia, Canada were investigated based on 26 females maintained in the laboratory and supplementary observations of live and preserved animals. The rate of embryonic development was determined by measuring the percentage area occupied by yolk in lateral views of eggs removed from brooding females throughout development. This measurement was also used to assay for a gradient in developmental rate within a single egg mass.* L. foraminatus *females exhibited biennial reproduction including an 18 month brooding period. Females molted, extruded eggs and mated in mid-summer and did not release larvae until late winter or early spring of the second year after fertilization. Embryogenesis included a 12 month diapause at the gastrula stage. Females released larvae for a mean of 69 days, the longest period reported for any lithodid. While the development stage of embryos was observed to be heterogeneous within a brood, no spatial gradient in development rate was detected, calling into question the oxygen limitation hypothesis of extended hatching. Biennial reproduction of* L. foraminatus *may be a consequence of occupying relatively low quality habitat. Relative to annual reproduction, biennial reproduction halves the potential rate of increase of a population. A reduced rate of increase may increase vulnerability to overharvest, suggesting that* L. foraminatus *is not a good candidate for commercial exploitation. The adaptive value of embryonic diapause is uncertain and warrants further research.*

INTRODUCTION

Knowledge of life history parameters is critical to the development of management strategies for commercially harvested marine organisms. Species with slow growth, late first reproduction, and a low potential rate of population increase may be more vulnerable to overfishing than those with rapid growth, early first reproduction, and a high potential rate of population increase (Adams, 1980). Commercially exploited representatives of the family Lithodidae (king crabs) have a history of stock collapse. Red king crab *(Paralithodes camtschaticus)* (Orensanz et al., 1998), blue king crab *(Paralithodes platypus)* (Stevens, 2006a), and southern king crab *(Lithodes santolla)* (Lovrich & Vinuesa, 1999) have all experienced dramatic fishing-related declines. In addition to directed fisheries, by-catch can also have devastating impacts on lithodid populations. The decimation of the female broodstock of the Bristol Bay red king crab population by groundfish trawling (Dew & McConnaughey, 2005) illustrates the danger of fisheries policy that is not predicated on adequate life history data.

Lithodid crabs exhibit a number of life history traits that separate them from the morphologically similar but phylogenetically distant true crabs (family Brachyura). Unlike brachyurans, female lithodids are incapable of sperm storage and must molt, extrude eggs and mate almost simultaneously. Eggs are fertilized externally. The inability to store sperm can result in females missing a reproductive cycle if the supply of males is disrupted during the breeding period (Sato et al., 2005). As in other decapods (with the exception of penaeid shrimp), fertilized eggs are attached to the pleopods of the female and are brooded for the duration of embryogenesis. Lithodids generally have larger eggs and lower fecundity than sympatric true crab species of similar size; for example, 50,000

- 300,000 eggs 1.0-1.2 mm in diameter for *Paralithodes platypus* (Somerton & MacIntosh, 1985) versus 938,000 eggs 0.44 mm in diameter for *Cancer magister* (Hines, 1991). Where data are available, lithodids also appear to mature later and live longer than sympatric brachyurans (Gulf of Alaska commercial species reviewed in Table 2 of Orensanz et al., 1998). Relative to other decapods, lithodid crabs exhibit a very extended duration of larval release, often 30 days or more (Paul & Paul, 2001; Thatje et al., 2003; Stevens, 2006b; Stevens & Swiney, 2007). Brachyuran crabs generally release larvae over a much shorter period, measured in hours or days rather than weeks or months (discussed by Stevens & Swiney, 2007). Some authors have suggested that extended hatching could be an adaptive strategy to ensure that at least some larvae emerge at the right time to exploit the spring plankton bloom (Stevens, 2006b; Stevens & Swiney, 2007). Others have proposed that it may be a consequence of differential development rates resulting from an oxygen gradient within the egg mass (Thatje, 2004; Thatje et al., 2003; Reid et al., 2007).

In addition to its management implications, life history data across a taxon allow for the formulation and testing of hypotheses regarding the evolution of life history strategies within that group. There is considerable variability in reproductive traits within the subfamily Lithodinae, which includes all of the large commercially harvested lithodids. Dramatic differences exist even between congenerics and morphologically or ecologically similar species. Some lithodines invest in few large eggs and produce lecithotrophic larvae, while others invest in many small eggs and produce planktotrophic larvae. For example, a female golden king crab *(Lithodes aequispinus)* of 120 mm carapace length produces 11,330 eggs 2.2 mm in diameter (Otto & Cummisky, 1985),

while a similar sized female red king crab produces 150,000 eggs 1 mm in diameter (Haynes, 1968). In addition, different lithodine species may spawn annually, biennially or asynchronously (Lovrich & Vinuesa, 1993), leading to differences in predicted lifetime fecundity. Differences occur even among species falling into each of these 3 categories. For species with biennial reproduction, embryogenesis may occupy 1 year of the 2 year cycle as in blue king crab (Jensen & Armstrong, 1989), or as much as 18 to 22 months as in false southern king crab *(Paralomis granulosa)* (Lovrich & Vinuesa, 1993). Even within a species, different age classes may exhibit different reproductive strategies. Small female blue king crab may produce broods in consecutive years while large females reproduce biennially (Jensen et al., 1985). The variation in reproductive traits among lithodids makes this family a candidate for research into life history evolution.

A major obstacle to the formulation of hypotheses regarding lithodid life history evolution is the narrow taxonomic focus of research on the reproductive biology of this family. Essentially all published work focuses on the three most commercially important genera: *Lithodes, Paralomis* and *Paralithodes.* Information on the reproduction of the other 6 genera of lithodines, and on the 5 genera in the subfamily Hapalogastrinae, is mostly limited to anecdotal accounts of the timing of mating or hatching (see Zaklan, 2002). Information on the reproductive biology of species in the genus *Lopholithodes* falls into this category despite their large size, coastal habitat, and commercial and recreational harvesting.

Lopholithodes mandtii (Brandt, 1849) (Puget Sound king crab) and *Lopholithodes foraminatus* (Stimpson, 1859) (brown box crab) occur in coastal waters on the West Coast of North America from Alaska to California. Both species grow quite large;

L. mandtii up to 30 cm carapace width (CW) and *L. foraminatus* up to 18.5 cm CW (Jensen, 1995). Recreational harvesters take some *L. mandtii* by SCUBA diving and *L. foraminatus* in traps (pers. obs.). The recreational limit for both species in British Columbia is 1 per day with no size restrictions (Fisheries and Oceans Canada, 2007). *Lopholithodes foraminatus* has also been the subject of experimental commercial fisheries in California, Oregon and British Columbia (reviewed by Zhang et al., 1999). In British Columbia a test fishery was conducted in 2001 to determine an optimal trap type and collect data on reproductive condition (Zhang, 2001); however, no subsequent fishery has developed. In Oregon *L. foraminatus* have been harvested commercially since 1982, with a peak catch of 272,000 lbs in 1984 (Zhang et al., 1999). The catch in 2007 was 2281 lbs (Oregon Fish and Wildlife Commission, 2008). *L. foraminatus* is also caught as by-catch in groundfish and shrimp trawl fisheries (Kato, 1992; Zhang et al., 1999).

Information on the reproduction of Puget Sound king crab is limited to reports of movement into shallow water to breed in late winter and early spring (Jensen, 1995). Larvae for developmental studies were also collected from individual females in Alaska in May (Haynes, 1993) and Washington (Puget Sound) in April (Crain & McLaughlin, 2000). Slightly more information is available regarding box crab life history. Fecundity for 4 female *L. foraminatus* captured in an Oregon test fishery ranged from 20,100 to 48,000 (Jean McCrae, Oregon Department of Fish and Game, pers. com. cited by Zhang et al., 1999). Based on data from test fisheries in California, Oregon (Goddard, 1997), and British Columbia, Zhang et al. (1999) concluded that *L. foraminatus* females probably reach functional maturity at a carapace length (CL) of 78-83 mm and release

larvae in the spring. The authors also speculated that embryogenesis probably requires 200-300 days. The April 2001 test fishery in BC confirmed that females achieve functional maturity at about 8 cm CL; 50% of 75-84 mm females bore eggs, as did more than 95% of females larger than 85 mm. It was also observed that both old and new shelled females were brooding apparently new egg clutches (identified by yellow eggs) (Zhang, 2001).

The present study seeks to combine field and laboratory observations to determine the reproductive cycle of *Lopholithodes foraminatus* in British Columbia. The timing of molting, egg extrusion, brooding and hatching by captive females are related to the reproductive status of females captured in the field and preserved specimens from the Royal B.C. Museum. Data on the reproductive timing of adult females are corroborated by qualitative and quantitative analysis of eggs sampled throughout the brooding period. Detailed data are presented on the duration and magnitude of larval release by females in the lab. In addition, a first attempt is made to detect a spatial gradient of developmental stages within the brood of a female lithodid crab. The presence of such a gradient would provide support for the oxygen limitation hypothesis of extended hatching in the Lithodidae.

The data presented here are a necessary step towards the development of management strategies to prevent targeted or incidental depletion of box crab populations. They also broaden our understanding of lithodid reproduction and allow progress towards workable hypotheses regarding the evolution of life history strategies in the Lithodidae.

METHODS

Adult capture:

Adult box crabs were captured in rectangular Dungeness crab traps at approximately 120m depth west of Twin Islands in the Northern Strait of Georgia, British Columbia, Canada (50˚ 01' 12" N, 124˚56'43" W) on March 12th and June 4th, 2006; and January 13th and March 4th, 2007. Crabs were transported to the University of Victoria in insulated boxes of seawater. Additional crabs were captured by a Department of Fisheries and Oceans research vessel on March 8, 2008 north of Double Island at the entrance to Toba Inlet (approximately 50˚19' N, 124˚ 47' W) at a depth of between 60 and 95 m. These crabs were held in the re-circulating seawater system at the Pacific Biological Station in Nanaimo until May 7th, 2008 when they were transported to the University of Victoria. All live female crabs were assigned a specimen number from 1-30.

Female reproductive status in the field

The reproductive status of live females was scored as pre-reproductive, post-brooding, brooding eyed eggs, or brooding un-eyed eggs. Post-brooding females could be distinguished from pre-reproductive females by the presence of a dark 'moss' of egg attachment filaments on the pleopods. Note was also made of the condition of the exoskeleton, including presence of staining and epizootic growth. Carapace width (CW) was measured at the widest point of the carapace including spines. To facilitate comparison with other studies, mean CW was converted to carapace length (CL) using the formula CL = (CW*.703) + 13.219 (Zhang et al., 1999).

Reproductive status was also determined for 16 females from the Royal British Columbia Museum invertebrate collection. These females were scored simply as pre-

reproductive, post-brooding, or egg bearing, as it was not possible to determine the developmental state of preserved eggs. Preserved female crabs for which reproductive status was determined were assigned a number from 31-46. Date of capture was known for all but one of these specimens.

Female reproductive status in the laboratory

Twenty six female *L. foraminatus* were maintained in the lab to determine the timing of key reproductive events. Crabs were held singly or in groups of up to 8 individuals in 150-230 L tanks with sand substrate in the University of Victoria re-circulating seawater systems. Where possible, at least 1 male crab was present in each tank at all times. Crabs were fed twice a week with frozen krill (*Euphausia* sp.) supplemented occasionally with pieces of fish, cracked sea urchins (*Stronglyocentrotus* spp.), and brittle stars (*Ophiopholis sp.*).

As it was not possible to maintain crabs in the same seawater system over the entire study period, there was some variation in temperature, light, and salinity regimes when individuals were switched between systems. However, all holding locations received at least some natural light from a window and water temperatures were generally between 9 and 10 °C. During the study period the mean temperatures and salinities (± standard deviations (SD)) for the three re-circulating seawater systems were: 10.3 ± 0.4 °C / 29.3 ± 1.0 ppt; 9.7 ± 0.5 °C /30.1 ± 1.6 ppt; and 9.4 ± 0.4 °C / 30.3 ± 2.0 ppt.

Females 16-24 and 27-30 were maintained in the same re-circulating system under natural photoperiod illumination for their entire time in the laboratory; Figure 2.1A illustrates the temperature regime experienced by these crabs. The temperature regime

experienced by female 1 from June 21st, 2006 to Sept 30th, 2008 is presented in Figure 2.1B.

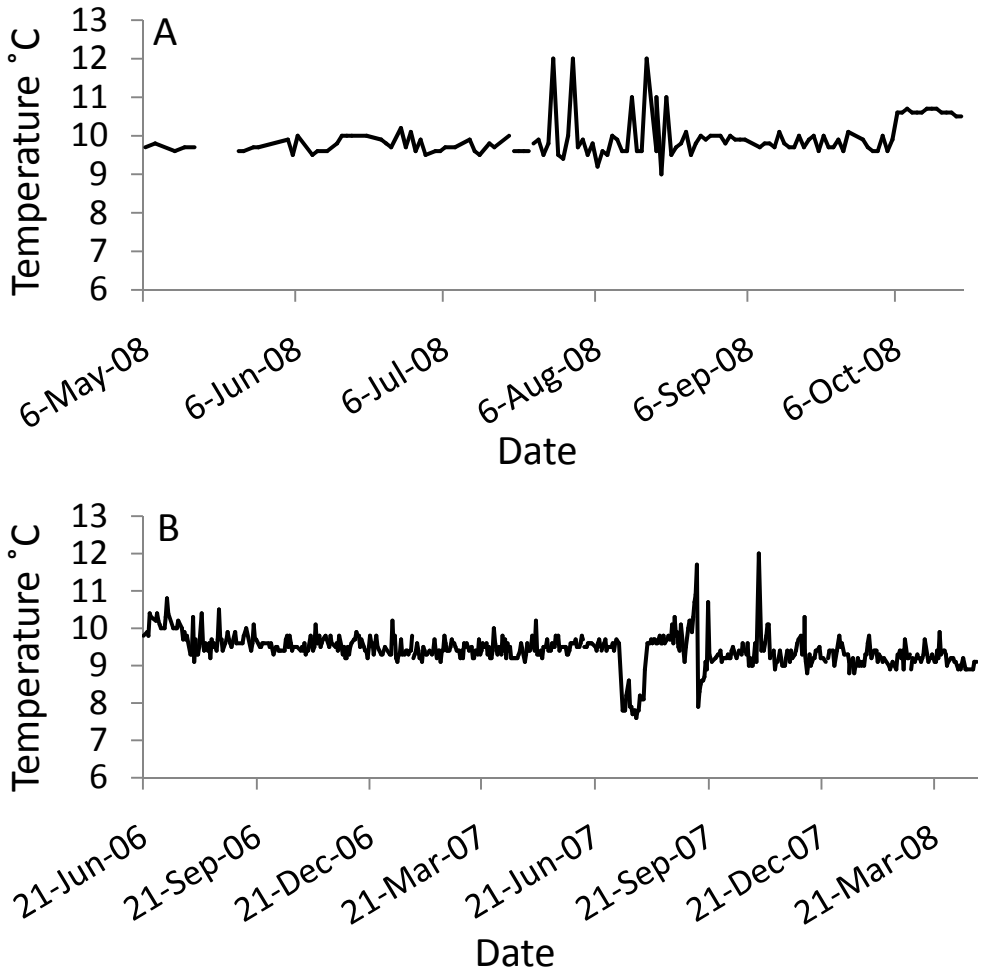


Fig.2.1. Temperature regimes experienced by female *Lopholithodes foraminatus* maintained in the University of Victoria re-circulating seawater systems: (A) females 16-24 and 26-30; (B) female 1.

Tanks were examined daily and molts and mortalities were removed. In cases where the exact date of molting was not noted (in some cases exuviae were mistaken for live crabs), it was taken to be the mid-point between confirmed observations. Mean molting dates were calculated separately for females originating from Twin Islands and

from Toba Inlet, and for all females combined. These dates were calculated for each category of females as:

$$\Sigma(\text{Day of the year} * \text{number of females molting that day}) / \text{total number of molts}$$

Mean molting dates of females from the two populations were compared using Student's t-test.

Qualitative and quantitative analysis of development rate

Eggs were removed from several females throughout development for qualitative observations of embryogenesis. Samples of eggs were obtained by gently prying the edge of the abdomen away from the underside of the thorax and inserting the tip of a pair of forceps. The timing of this sampling is indicated in Figure 2.2.

Quantitative analysis of development rate was primarily based on a female (1) that was captured in a pre-reproductive state on March 12th, 2006 and molted, extruded eggs and mated in the lab in the last week of July 2006. A sample of at least 10 eggs was removed from this female every 1-2 months beginning in March 2007. Photographs were taken of individual eggs in a drop of seawater on a glass slide at 50 X magnification using a Sony PowerHAD digital video camera mounted on an Olympus SZX9 dissecting microscope. Eggs were photographed in a lateral orientation under dark field illumination from a fiber optic source (Stevens, 2006a).

Photographs were analyzed using Northern Eclipse software calibrated with a slide micrometer. Egg area and yolk area were outlined manually using the polygon tool. Percentage yolk area in lateral view (PYA) was calculated as the ratio of yolk area to total egg area, and mean values (± 95% confidence intervals) were calculated for samples of at least 10 eggs. The green and red dashed lines in Figure 2.3L illustrate the measurements of yolk area and egg area used to calculate PYA.

Photographs of a group of 14 eggs removed from female 14 on November 15th, 2007 were measured a second time and mean measurement error was calculated as:

$$\Sigma \left(\sqrt{(1^{st} \text{ measurement} - 2^{nd} \text{ measurement})^2} / ((1^{st} \text{ measurement} + 2^{nd} \text{ measurement})/2)\right) / 14$$

This formula calculates the error as a percentage of the PYA measured, not as ± error in the PYA values themselves.

Decrease in PYA of embryos brooded by female 1 was related to days since extrusion and days before the mid-point of hatching (calculated as described under the following heading). Since qualitative observation did not indicate any development of differentiating larval tissues until August 2007, no quantitative analysis was made of photographs taken prior to July 2007.

Supplementary mean PYA measurements were obtained in the same manner from 3 other female crabs (8, 9, and 14). As dates of egg extrusion were not known for these females, average PYA values were related to the number of days before the mid-point of hatching for each female. The timing of quantitative egg sampling for each female is indicated in Figure 2.2.

For comparison, average PYA values were also determined on Oct 9, 2008 for samples of eggs removed from females 27-30. These females had been trapped brooding un-eyed eggs on March 8th, 2008.

Timing, duration and magnitude of larval release

Female crabs were moved to individual flow-through containers before the anticipated start of hatching (assessed from developmental stage of embryos). Suspended zoeae released by female 2 in spring 2006 were collected from a 170 L tank with a screened outflow using either a 5mm diameter pipette or a fine mesh net. All suspended larvae were counted daily. Zoeae on the bottom of the tank were siphoned out and

discarded. In the subsequent two seasons, females releasing zoeae were maintained in partially covered 20 L plastic tubs supplied with a constant flow of seawater. Larvae passed through a 90˚ overflow pipe that ended in a T at the bottom of a 750 mL plastic container with a 400 μm Nitex® mesh bottom. This container was seated in a 1L glass beaker. Healthy zoeae were collected from the 750 mL containers each morning and counted. Virtually all zoeae remaining on the bottom of the plastic tubs in the morning had morphological abnormalities and were unable to suspend themselves in the water column. These non-viable zoeae were not enumerated.

The mid-point of hatching for each female was calculated as:

*(day of the year) * (# of zoeae collected) / (total # of zoeae collected for that female)*

In the one case where hatching began before January 1st (female 1), days in December were numbered in descending order beginning with day -1 (December 31st). On the few occasions where zoeae were not collected on a daily basis the number of zoeae hatched on each day was calculated as:

(total # of zoeae collected) / (# of days since last collection)

The zoeae released by females 25 and 26 in 2008 were not enumerated but the duration of hatching was noted.

Spatial patterning of development rate

One brooding female from the group trapped on March 8th, 2008 was euthanized on October 11, 2008 for analysis of spatial patterns of development rate within the egg mass. Sixty four pairs of eggs adjacent to each other in the egg mass (in physical contact) were removed using fine forceps and isolated in the wells of a tissue culture plate. Care was taken to remove pairs of eggs from throughout the egg mass. An additional 138 eggs were removed from throughout the egg mass and pooled together in a dish of seawater.

After thorough mixing, haphazard pairs of eggs from this pooled group were isolated in the wells of a tissue culture plate. These procedures were completed without the aid of magnification to prevent inadvertent bias based on visible differences in developmental stage.

All eggs were photographed and PYA was calculated as described previously. The two eggs in each pair were photographed and measured non-sequentially to prevent inadvertent bias. The absolute value of the difference in PYA between pairs of adjacent individuals was compared to that between randomly paired individuals using a Mann-Whitney U test (Sigma Stat 2.03).

RESULTS

Female reproductive status in the field

Reproductive status was determined for a total of 30 live female *Lopholithodes foraminatus* (Table 2.1) collected in the field and 16 preserved females (Table 2.2) from the Royal BC Museum collection. Twelve of the live females were pre-reproductive, 5 were post-brooding, and 13 were brooding eggs and/or releasing zoeae. Two of the preserved females were pre-reproductive, 1 was post-brooding and 13 were brooding eggs. The mean (± SD), maximum and minimum measured CW and calculated CL of pre-reproductive and brooding or post-brooding females are presented in Table 2.3. The largest pre-reproductive female had a CW of 11.1 cm (CL = 9.2cm) while the smallest reproductive female had a CW of 8.8 cm (CL = 7.5 cm)

Table 2.1. Origin, size, reproductive status upon collection, and types of data collected for live female *Lopholithodes foraminatus* examined in this study.

Spec. #	Capture Date	Capture location	Carapace width (incl. spines)	# zoeae released	Reproductive state	Held in lab?	Use in study
1	3/12/06	Twin Isl.	9.0 cm	2170	Pre-reproductive	Yes	H, PYA
2	3/12/06	Twin Isl.	10.0cm	4682	Eyed eggs	Yes	H
3	3/12/06	Twin Isl.	13.0 cm	N/A	Un-eyed eggs	Yes	H
4	3/12/06	Twin Isl.	< 9 cm	N/A	Pre-reproductive	No	N/A
5	3/12/06	Twin Isl.	< 9 cm	N/A	Pre-reproductive	No	N/A
6	3/12/06	Twin Isl.	< 8 cm	N/A	Pre-reproductive	No	N/A
7	6/4/06	Twin Isl.	9.3 cm	N/A	Pre-reproductive	No	N/A
8	1/13/07	Twin Isl.	10.2 cm	N/A	Eyed eggs	Yes	H, PYA
9	1/13/07	Twin Isl.	11.6 cm	2962	Un-eyed eggs	Yes	H, PYA
10	3/4/07	Twin Isl.	11.1 cm	13400	Pre-reproductive	Yes	N/A
11	3/4/07	Twin Isl.	9.5 cm	N/A	Pre-reproductive	Yes	N/A
12	3/4/07	Twin Isl.	11.1 cm	N/A	Post-brooding	Yes	N/A
13	3/4/07	Twin Isl.	10.4 cm	10968	Releasing larvae	Yes	H
14	3/4/07	Twin Isl.	10.4 cm	N/A	Un-eyed eggs	Yes	H, PYA
15	3/4/07	Twin Isl.	9.7 cm	N/A	Un-eyed eggs	Yes	N/A
16	3/8/08	Toba In.	8.6 cm	N/A	Pre-reproductive*	Yes	N/A
17	3/8/08	Toba In.	9.3 cm	N/A	Pre-reproductive*	Yes	N/A
18	3/8/08	Toba In.	8.9 cm	N/A	Pre-reproductive*	Yes	N/A
19	3/8/08	Toba In.	7.9 cm	N/A	Pre-reproductive*	Yes	N/A
20	3/8/08	Toba In.	10.4 cm	N/A	Pre-reproductive*	Yes	N/A
21	3/8/08	Toba In.	9.9 cm	N/A	Post-brooding*	Yes	N/A
22	3/8/08	Toba In.	9.9 cm	N/A	Post-brooding*	Yes	N/A
23	3/8/08	Toba In.	14.2 cm	N/A	Post-brooding*	Yes	N/A
24	3/8/08	Toba In.	9.5 cm	N/A	Post-brooding*	Yes	N/A
25	3/8/08	Toba In.	11.1 cm	N/A	Releasing larvae*	Yes	H
26	3/8/08	Toba In.	11.2 cm	N/A	Releasing larvae*	Yes	H
27	3/8/08	Toba In.	10.5 cm	N/A	Un-eyed eggs*	Yes	PYA, SP
28	3/8/08	Toba In.	10.6 cm	N/A	Un-eyed eggs*	Yes	PYA
29	3/8/08	Toba In.	10.6 cm	N/A	Un-eyed eggs*	Yes	PYA
30	3/8/08	Toba In.	12.1 cm	N/A	Un-eyed eggs*	Yes	PYA

Twin Isl. indicates crabs caught at approximately 120m depth west of Twin Islands in the Northern Strait of Georgia, British Columbia, Canada (50˚ 01’ 12” N, 124˚56’43” W); **Toba In.** indicates crabs caught at a depth of between 60 and 95 m, north of Double Island at the entrance to Toba Inlet (approximately 50˚19’ N, 124˚ 47’ W). **Post-brooding** crabs were identified by a moss of egg attachment filaments on their pleopods, indicating that they had carried a brood of eggs since their last molt, **pre-reproductive** crabs lacked this moss and had not carried a brood since their last molt. Use in study: **(H)** data collected on duration and/or magnitude of larval release; **(PYA)** subsamples of eggs removed for calculation of mean percentage lateral yolk area; **(SP)** eggs removed for analysis of the spatial patterning of development in the egg mass.

* Crabs captured on March 8, 2008; reproductive status recorded on May 7, 2008.

Table 2.2. Origin, size, and reproductive status of female *Lopholithodes foraminatus* in the Royal British Columbia Museum (RBCM) invertebrate collection. Data were not recorded for individuals smaller than 3.5 cm or for individuals in which reproductive status could not be determined (dry specimens).

Spec. # (this study)	RBCM Catalogue #	Capture date	Capture location	Carapace width (incl. spines)	Reproductive status
31	?	?	?	> 14 cm*	Brooding eggs
32	973-219-1	9/12/73	Queen Charlotte Sound	9.8 cm	Brooding eggs
33	973-219-1	9/12/73	Queen Charlotte Sound	> 12.4 cm*	Brooding eggs
34	976-8-2	5/3-12/61	Goose Isl., Queens Sound	9.7 cm	Brooding eggs
35	978-225-1	5/30/72	?	8.9 cm	Pre-reproductive
36	980-699-4	6/1/72	West of La Perouse Bank	?*	Brooding eggs
37	983-1636-1	11/18/73	Cliffe Pt. Quatsino Sound	6.8 cm	Pre-reproductive
38	985-28-5	9/15/81	?	12.1 cm	Brooding eggs
39	985-28-5	9/15/81	?	10.1 cm	Post-brooding
40	985-477-1	9/22/65	?	9.43 cm	Brooding eggs
41	985-477-1	9/22/65	?	9.88 cm	Brooding eggs
42	985-477-1	9/22/65	?	9.09 cm	Brooding eggs
43	985-477-1	9/22/65	?	10.19 cm	Brooding eggs
44	985-477-1	9/22/65	?	8.82 cm	Brooding eggs
45	985-534-2	9/30/85	Hecate Strait	11.38 cm	Brooding eggs
46	991-387-2	3/1/53	Dallas Rd. Victoria	14.4 cm	Brooding eggs

Post-brooding crabs were identified by a moss of egg attachment filaments on their pleopods indicating that they had carried a brood of eggs since their last molt, **pre-reproductive** crabs lacked this moss and had not carried a brood since their last molt.

* Carapace width could not be measured due to damage.

Table 2.3. The mean (± standard deviation), maximum, and minimum measured CW and calculated CL of pre-reproductive, and brooding and post-brooding female *Lopholithodes foraminatus*. All units are centimetres and CL = (CW*.703) + 13.219.

	Measured				Calculated			
	Mean CW	±SD	Min	Max	Mean CL	±SD	Min	Max
Pre-reproductive females (n=11)	N/A	N/A	6.8	11.1	N/A	N/A	6.1	9.2
Brooding and post-brooding females (n=29)	10.7	1.4	8.8	14.4	8.9	1.0	7.5	11.4

The 13 live brooding females fell into two distinct categories based on developmental stage of the embryos and condition of the exoskeleton. Eight were brooding embryos consisting of a bright orange ball of yolky cells lacking any differentiating larval tissues. These females had hard exoskeletons with slight epizootic growth and little staining. The other 6 females were brooding orange/brown eggs at an advanced stage of development. These eggs exhibited black pigmented eyespots, red/orange chromatosomes, and development of transparent differentiating larval tissue. Yolk volume in these embryos was also greatly reduced. The exoskeletons of females in this second category had extensive epizootic growth including tube building polychaetes, hydrozoan polyps, and minute bivalves. The distal segments of the legs, undersides of the chelae, and portions of the ventral surface were also stained black and the denticulations of the chelae were eroded. A similar shell condition was observed in post-brooding females, but never in males or pre-reproductive females. Females in both categories were caught at the same time on three occasions at Twin Islands and were present at the same time in the group of crabs captured near Toba Inlet (Table 2.1).

Female reproductive status in the laboratory

Eight pre-reproductive females, 5 post-brooding females, 8 females with un-eyed eggs, and 5 females with eyed or hatching eggs were maintained in the laboratory to determine the timing of reproductive events. Sixteen of these females molted in the laboratory and almost all died shortly after molting. Only female 1 molted twice, completing an entire reproductive cycle in captivity (Figure 2.2). This cycle consisted of molting, extrusion of eggs and mating in late July of 2006; brooding for just over 17 months; releasing larvae for 3 months in the late winter of 2008; and finally 5 months of

post-brooding status before molting, extruding eggs and mating for a second time in August 2008.

Females 14 and 19 were the only individuals to not extrude eggs immediately after molting. Female 14 was the only female to molt in a tank that did not contain a male and no male was introduced to the tank until 6 days post-molt. Female 14 died 7 days after molting and mature eggs were found in her oviducts.

Mate guarding by males was observed for up to 7 days before and one day after female ecdysis. In all instances of pre-copulatory mate guarding, the male held the female by the merus of her major (right) cheliped with his minor (left) cheliped. In some cases this position was observed in post copulatory mate guarding while in others the male positioned himself with both chelae spread over the dorsal surface of the female. Egg extrusion and fertilization apparently occurred within 48 hours of ecdysis, and in some cases within 24 hours. The act of copulation was never observed.

All 17 instances of female ecdysis occurred between June 28th and August 28th. The mean molting date for all females (± SD) was August 2nd ± 13 days. The mean molting date of females collected at Twin Islands (July 29th ± 17 days) was not significantly different from that of females collected in Toba Inlet (August 5th ± 12 days) (Student's t-test, $t = -0.952$, $df = 15$, $P = 0.356$).

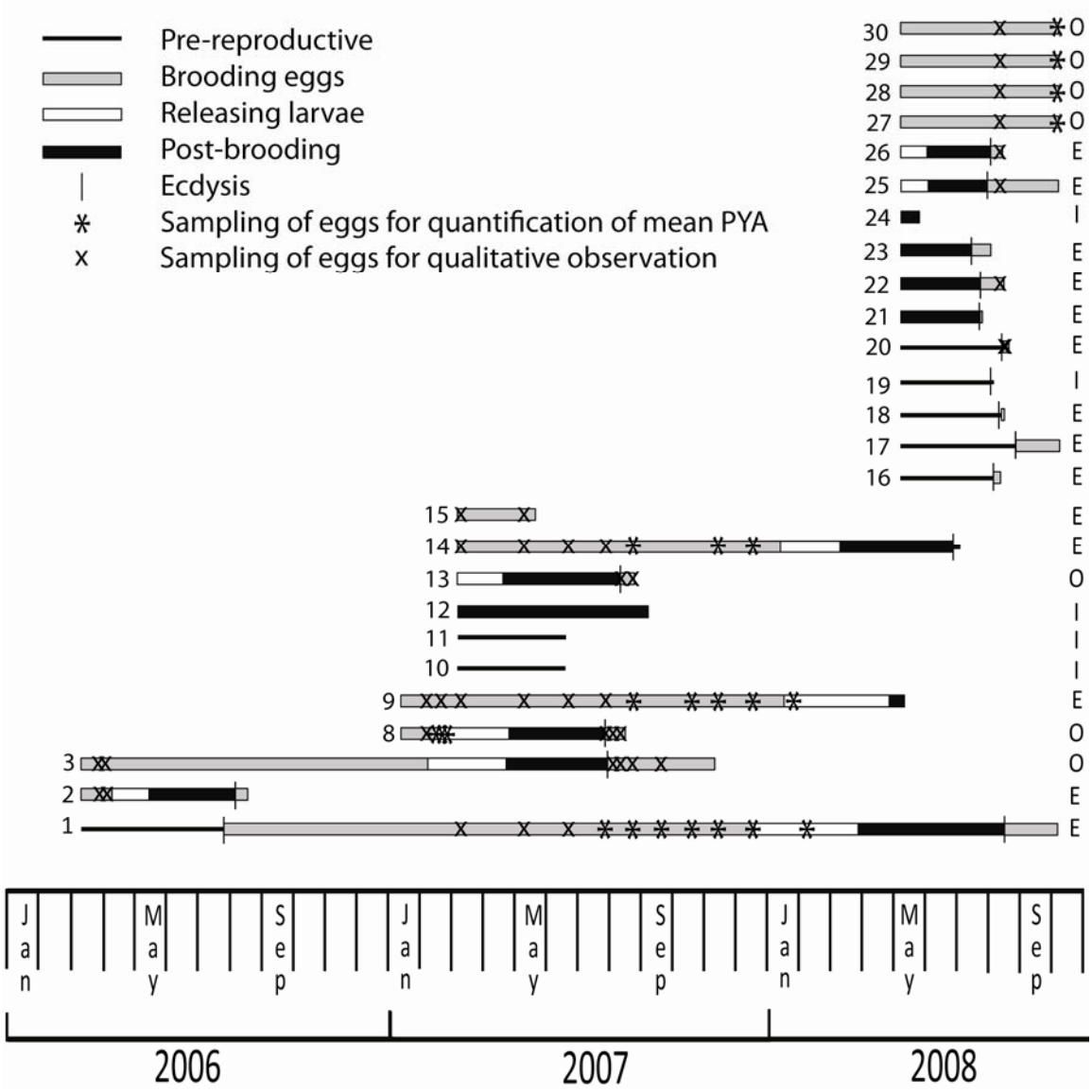


Fig. 2.2. Reproductive state of female *Lopholithodes foraminatus* maintained in group tanks in the laboratory in the presence of at least one male. Each numbered horizontal line represents a single crab, beginning with capture and terminating with death **Post-brooding** crabs were identified by a moss of egg attachment filaments on their pleopods indicating that they had carried a brood of eggs since their last molt, **pre-reproductive** crabs lacked this moss and had not carried a brood since their last molt. Females 16-30 were captured north of Double Island at the entrance to Toba Inlet, all others were captured west of Twin Islands in the Northern Strait of Georgia (see Methods). Females were apparently releasing zoeae, molting and mating either in even years (E), or in odd years (O). The reproductive timing of pre-reproductive and post-brooding females that did not extrude eggs in the lab was indeterminate (I). Samples of eggs were removed from females to calculate decrease in mean percentage yolk area in lateral view (PYA), the timing of this sampling is indicated by asterisks. The timing of egg sampling for qualitative observation is indicated by (x).

Qualitative and quantitative analysis of development rate

The color of newly extruded eggs ranged from bright yellow to bright orange. On the day of extrusion and fertilization the yolky eggs showed no sign of cleavage. Polar bodies were visible for some eggs by the second day post-extrusion. By the 3rd or 4th day after extrusion, some embryos had begun cleavage (Figure 2.3A). Approximately 10 days post-extrusion, individual cells appeared polygonal and were generally bordered in two dimensions by 5-7 other cells (Figure 2.3B). Nuclei were clearly apparent as irregularities within the homogenous yolky cytoplasm. By 2 to 3 weeks post-extrusion, embryonic cells appeared small, irregular, and yolky; nuclei were no longer apparent (Figure 2.3C).

Very little qualitative change in this state was observed over the subsequent 11-12 months of embryogenesis (Figure 2.3D). Development of the differentiating larva became apparent in some eggs approximately 12-13 months after fertilization as a small transparent indentation into the yolk mass (Figure 2.3E). By 14 months post fertilization, some differentiating larvae had clearly developed optic lobes and appendages. At this stage, transparent larval tissues occupied as much as 20% of the egg area when viewed laterally; the balance consisted of opaque yolk (Figure 2.3F). Black eye pigmentation and red chromatosomes became evident in some eggs by 15 months post fertilization; and as eye and chromatosome pigmentation increased the colour of the remaining yolk became lighter (Figure 2.3G-I). By 16 months post fertilization all embryos had at least some eye pigment (Figure 2.3J). At this stage the most advanced differentiating larvae occupied almost half of the egg area in lateral view. Over the subsequent 2 months the size of the pigmented portion of the eye and degree of chromatosome pigmentation

continued to increase while yolk volume decreased; by the mid-point of hatching most eggs had little yolk remaining (Figure 2.3K).

Eggs in close proximity to each other within the egg mass were observed to be at different developmental stages during the entire period of rapid development from 13 to 18 months post fertilization. This heterogeneity in developmental stage is illustrated in Figure 2.3G-I by the difference between 3 eggs removed from female 1 in October of 2007, 15 months post fertilization. At this stage some differentiating larvae were completely transparent with small optic lobes (Figure 2.3G) while others had pigmented eyes, red chromatosomes, and well developed appendages (Figure 3I). In clumps of eggs at 16 months post-extrusion, embryos with barely evident eye pigmentation (Figure 2.3Ja) were directly adjacent to embryos at a much more advanced stage of development (Figure 2.3Jb).

Percentage lateral yolk area remained between 95% and 100% until about 13 months post fertilization (5 months before the mid-point of hatching). Yolk area was not 100% during this period due to a small separation between the embryo and egg membrane. The size of this separation apparently varied between eggs and between females. A rapid decrease in mean PYA began approximately 5 months before the mid-point of hatching (Figure 2.4). Mean PYA decreased steadily to about 50% by 50 days before the mid-point of hatching and to just over 25% at the mid-point of hatching.

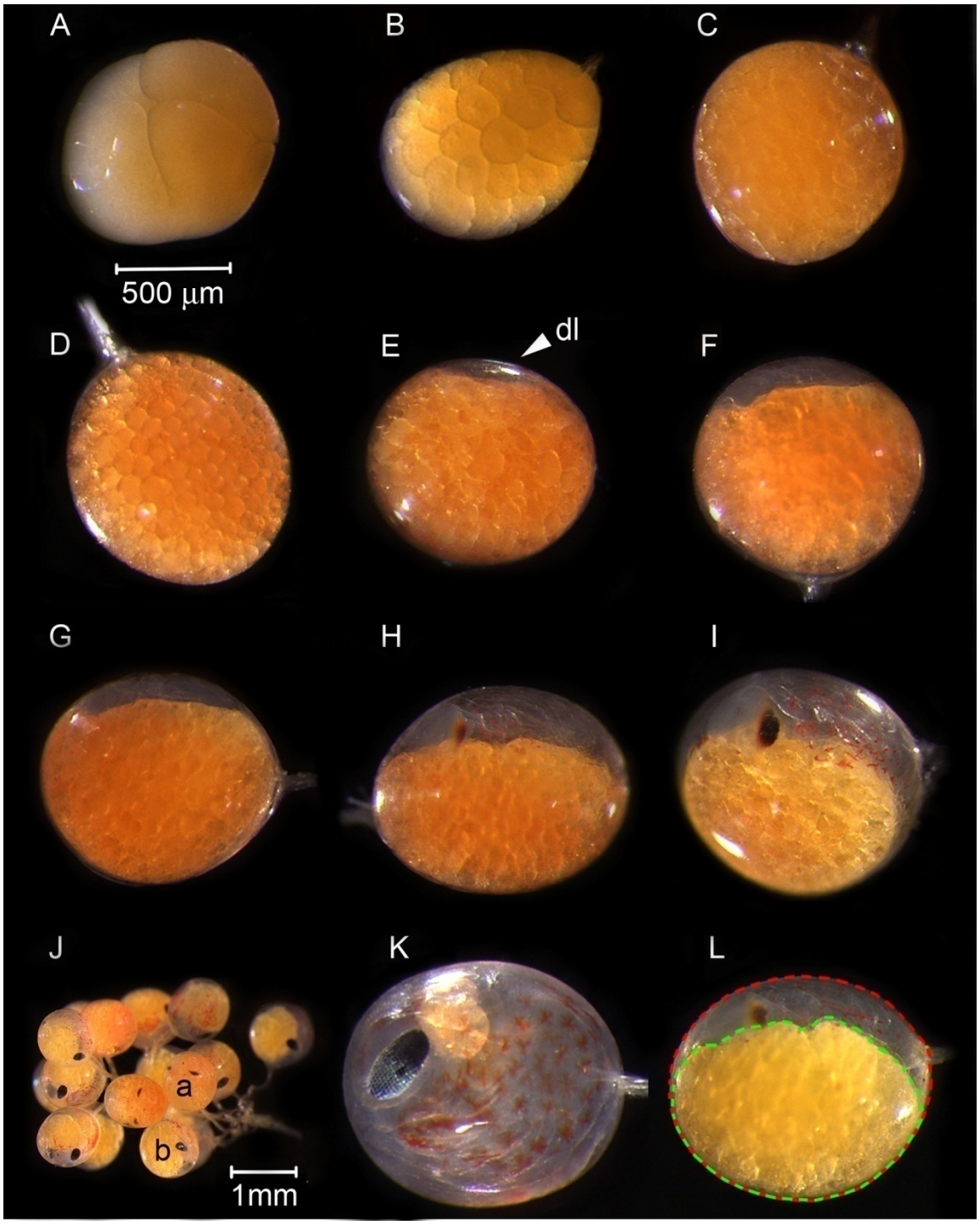


Fig. 2.3. Eggs removed from the broods of female (♀) *Lopholithodes foraminatus* throughout embryogenesis: (A) ♀20, 4 days post-extrusion (p-e); (B) ♀ 3, removed 4 days p-e, photographed 11days p-e; (C) ♀ 25, 13 days p-e; (D) ♀ 1, approx. 11 months p-e; (E) ♀ 1, approx. 13 months p-e, **dl** differentiating larva; (F) ♀ 1, approx. 14 months p-e; (G-I) ♀ 1, three eggs from the same sample, approx. 15 months p-e; (J) ♀ 1, clump containing (a) less developed and (b) more developed eggs approx. 16 months p-e; (K) ♀ 1, approx. 18 months p-e, one day after the mid-point of hatching; (L) ♀ 27, October 9, 2008, illustrates measurements used to calculate percentage yolk area in lateral view (PYA).

On October 9th 2008, some of the eggs removed from females 28-30 were beginning to show development of differentiating larval structures, and in a few cases optic lobes were evident. The eggs removed from female 27 were at a more advanced stage of development; most exhibited eye and chromatosome pigmentation (Figure 2.3L). They were at a similar stage to those removed from female 1 on October 19th, 2007 (Figure 2.3G-I). The mean PYA (± 95% confidence intervals) of the samples of eggs removed from females 27-30 were 74 ± 3%, 94 ± 2%, 94 ± 2%, and 91 ± 2% respectively. Based on the PYA observed at the same time of year for females 1, 9 and 13 it is likely that females 27-30 would have released larvae in the late winter or early spring of 2009.

The mean percentage error (± SD) in the measurement of PYA from photographs was calculated to be 1.2% ± 0.9% of the PYA values measured.

Duration and magnitude of larval release

Daily release of zoeae was quantified for 7 females, data for 5 of these females are presented in Figures 2.5 and 2.6. Females 3 and 14 released very few zoeae as they had dropped the majority of their eggs prior to the completion of embryogenesis (> 6 months and 3 months before the mid-points of hatching respectively). Female 3 released 34 zoeae between February 5th and April 20th, 2007 (mid-point of hatching March 7th); female 14 released 31 zoeae between January 13th and March 10th, 2008 (mid-point of hatching February 13th). The mean mid-point of hatching for all 7 females ± SD was March 10th ± 26 days (all 7 females weighted equally).

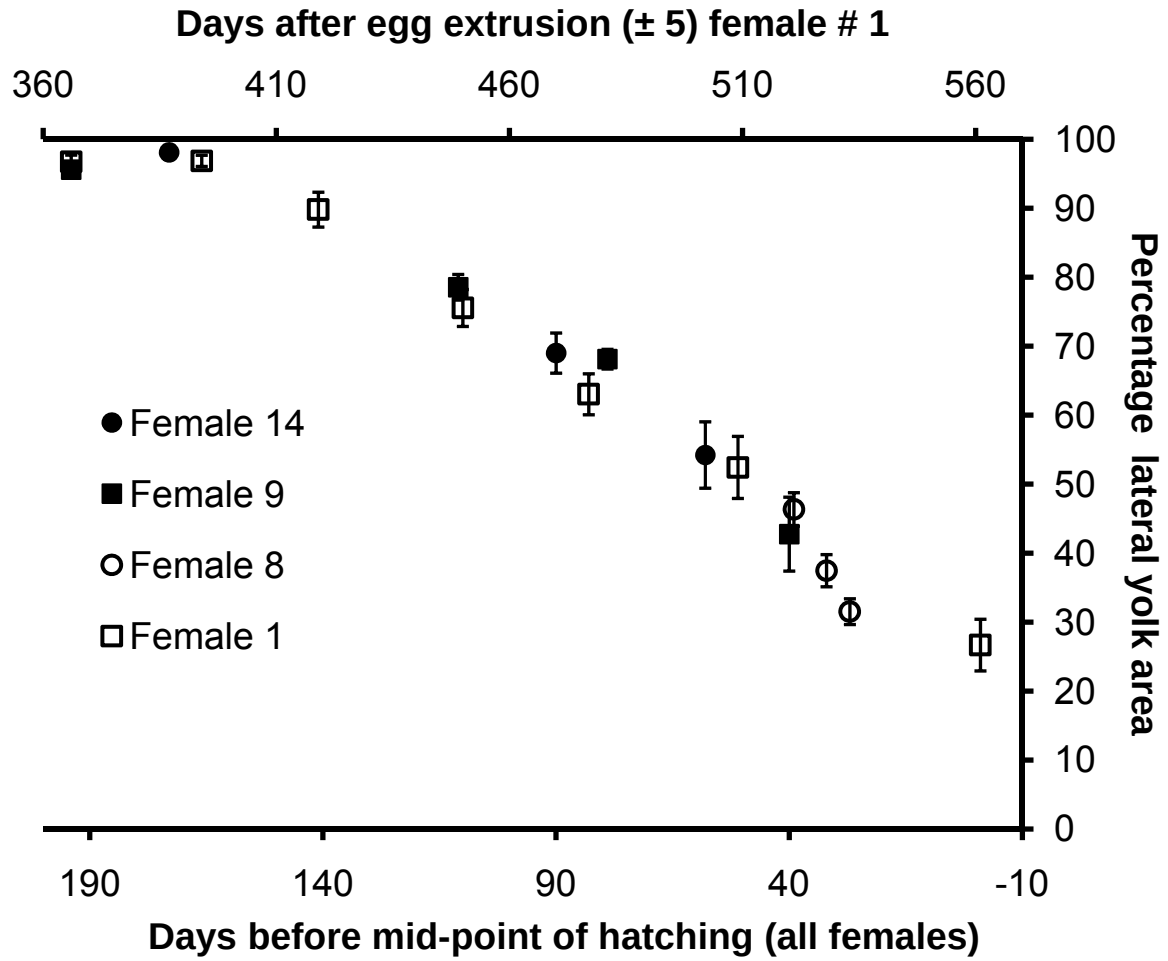


Fig 2.4. Decrease in mean percentage yolk area in lateral view (PYA) of subsamples of at least 10 eggs (maximum 58, mean 20) removed from the pleopods of female *Lopholithodes foraminatus* during the final 200 days of embryogenesis. Error bars indicate ± 95% confidence intervals. The time of year of sampling is indicated in Figure 2.2.

Hatching for all females was very protracted, with a relatively small number of larvae released each day (Figures 2.5 & 2.6). Duration of hatching ranged from 38 days to 106 days (mean {± SD} = 69 {± 25} days).

Females 25 and 26 were releasing zoeae when brought into the lab on May 7th, 2008. They continued to release a few zoeae until approximately June 2nd.

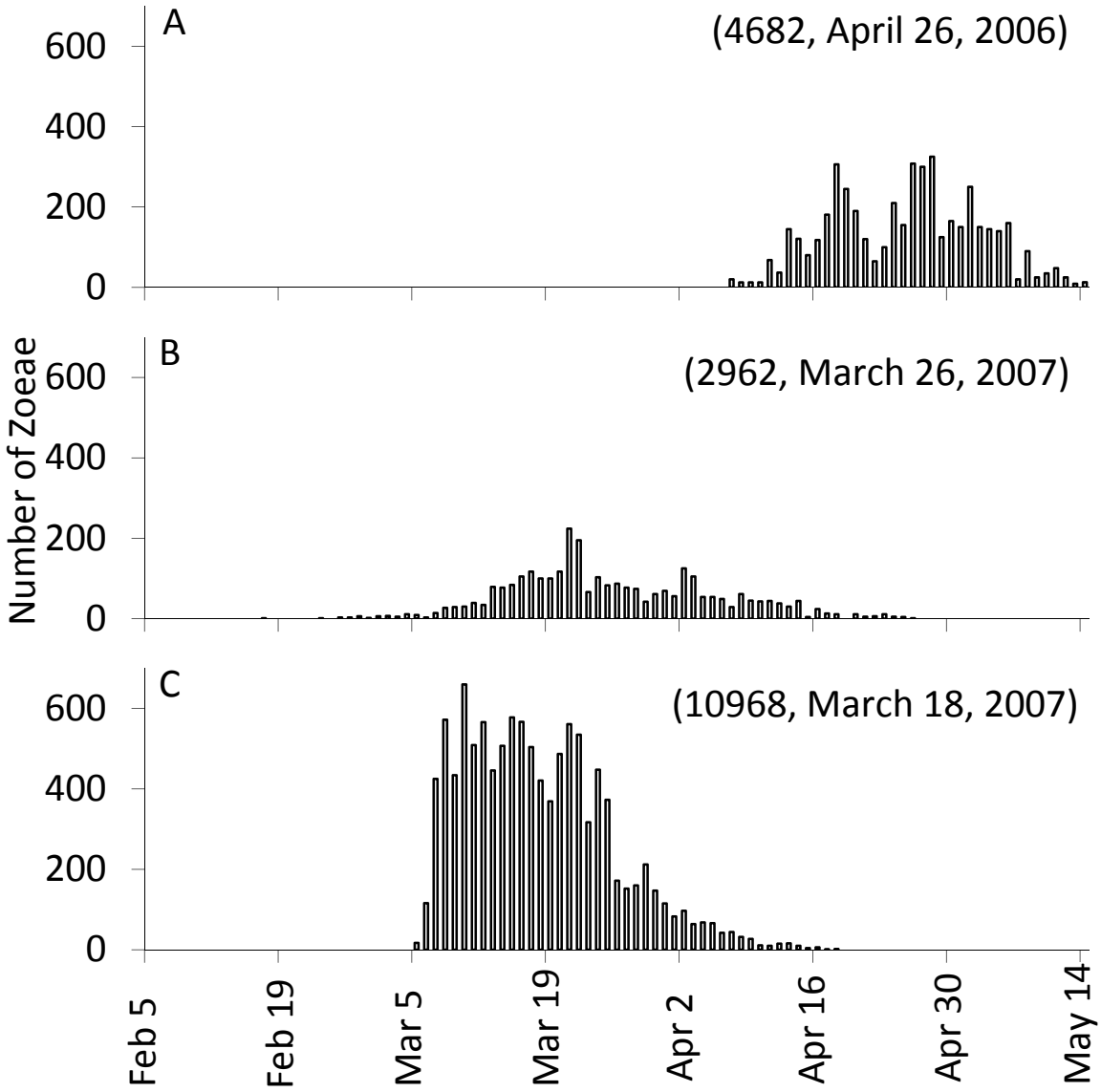


Fig. 2.5. Number of healthy zoeae released daily by *Lopholithodes foraminatus* females 2 (A), 8 (B), and 13 (C) in the spring of 2006 (A) and 2007 (B and C). The total number of larvae released by each female and the mid-point of hatching are indicated in parentheses.

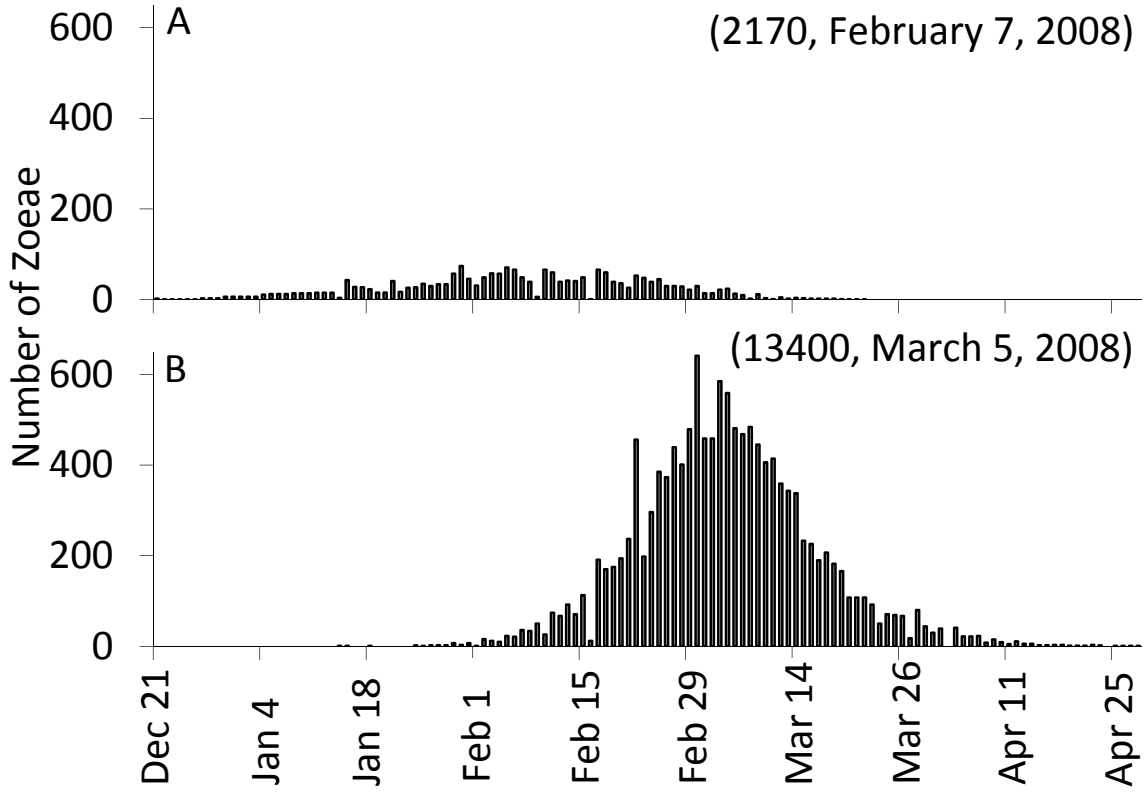


Fig. 2.6. Number of healthy zoeae released daily by *Lopholithodes foraminatus* females 1 (A) and 9 (B) in the spring of 2008. The total number of larvae released by each female and the mid-point of hatching are indicated in parentheses.

Spatial patterning of development rate

The mean difference in PYA (± SD) between eggs adjacent to each other in the egg mass of female 27 on Oct 11, 2008 was 6.2 ± 4.4% (64 pairs). The mean difference between randomly paired eggs was 6.4 ± 4.7% (69 pairs). The difference in PYA between adjacent eggs was not significantly different from that between randomly paired eggs (Mann-Whitney rank sum test; $U = 4223$, $P = 0.771$). A box plot of these data is presented in Figure 2.7.

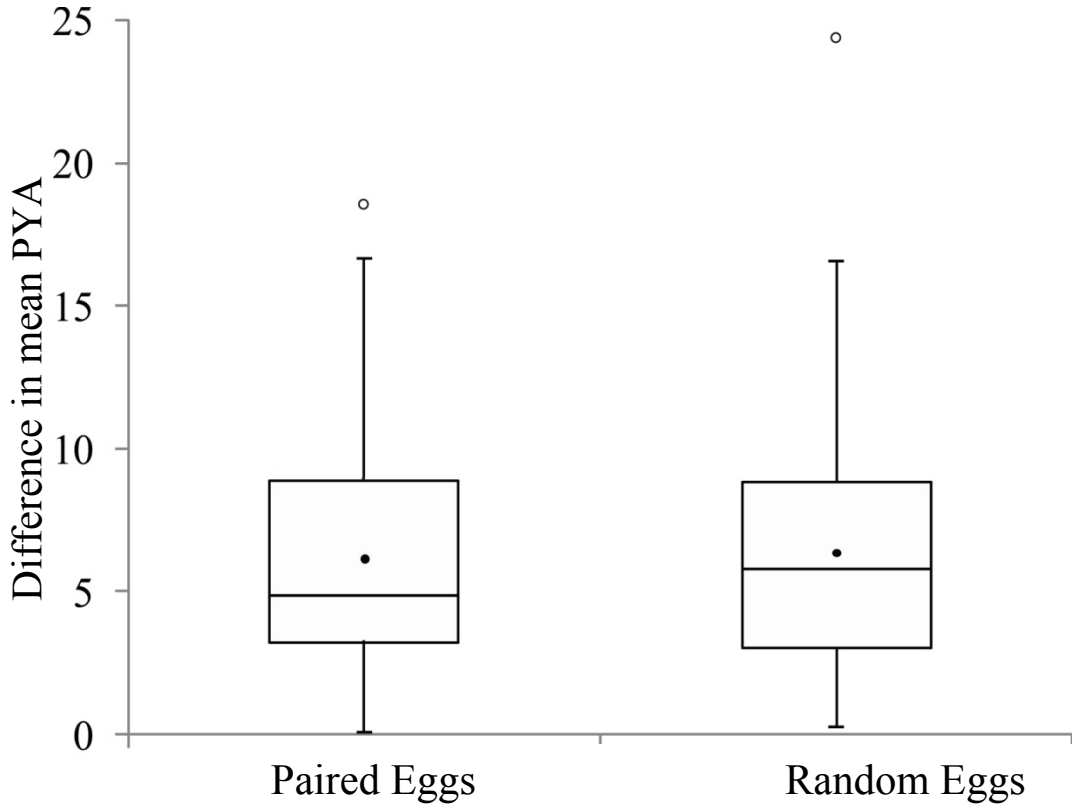


Fig.2.7. Box plot of the difference in percentage yolk area in lateral view (PYA) between *Lopholithodes foraminatus* eggs in contact with each other in the egg mass (paired eggs), and the difference in PYA between haphazardly paired eggs from throughout the egg mass (random eggs).

DISCUSSION

Biennial reproductive cycles

Results of this study suggest that brown box crabs in British Columbia waters have a biennial reproductive cycle. Females molt, extrude eggs and mate in mid-summer, brood eggs for approximately 18 months, and release larvae in the late winter or early spring of the second year after mating. The fact that box crabs collected on 4 separate occasions during the spring included brooding females with eggs in either an early or late stage of development could be interpreted as evidence for asynchronous reproduction or for synchronous reproduction with a cycle lasting more than 1 year. However, the almost complete lack of post-brooding females outside of the late spring to mid-summer period

suggests that an asynchronous reproductive cycle is unlikely (the post-brooding status of females 12 and 39 in early spring and fall respectively could be due to premature brood loss, as observed in females 3 and 14). Further support for synchronous reproduction in *L. foraminatus* is provided by zoeal planktotrophy (see chapter 3) and evidence that zoeae are released only in late winter to early spring. Where data exist, all lithodids with planktotrophic zoeae also release larvae in the spring (summarized up to 2002 in Table 5 of Zaklan, 2002 and see Hong et al., 2005).

The strongest support for a relatively synchronous biennial reproductive cycle was provided by the timing of reproductive events for female *L. foraminatus* maintained in the laboratory. While only female 1 underwent a complete reproductive cycle, the status of all other females maintained in captivity corresponded to the same biennial pattern. All 17 female molting events occurred during a 2 month window in mid-summer (Figure 2.2). This was the case regardless of whether females had been caught the same year (females 1, 2, 8, 13, 16-23 & 25-26) or in a previous year (females 1, 3 & 14). The mean date of molting for crabs from Twin islands (July 29th) was not significantly different from that for females from Toba inlet (August 5th) ($P = 0.356$). All individuals, with the exception of females 14 and 19, extruded eggs after molting. Female 19 was only 7.4 cm CL after molting and was probably not functionally mature. Female 14 had mature oocytes in her oviducts and presumably would have extruded eggs if a male had been present in her tank at the time of molting.

The three females caught in the winter and early spring with un-eyed eggs and held in the lab until hatching (females 3, 9 & 4) did not release larvae until the following late winter or early spring. Similarly females 27-30 were caught in March of 2008 with

un-eyed eggs and began to show signs of rapid embryonic development in the fall of 2008. They would almost certainly have released larvae in the late winter and spring of 2009. Given the timing of female ecdysis in the laboratory it seems very likely that all females captured with un-eyed eggs had molted, extruded eggs and mated the previous summer.

Interpretations of life history parameters from captive animals may be subject to error due to unnatural conditions experienced in the laboratory (Reid et al., 2007). The temperature experienced by brooded embryos in this study was generally between 9.5 and 10 ˚C while the mean temperature in the Northern Strait of Georgia at 120 dbar (approximately 120 m) ranges from 8.5 - 9.5 ˚C over the course of the year (Fisheries and Oceans Canada, 2008). Elevated temperature during embryogenesis has been shown to lead to earlier hatching in red king crab (Shirley et al., 1990) and blue king crab (Stevens et al., 2008). The mid-points of hatching for females that brooded in the laboratory for more than 10 months (females 1 [Feb. 7th], 3 [March 7th], 9 [March 5th], & 14 [Feb 13th]), were all earlier than those for females that released larvae within 2 months of capture (Females 2 [April 26th], 8 [March 26th], & 13 [March 18th]). It is possible that elevated incubation temperatures in the lab may have accelerated embryogenesis. However, Stevens et al. (2008) found that a difference in mean temperature during brooding of 3.8 ˚C (between females held at 2.3˚C and 6.1˚C) resulted in only a 23 day increase in the time required for blue king crab embryogenesis (410 days to 433 days). It seems unlikely that the slightly elevated temperatures experienced by female box crabs in the present study were sufficient to lead to an incorrect interpretation of the reproductive cycle. Nevertheless, the mean mid-point of hatching observed in this study (March 10th) may be

slightly earlier than what occurs in nature. Also, the estimate of time required for embryogenesis (560 days based on Female 1, mid-point of hatching February 7th) may underestimate the period of embryogenesis in the field by 1-2 months.

The occurrence of both new shell and old shell female *L. foraminatus* bearing apparently new (yellowish) broods in the 2001 British Columbia test fishery poses a problem for the biennial reproduction hypothesis. This observation was interpreted as evidence that mating might be possible for both hard and soft shelled females (Zhang, 2001). However, as no reports of hard shell mating by lithodid crabs exist in the literature, this seems an unlikely explanation. In the present study, females bearing eyed eggs in the winter and spring exhibited extremely worn, stained exoskeletons with considerable growth of encrusting organisms. The exoskeletons of females bearing un-eyed eggs were considerably cleaner but were not "new" and bore some epizootic growth. If females had molted and extruded eggs in mid-summer their exoskeletons would already be 8 months old by April. Zhang (2001) grouped together "old shell" and "real old shell [sic]" into a single "old shell" category. It is possible that females in the first year of brooding and at the end of the second year of brooding (likely releasing larvae at the time of the 2001 study) were placed in the same shell condition category. Very old shells were only observed in the present study for females brooding eyed-eggs or in a post-brooding state. This would suggest that pre-reproductive females and males molt more frequently while brooding females molt only every two years as part of a biennial reproductive cycle.

It is difficult to relate the stage of development of eggs in the 2001 test fishery to the present study. Zhang (2001) classified eggs by colour through yellow, orange, red,

brown and black. In the present study, eggs from different females were observed to vary from bright yellow to bright orange through most of the developmental period (compare Figures 2.3H and 2.3L; different yolk colour at the same developmental stage). Only in the final 3 months of development did the brood slowly change to an orange/brown colour as the yolk volume decreased and eye and chromatosome pigmentation increased. Red or black eggs were never observed.

Based on the smallest reproductive and largest pre-reproductive females observed in this study (Table 2.3), mating likely occurs for the first time at between 7.5 and 9.2 cm CL. This is similar to previous estimates of size at functional maturity for female *L. foraminatus* of approximately 8 cm CL (Zhang et al., 1999; Zhang, 2001).

Rate of embryonic development

The timing of early egg cleavages observed in this study was similar to that observed for blue (Stevens, 2006a) and red king crab (Nakanishi, 1987). By three weeks post fertilization, *L. foraminatus* embryos had reached a stage characterized by small irregular-shaped yolky cells. This stage was similar to Stage 2 (blastula-gastrula) of *Paralithodes platypus* (Stevens 2006b), Embryonic Stage II (although without a whitish mass of cells on the yolk surface) of *Paralomis granulosa* (Lovrich & Vinuesa, 1993), and Stages 3/4 (cleavage and blastula/gastrula) of the brachyuran *Chionoecetes opilio* (Moriyaso & Lanteigne, 1998).

Embryos did not begin to show signs of yolk area reduction and development of transparent, differentiating larval structures until about 12-13 months post fertilization (female 1) or 5-6 months before the mid-point of hatching (Figure 2.2). The stage at which an indentation in the yolk first became apparent was similar to Stage 4 (pre-

nauplius) of *Paralithodes platypus* embryos (Stevens 2006b) and Stage 6 (pre-nauplius) of *Chionoecetes opilio* embryos (Moriyaso & Lanteigne, 1998). When diapause early in embryogenesis has been described for other decapod species, it occurs during the gastrula stage (Wear, 1974; Lovrich & Vinuesa, 1993; Petersen & Anger, 1997; Moriyasu & Lanteigne, 1998; Swiney, 2008). Similarly, my observations suggest that *Lopholithodes foraminatus* embryos experience a diapause at the gastrula stage which lasts approximately 12 months. Females 3, 9, 14, 15, and 27-30 were obtained from the field while their broods were in this period of diapause.

Significance of biennial reproduction with embryonic diapause

It is difficult to understand what adaptive significance biennial reproduction and extended diapause could have in the life history of *L. foraminatus*. Red king crabs, which also produce planktotrophic zoeae, have an annual reproductive cycle and complete embryogenesis within a single year at 7.2 ˚C (Stevens & Swiney, 2007). Even some lithodid species with large eggs and lecithotrophic larvae complete embryogenesis within a year, e.g. *Lithodes santolla* (Lovrich & Vinuesa, 1999) and *Lithodes aequispinus* (Paul & Paul, 2001). *Paralithodes platypus* has a biennial reproductive cycle (Sasakawa, 1975; Somerton & MacIntosh, 1985; Jensen & Armstrong, 1989) but still completes embryogenesis in less time than *L. foraminatus,* despite brooding at a lower temperature (395 days at 5.2 ˚C, Stevens 2006b; 410 days at 6.1 ˚C, Stevens et al., 2008). These results suggest that there is no unusual biochemical or physiological constraint on the rate of embryonic development in lithodid crabs. Comparison between the reproductive cycles of *Lopholithodes foraminatus* and other lithodids leads to 2 outstanding questions:

1. Why do box crabs have biennial reproduction?

2. Given biennial reproduction, and no apparent biochemical or physiological constraint on developmental rate, why does embryogenesis involve a 1 year diapause?

The question of reproductive timing has been addressed for a number of commercially important lithodid species. Synchronized annual larval release is easily explained for relatively shallow water species with either planktotrophic or lecithotrophic larvae as it facilitates larval access to phytoplankton (Shirley & Shirley, 1989; Shirley et al., 1990; Stevens & Swiney, 2007) and juvenile access to seasonal benthic production (Reid et al., 2007) respectively. An equally clear explanation exists for larval release throughout the year by relatively deep water representatives of the genera *Lithodes* and *Paralomis.* It has been assumed that synchrony of hatching with seasonal phytoplankton availability is not necessary for these species due to their non-feeding larvae (Somerton, 1981; Sloan, 1985; Paul & Paul, 2001, Reid et al., 2007).

Explaining synchronous biennial reproduction of large female *Paralithodes platypus* has been more problematic given that a sympatric congener (*Paralithodes camtschaticus*) exhibits annual reproduction. Somerton & MacIntosh (1985) suggested that biennial reproduction in blue king crab represents an alternative "low frequency reproduction (LFR)" strategy as described by Bull and Shine (1979). Greater energy investment per brood, larger larvae, and a longer reproductive life associated with less frequent molting compensates for reduced frequency of reproduction. Jensen & Armstrong (1989) disagreed with the hypothesis of an adaptive LFR strategy and proposed that biennial reproduction in *P. platypus* results from the inability of large females to acquire adequate energy to produce a brood within a single year. They

suggested that this could be due to blue king crab occupying poorer quality habitat or colder water than red king crab within the same range. They supported this hypothesis by highlighting inaccuracies in the brood investment calculations of Somerton and MacIntosh (1985) and by pointing out that small *P. platypus* females can reproduce in successive years.

The biennial reproductive cycle of *Paralomis granulosa* (Lovrich & Vinuesa, 1993) has also proved difficult to interpret. A sympatric species (*Lithodes santolla*) not only has annual molting and reproduction but also higher fecundity (Lovrich & Vinuesa, 1999). Lovrich and Vinuesa (1999) suggested that biennial reproduction in *Paralomis granulosa* could be due to occupation of relatively poor quality habitat.

It is not possible to attribute biennial reproduction of *Lopholithodes foraminatus* in Southern British Columbia to a harsh environment characterized by extremely low temperatures and a short growing season as suggested for blue king crab by Jensen and Armstrong (1989). However, it is possible that the relatively deep soft substrate habitat occupied by *L. foraminatus* (Jensen, 1995; Zhang, 2001) has limited food resources relative to shallower, hard substrate habitats. The Pacific Northwest has a high diversity of large predatory crabs such as *Cancer magister*, *C. productu*s and *C. antennarius.* It has been suggested that the presence of these cancrids excludes the introduced crab *Carcinus maenas* from habitat it might otherwise occupy (Jensen et al., 2007). The presence of these predatory crabs presumably also influences the niche space occupied by native decapods. It is possible that *L. foraminatus* occupies an ecological niche of lower productivity, lower competition and reduced predation risk at the expense of high reproductive output.

Lopholithodes mandtii is found on hard substrates in high current areas in generally shallower water than *L. foraminatus*. It also grows considerably larger; up to 30 cm CW. The complete reproductive cycle of *L. mandtii* is not known, but it has been observed to breed in shallow water in the late winter or early spring (Jensen, 1995). This reproductive timing is similar to that of *Paralithodes camtschaticus*, which exhibits annual reproduction (Stevens & Swiney, 2007). If *L. mandtii* is found to have annual reproduction it would be the 3rd known instance where sympatric pairs of lithodid species with the same type of larval development exhibit divergent reproductive periodicity (and the 2nd instance where they are congenerics). This illustrates how, as our knowledge of lithodid reproduction becomes broader, we will be better placed to address questions about the life history adaptations of this group.

The second outstanding question regarding the reproductive cycle of *L. foraminatus* is why embryos should undergo a 12 month diapause early in development. Embryonic diapause in decapods may serve to synchronize hatching with seasonal food availability (Wear, 1974; Petersen & Anger, 1997; Stevens et al., 2008; Swiney, 2008). For example, Stevens et al. (2008) found that blue king crab embryos experienced a period of diapause late in embryogenesis when brooded at 4˚C and 6 ˚C but not at 2˚C. The authors suggested that this diapause may allow *P. platypus* to compensate for elevated water temperature that could otherwise lead to inappropriate time of hatching. An opposite effect was observed in the development of the brachyuran *Chionoecetes opilio* in the cold water of the Gulf of St. Laurence (Moriyaso & Lanteigne, 1998). Two periods of diapause (during the gastrula stage and eye pigment formation stage) apparently prolonged embryogenesis by 9 to 10 months leading to a biennial reproductive

cycle. In warmer water, embryogenesis is completed in a single year. Both of these results disagree with those of Wear (1974), who found that while temperature had a major effect on the rate of embryogenesis, it had no effect on the duration of diapause for 4 species of brachyurans.

Diapause during embryogenesis of *Lopholithodes foraminatus* presumably has different purpose than in either of the examples above. As discussed earlier it does not seem likely that embryogenesis cannot be completed within 1 year due to biochemical or physiological constraints. It therefore seems very surprising that *L. foraminatus* does not brood eggs for 1 year of the two year cycle as is the case for *Paralithodes platypus* (Jensen & Armstrong, 1989; Stevens, 2006a; Stevens et al., 2008). Brooding eggs has been shown to entail energetic expenses for female crabs (Baeza & Fernández, 2002; Fernández & Brante, 2003). In addition, embryos that are brooded for an extended period of time may use up more of their own metabolic reserves than those brooded for a shorter period (Petersen & Anger, 1997). A long brooding period also increases the exposure time of embryos to pathogens and egg parasites. A period of embryonic diapause similar to that observed in the present study occurs during biennial reproduction of *Paralomis granulosa* off the southern tip of South America (Lovrich & Vinuesa, 1993; Lovrich & Vinuesa, 1999). I am not aware of any hypotheses that have been advanced to explain this phenomenon. Hopefully the acquisition of more life history data will shed light on the adaptive significance of this enigmatic life history character.

Extended Hatching

Hatching has been found to occur over a very extended period for all lithodid species investigated: 34 days (mean) for *Lithodes aequispinus* (Paul & Paul, 2001); 13-61

days for *Paralomis granulosa* and 35-41 days for *Lithodes santolla* (Thatje et al. 2003); 29 (mean) (Stevens, 2006b) or 46.7 days (mean) (Stevens et al., 2008) for *Paralithodes platypus*; and 31.6 days (mean) for *Paralithodes camtschaticus* (Stevens & Swiney, 2007). The mean duration of hatching observed in this study (69 days), is to my knowledge the longest reported for any decapod, although other authors indicate that they may have underestimated hatching duration (Thatje et al., 2003; Reid et al., 2007).

Two hypotheses have been advanced to explain extended hatching in lithodid crabs. These hypotheses are not necessarily mutually exclusive. It has been suggested that oxygen gradients within the egg mass might result in differential rates of embryonic development, and in turn extended hatching. This hypothesis was proposed for southern ocean lithodids with lecithotrophic development, for which larval release need not be synchronous with plankton availability (Thatje et al., 2003; Thatje, 2004; Reid et al., 2007). Egg mass oxygen gradients have indeed been reported for other marine invertebrates including gastropods (e.g. Cohen & Strathmann, 1996), and brachyuran crabs (Naylor et al., 1999; Baeza & Fernández, 2002). Fernández et al. (2003) demonstrated a correlation between low partial pressure in the center of egg mass of two brachyuran species and evidence of slowed development rate (indicated by percentage yolk volume).

Stevens (2006b) pointed out that extended hatching is not limited to lithodid crabs with lecithotrophic development, and that lithodids, like brachyurans, are capable of ventilating their egg mass through abdominal flapping. Stevens suggested that extended hatching in lithodids could be a “diversified bet-hedging” strategy (Slatkin, 1974) to ensure that at least some larvae encounter optimum conditions. This hypothesis has

subsequently been developed by Stevens and Swiney (2007) and Stevens et al. (2008). It should be noted that an adaptive diversified bet-hedging strategy need not be independent of an oxygen availability cause.

The extended hatching observed in the present study confirms that this phenomenon is not just a characteristic of high latitude, lecithotrophically developing lithodid species as implied by Thatje et al., (2003). Two results of the present study also call into question the oxygen gradient theory of extended hatching in lithodid crabs.

The first of these derives from an unintentional experiment resulting from the near total loss of the broods of females 3 and 14 (by 6 and 3 months before the mid-point of hatching respectively). If extended hatching was a consequence of an oxygen gradient in the egg mass we would expect that the reduction of that gradient in these two females would have resulted in an abbreviated duration of hatching. Although the period during which most of the brood was absent was relatively short, it is this latter part of the brooding period that is characterized by the highest embryonic oxygen consumption (Naylor et al., 1999; Baeza & Fernández, 2002). In fact the durations of hatching for females 3 and 14 were 75 and 57 days respectively, well within the range of durations for females with full broods.

A second result which called into question the oxygen gradient hypothesis was the lack of evidence of a spatial gradient of developmental stages within the egg mass. This was confirmed by both qualitative observations of multiple broods and comparison of the differences in PYA between pairs of adjacent eggs and haphazardly paired eggs in the brood of female 27. This latter result should be treated cautiously as it includes data from only a single female. If the variability in development rate of lithodid embryos within a

brood is indeed independent of oxygen consumption, then the mechanism underlying this variability warrants further investigation.

Management implications of biennial reproduction

Biennial reproduction of *Lopholithodes foraminatus* off the coast of British Columbia has implications for the management of both directed fisheries and by-catch impacts. Recent work on skipped spawning cycles in iteroparous marine fish has highlighted the importance of reproductive periodicity to fisheries management models. The presence of non reproductive individuals in a population can lead to an overestimation of spawning stock biomass (SSB) (e.g. Rideout et al., 2005; Jørgensen et al., 2006). If not recognized, biennial reproduction could have the same effect, as estimates of the potential rate of increase of the population based on annual reproduction would be double the actual values.

Marine species exhibiting late maturation, long life, and slow potential rate of increase are particularly vulnerable to overfishing (Adams, 1980; Jennings et al., 1998; Jennings et al., 1999). These life history characters may be linked to occupation of low productivity habitats, as is the case for deep sea fish species (Koslow et al., 2000). While the inshore waters of the Pacific Northwest are not a low productivity system, it is possible that biennial reproduction in *L. foraminatus* is a response to occupation of a lower productivity niche within this system. Given the diversity of reproductive traits among lithodid crabs, and their history of fishing related stock collapse, caution is clearly warranted in the development of new lithodid fisheries.

While recreational harvest of *L. foraminatus* within current limits (1 crab per person per day, Fisheries and Oceans Canada, 2007) seems unlikely to have a major

impact on stocks, development of a commercial fishery in British Columbia would not be advisable. Other jurisdictions in the Pacific Northwest should also be wary of permitting commercial harvest. Despite the fact that most commercial crab fisheries are male-only, the reproductive potential of a stock can still be impacted due to inability of females to find mates and inability of males to produce adequate sperm for multiple matings (Sato et al., 2007). This is particularly true for lithodid crabs, which have only a narrow window after female moulting during which eggs can be fertilized (Powell et al., 1974, Sato et al., 2005). A more immediate threat to *L. foraminatus* stocks may be by-catch mortality in groundfish and shrimp trawl fisheries. Such fisheries may impact both males and females. Direct by-catch mortality caused by trawl gear (e.g. Broadhurst et al., 2006) and ecosystem level effects of trawling on biodiversity (e.g. Thrush & Dayton, 2002) are subjects of global concern. *Lopholithodes foraminatus* is just one of many species threatened by the continued prosecution of trawl fisheries in the Pacific Northwest.

REFERENCES

Adams P.B. (1980) Life history patterns in marine fishes and their consequences for fisheries management. *Fishery Bulletin*, 78, 1-12.

Baeza J.A. and Fernández M. (2002) Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding. *Functional Ecology*, 16, 241-251.

Broadhurst M.K., Suuronen P. and Hulme A. (2006) Estimating collateral mortality from towed fishing gear. *Fish and Fisheries*, 7, 180-218.

Bull J.J. and Shine R. (1979) Iteroparous animals that skip opportunities for reproduction. *The American Naturalist*, 114, 296-303.

Cohen S.C. and Strathmann R.R. (1996) Embryos at the edge of tolerance: effects of environment and structure of egg masses on supply of oxygen to embryos. *Biological Bulletin*, 190, 8-15.

Crain J.A. and McLaughlin P.A. (2000) Larval and early juvenile development in the Lithodidae (Decapoda: Anomura: Paguroidea) reared under laboratory conditions. 1. subfamily Lithodinae: *Lopholithodes mandtii* Brandt, 1848. *Invertebrate Reproduction and Development*, 37, 43-59.

Dew C.B. and McConnaughey R.A. (2005) Did trawling on the brood stock contribute to the collapse of Alaska's king crab? *Ecological Applications*, 15, 919-941.

Fernández M. and Brandte A. (2003) Brood care in Brachyuran crabs: the effect of oxygen provision on reproductive costs. *Revista Chilena de Historia Natural,* 76, 157-168.

Fernández M., Ruiz-Tagle N., Cifuentes S., Pörtner H.-O. and Arntz (2003) Oxygen-dependant asynchrony of embryonic development in embryo masses of brachyuran crabs. *Marine Biology*, 142, 559-565.

Fisheries and Oceans Canada (2007) *2007-2009 British Columbia Tidal Waters Sport Fishing Guide*. pp 28.

Fisheries and Oceans Canada (2008) Inside Passage: Northern Strait of Georgia: winter, spring, summer, and fall. In *Range Profiles Data Page*. Retrieved Jan 22, 2009, from: http://www-sci.pac.dfo-mpo.gc.ca/osap/data/SearchTools/rangeprofiles_e.htm

Goddard J. (1997) Size at maturity in box crabs, *Lopholithodes foraminatus*, from Oregon. Report prepared for the Oregon Department of Fish and Wildlife. 7 pp.

Haynes E.B. (1968) Relation of fecundity and egg length to carapace length in the king crab, *Paralithodes camtschatica*. *Proceedings of the National Shellfisheries Association*, 58, 60-62.

Haynes E.B. (1993) Stage-I zoeae of laboratory-hatched *Lopholithodes mandtii* (Decapoda, Anomura, Lithodidae). *Fishery Bulletin*, 91, 379-381.

Hines A.H. (1991) Fecundity and reproductive output in nine species of *Cancer* crabs (Crustacea, Brachyura, Cancridae). *Canadian Journal of Fisheries and Aquatic Science*, 48, 267-275.

Hong S.Y., Perry R.I., Boutillier J.A. and Kim M.H. (2005) Larval development of *Acantholithodes hispidus* (Stimpson) (Decapoda: Anomura: Lithodidae) reared in the laboratory. *Invertebrate Reproduction and Development*, 47, 101-110.

Jennings S., Reynolds J.D. and Mills S.C. (1998) Life history correlates of responses to fisheries exploitation. *Proceedings of the Royal Society of London, Series B*, 265, 333-339.

Jennings S., Greenstreet S.P.R. and Reynolds J. (1999) Structural change in an exploited fish community: a consequence of differential fishing effects on species with contrasting life histories. *The Journal of Animal Ecology*, 68, 617-627.

Jensen G.C. (1995) *Pacific Coast Crabs and Shrimps*. Monterey, California: Sea Challengers.

Jensen G.C. and Armstrong D.A. (1989) Biennial reproductive cycle of blue king crab, *Paralithodes platypus*, at the Pribilof Islands, Alaska and comparison to a congener, *P. camtschatica*. *Canadian Journal of Fisheries and Aquatic Science*, 46, 932-940.

Jensen G.C., Armstrong D.A. and Williams G. (1985) Reproductive biology of blue king crab *Paralithodes platypus*, in the Pribilof Islands. In *Proceedings of the International King Crab Symposium*. Anchorage, AK. Report No. **AK-SG-85-12**. pp. 109-120.

Jensen G.C., McDonald P.S. and Armstrong D.A. (2007) Biotic resistance to green crab, *Carcinus maenas*, in California bays. *Marine Biology*, 151, 2231-2243.

Jørgensen C., Ernande B., Fiksen Ø. and Dieckmann U. (2006) The logic of skipped spawning in fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 63, 200-211.

Kato S. (1992) Box crab. In Leet W.S., Dewees C.M. and Haugen C.W. (eds) *California's living marine resources and their utilization*. Davis CA: Sea Grant Extension, pp. 192.

Koslow J.A., Boehlert G.W., Gordon J.D.M., Haedrich R.L., Lorance P. and Parin, N. (2000) Continental slope and deep-sea fisheries: implications for a fragile ecosystem. *ICES Journal of Marine Science*, 57, 548-557.

Lovrich G.A. and Vinuesa J.H. (1993) Reproductive biology of the false southern king crab (*Paralomis granulosa*, Lithodidae) in the Beagle Channel, Argentina. *Fishery Bulletin*, 91, 664-675.

Lovrich G.A. and Vinuesa J.H. (1999) Reproductive potential of the the lithodids *Lithodes santolla* and *Paralomis granulosa* (Anomura, Decapoda) in the Beagle Channel, Argentina. *Scientia Marina,* 63, 355-360.

Moriyasu M. and Lanteigne C. (1998) Embryo development and reproductive cycle in the snow crab, *Chionoecetes opilio* (Crustacea: Majidae), in the southern Gulf of St. Lawrence, Canada. Canadian Journal of Zoology, 76, 2040-2048.

Nakanishi, T. (1987) Rearing condition of eggs, larvae and post-larvae of king crab. *Bulletin of the Japan Sea Regional Fisheries Research Laboratory*, 37, 57-161. (in Japanese with English summary)

Naylor J.K., Taylor E.W. and Bennet D.B. (1999) Oxygen uptake of developing eggs of *Cancer pagurus* (Crustacea: Decapoda: Cancridae) and consequent behaviour of the ovigerous females. *Journal of the Marine Biological Association of the United Kingdom*, 79, 305-315.

Oregon Fish and Wildlife Commission (2008) Meeting material exhibit C Attachment 2: Annual Review of the Developmental Fisheries. In *Oregon Fish and Wildlife Commission minutes*, January 11, 2008.

Orensanz J.M., Armstrong J., Armstrong D. and Hilborn R. (1998) Crustacean resources are vulnerable to serial depletion – the multifaceted decline of crab and shrimp fisheries in the Greater Gulf of Alaska. *Reviews in Fish Biology and Fisheries*, 8, 117-176.

Otto R.S. and Cummiskey P.A. (1985) Observations on the reproductive biology of golden king crab (*Lithodes aequispina*) in the Bering Sea and Aleutian Islands. In *Proceedings of the International King Crab Symposium*. Anchorage, AK. Report No. **AK-SG-85-12**. pp. 123-135.

Paul A.J. and Paul J.M. (2001) The reproductive cycle of golden king crab *Lithodes aequispinus* (Anomura: Lithodidae). *Journal of Shellfish Research*, 20, 369-371.

Petersen S. and Anger K. (1997) Chemical and physiological changes during the embryonic development of the spider crab, *Hyas araneus* L. (Decapoda: Majidae). *Comparative Biochemistry and Physiology B – Biochemistry and Molecular Biology*, 117, 299-306.

Powell G.C., James K.E. and Hurd C.L. (1974) Ability of male king crab, *Paralithodes camtschcatic*a, to mate repeatedly, Kodiak, Alaska, 1973. *Fishery Bulletin*, 72, 171-179.

Reid W.D.K., Watts J., Clarke S., Belchier, M. and Thatje S. (2007) Egg development, hatching rythm and moult patterns in *Paralomis spinosissima* (Decapoda: Anomura: Paguroidea: Lithodidae) from South Georgia waters (Southern Ocean). *Polar Biology*, 30, 1213-1218.

Rideout R.M., Rose G.A., and Burton M.P.M. (2005) Skipped spawning in female iteroparous fishes. *Fish and Fisheries*, 6, 50-72.

Sasakawa Y. (1975) Studies on blue king crab resources in the Western Bering Sea II. Verification of spawning cycle and growth by tagging. *Bulletin of the Japanese Society of Scientific Fisheries*, 41, 937-944. (in Japanese with English summary)

Sato T., Ashidate M. and Goshima S. (2005) Negative effects of delayed mating on the reproductive success of female spiny king crab, *Paralithodes brevipes*. *Journal of Crustacean Biology*, 25, 105-109.

Sato T., Ashidate M., Jinbo T. and Goshima S. (2007) Does male-only fishing influence reproductive success of the female spiny king crab, *Paralithodes brevipes*? *Canadian Journal of Fisheries and Aquatic Science*, 64, 735-742.

Shirley S.M. and Shirley T.C. (1989) Interannual variability in density, timing and survival of Alaskan red king crab *Paralithodes camtschatica* larvae. *Marine Ecology Progress Series*, 54, 51-59.

Shirley T.C., Shirley S.M. and Korn S. (1990) Incubation period, molting, and growth of female red king crabs: effects of temperature. In Meltef B. (ed), *Proceedings of the International Symposium on King and Tanner Crabs, November 28-30, 1989*. Anchorage, AK. University of Alaska Sea Grant Program. Report No. **AK-SG-90-04.** pp. 123-135.

Slatkin M. (1974) Hedging one's evolutionary bets. *Nature* 250, 704-705.

Sloan N.A. (1985) Life history characteristics of fjord-dwelling golden king crabs *Lithodes aequispinus. 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. *Fishery Bulletin*, 79, 259-269.

Somerton D.A. and MacIntosh R.A. (1985) Reproductive biology of the female blue king crab *Paralithodes platypus* near the Pribilof Islands, Alaska. *Journal of Crustacean Biology*, 5, 365-376.

Stevens B.G. (2006a) Embryo development and morphometry in the blue king crab *Paralithodes platypus* studied by using image and cluster analysis. *Journal of Shellfish Research* 25, 569-576.

Stevens B.G. (2006b) Timing and duration of larval hatching for blue king crab *Paralithodes platypus* Brandt, 1850 held in the laboratory. *Journal of Crustacean Biology*, 26, 495-502.

Stevens B.G. and Swiney K.M. (2007) Hatch timing, incubation period, and reproductive cycle of captive primiparous and multiparous red king crab, *Paralithodes camtschaticus*. *Crustacean Biology*, 27, 37-48.

Stevens B.G., Swiney K.M. and Buck L. (2008) Thermal effects on embryonic development and hatching for blue king crab *Paralithodes platypus* (Brandt, 1850) held in the laboratory, and a method for predicting dates of hatching. *Journal of Shellfish Research*, 27, 1255-1263.

Swiney K.M. (2008) Egg extrusion, embryo development, timing and duration of eclosion, and incubation period of primiparous and multiparous tanner crabs (*Chionoecetes bairdi*). *Journal of Crustacean Biology,* 28, 334-341.

Thatje S. (2004) Reproductive trade-offs in benthic decapods crustaceans of high southern latitudes: tolerance of cold and food limitation. *Berichete zur Polar-und Meeresforschung*, 483, 1-183.

Thatje S., Calcagno J.A., Lovrich G.A., Sartoris F.J. and Anger K. (2003) Extended hatching periods in the subantarctic lithodid crabs *Lithodes santolla* and *Paralomis granulosa* (Crustacea: Decapoda: Lithodidae*). Helgoland Marine Research*, 57, 110-113.

Thrush S.F. and Dayton P.K. (2002) Disturbance to marine benthic habitats by trawling and dredging: implications for marine biodiversity. *Annual Review of Ecology and Systematics*, 33, 449-473.

Wear R.G. (1974) Incubation in British decapod Crustacea, and the effects of temperature on the rate and success of embryonic development. *Journal of the Marine Biological Association of the United Kingdom,* 54, 745-762.

Zaklan S.D. (2002) Review of the family Lithodidae (Crustacea: Anomura: Paguroidea): Distribution, biology, and fisheries. In Paul A.J., Dawe E.G., Elner R., Jamieson G.S., Kruse G.H., Otto R.S., Sainte-Marie B., Shirley T.C. and Woodby D. (eds), *Crabs in Cold Water Regions: Biology, Management, and Economics*. Fairbanks: University of Alaska Sea Grant College Program, pp. 751-845.

Zhang Z.Y. (2001) Evaluation of a box crab (*Lopholithodes foraminatus*) trap test in British Columbia. *Canadian Science Advisory Secretariat Research Document* 2001/158. Ottawa: Fisheries and Oceans Canada, 41 pp.

Zhang Z.Y., Workman G. and Phillips A.C. (1999) A review of the biology and fisheries of the box crab (*Lopholithodes foraminatus* Stimpson) in British Columbia. *Canadian Stock Assessment Secretariat Research Document* 99/131. Ottawa: Fisheries and Oceans Canada, 38 pp.

Chapter 3: Larval and early post-larval morphology, growth, and behaviour of laboratory reared *Lopholithodes foraminatus* (brown box crab)

ABSTRACT

*The larval and post-larval behaviour, growth, colour, and morphology of the brown box crab (*Lopholithodes foraminatus*) are described for the first time based on laboratory reared animals. A detailed morphological description is provided for 4 zoeal stages, the glaucothoe, and the first crab instar. Selected morphological changes over the remainder of the first year of development are also described. Data are presented on larval growth at 11 ˚C and on zoeal stage durations at approximately 8 ˚C, 12 ˚C, and 16 ˚C.*
While the 4 zoeal stages are planktotrophic, the glaucothoe does not feed; a life history character that has been termed 'secondary lecithotrophy'. Growth of L. foraminatus *larvae and post-larvae is generally similar to that of other North Pacific lithodids with planktotrophic zoeae. All zoeal stage durations decrease with increasing temperature. This relationship levels off between 12 ˚C and 16 ˚C, a higher temperature than in lithodid species from colder regions. Carapace morphology is suggested as a diagnostic character of larval and post-larval stages of* Lopholithodes foraminatus*. Secondary lecithotrophy may be wide spread or even universal among lithodids and also occurs in pagurid hermit crabs. If the family Lithodidae is indeed nested within the Paguridae, as suggested by recent phylogenetic hypotheses based on molecular evidence, secondary lecithotrophy may be plesiomorphic in lithodids.*

INTRODUCTION

Many marine invertebrates have complex life histories which include planktonic larval forms. The growth, behaviour and morphology of these larvae are often not well studied, even for ecologically or economically important species. Laboratory rearing allows for description of the morphology of ontogenetic stages and collection of developmental data critical for phylogenetic, ecological, and applied fisheries research.

The larval and early juvenile development of king crabs (Anomura: Paguroidea: Lithodidae) has been the subject of several recent studies. Much of this work has focused on economically important members of this group including: *Lithodes aequispinus* (e.g. Shirley & Zhou, 1997); *Lithodes santolla* and *Paralomis granulosa* (e.g. Calcagno et al., 2004); *Paralithodes camtschaticus* (e.g. Epelbaum et al., 2006) and *Paralithodes platypus* (e.g. Stevens et al., 2008). Interest has also been generated by debate surrounding the hypothesized evolution of the Lithodidae from a pagurid hermit crab ancestor (reviewed in McLaughlin & Lemaitre, 1997 & McLaughlin et al., 2007). This hypothesis has recently been supported by both morphological (Richter & Scholtz, 1994) and molecular evidence (Cunningham et al., 1992; Morrison et al., 2002; Tsang et al., 2008). While similarity between lithodid and pagurid zoeae has provided additional support for this hypothesis (Macdonald et al., 1957; Gould, 1992), a pagurid ancestry for the Lithodidae has also been questioned by interpretations of lithodid post-larval abdominal development (McLaughlin & Paul, 2002; McLaughlin et al., 2003; McLaughlin et al., 2004).

To date, complete descriptions of development have been published for 17 lithodid species in 9 genera. A summary of developmental studies can be found in Zaklan

(2002), and descriptions of development have been subsequently produced for *Acantholithodes hispidus* (Hong et al., 2005) and *Paralomis spinosissima* (Watts et al., 2006). Epelbaum et al. (2006) expanded on basic morphological description to include colour, growth and behavioural data for *Paralithodes camtschaticus* reared in the laboratory. The inclusion of such data increases the applicability of developmental studies to ecological and oceanographic research.

In the genus *Lopholithodes*, development has been described only for *Lopholithodes mandtii* (Puget Sound king crab) (zoea I by Haynes, 1993; all stages by Crain & McLaughlin, 2000a). Three distinct larval series obtained off the Oregon Coast in 1970-71 were also tentatively identified as *Lopholithodes* by Lough (1975). *Lopholithodes foraminatus* (Simpson, 1859) (brown box crab) occur sympatrically with *L. mandtii* along the Northwest Coast of North America, although *L. foraminatus* are generally deeper and live on soft rather than hard substrates (Jensen, 1995).

L. foraminatus has been the subject of a small scale commercial fishery in Oregon since 1982 and has been investigated as a commercial species in British Columbia (Zhang et al., 1999; Zhang, 2001). Box crabs are also caught as bycatch in shrimp and groundfish trawl fisheries (Zhang et al., 1999). Commercial fishing activity has apparently been the cause of local stock collapses in the lithodids *Paralithodes camtchaticus* (Dew & McConnaughey, 2005)*, Paralithodes platypus* (Stevens, 2006)*, Lithodes santolla* and *Paralomis granulosa* (Calcagno et al., 2005). Zhang et al. (1999) identified the lack of early life history data as an obstacle to the development of management strategies for *Lopholithodes foraminatus*.

This study describes the morphological development of laboratory reared zoeae, glaucothoe, and early juvenile stages of *Lopholithodes foraminatus*. Data are also presented on behaviour, length and weight at stage, and variation in stage duration with temperature. The implications of selected developmental characters, in particular secondary lecithotrophy, to the biology of the Lithodidae are discussed. Key morphological features useful in the identification of the larvae and post larvae of *L. foraminatus* are also described. These data will be applicable not only for species identification purposes but also for phylogenetic, ecological, and fisheries research on lithodid crabs.

METHODS

Obtaining and rearing larvae

Zoeae released by 4 female *L. foraminatus* were reared for use in different aspects of this study (Table 3.1, and see Chapter 2, Table 2.1 & Figure 2.2). Adult crabs were captured in rectangular Dungeness crab traps at approximately 120m depth on the north side of Baker Passage in the northern Strait of Georgia, British Columbia, Canada (50˚ 01’ 12” N, 124˚ 56’ 43” W). Adult crabs were maintained in the recirculating seawater systems at the University of Victoria. Females 2 and 8 began releasing larvae within 2 months of capture. The mean water temperatures (± SD) experienced by these females between the date of capture and completion of hatching were 10.5 ± 0.1 ˚C and 9.4 ± 0.2 ˚C respectively. Female 13 was captured already releasing larvae and female 9 was held in the lab for 1 year before the beginning of hatching. In spring 2006 female 2 released zoeae in a 170 L tank with a screened outflow. All suspended individuals were collected every morning with either a 5 mm diameter pipette or a fine mesh net. In subsequent

seasons, females releasing larvae were maintained in partially covered 20 L plastic tubs supplied with a constant flow of seawater. Larvae passed through a 90˚ overflow pipe that ended in a T at the bottom of a 750 mL plastic container with a 400 μm Nitex® mesh bottom. This container was seated in a 1L glass beaker. This arrangement allowed zoeae to swim to the top of the 750 mL container and avoid being damaged by turbulence. In all cases hatching occurred in the first hours of darkness and healthy zoeae for rearing experiments were collected the following morning. Virtually all zoeae remaining on the bottom of the tub in the morning had morphological abnormalities and were unable to suspend themselves in the water column. For this reason no effort was made to clean out these zoeae on a daily basis as in previous studies (e.g. Crain and McLaughlin, 2000a).

Seawater used for all rearing was taken from taps in the University of Victoria recirculating seawater system. Water in this system is collected from the ocean and is re-circulated through chillers, sand filters, and UV sterilization tubes.

Zoeae were maintained in 1 L glass beakers or 4 L plastic buckets at densities of 15-50 individuals / litre in either glass-fibre filtered seawater or seawater drawn directly from the system (details of rearing methodologies in different aspects of this study are outlined in Table 3.1). Cultures were fed daily with a combination of newly hatched *Artemia sp.* nauplii and > 24 hr old *Artemia* nauplii that had been fed with *Isochrysis sp.* paste (Reed Mariculture, Campbell, CA). An attempt was made to provide nauplii at a density such that a few would still be present on the subsequent day. When all individuals in a culture vessel had molted to the glaucothoe stage, a piece of nylon mesh was provided as a settlement substrate. In 2006, glaucothoe were initially fed with *Artemia*.

However; after experiments showed that this was a non-feeding stage (see below), no food was provided to glaucothoe during subsequent rearing work.

Table 3.1. Sources of larvae and culture methodologies utilized in describing the development of *Lopholithodes foraminatus.*

♀	**Capture date**	**Carapace width (incl. spines)**	**Larval hatching dates**	**Utilization in the present study and culture methodology.**
2	Mar 12, 2006	10.0 cm	Apr 7- May 14, 2006	Zoeae, glaucothoe, and juveniles dissected, examined, and photographed to produce the morphological description (F, GB (1), 15/L, D, ST); also used for 11 ˚C stage duration determination (25/L) and to test for lecithotrophy (15/L). Glaucothoe used to test for secondary lecithotrophy (GB (0.5); 8-12/L).
8	Jan 13, 2007	10.24 cm	Feb 17-Apr 26, 2007	First stage zoeae used for length / weight measurements.
13	Mar 4, 2007 *	10.42 cm	Mar 4-Apr 18, 2007	Zoeae, glaucothoe, and juveniles used for length / weight measurements (F, GB (1), 40/L, D, I); also used for 8 ˚C and 16 ˚C stage duration determination (F, GB (1), 25/L, ST/I). Glaucothoe and juveniles used as supplementary material for morphological description.
9	Jan 13, 2007	11.57 cm	Jan 14-Apr 28, 2008	Zoeae used to determine effect of temperature on stage duration (UF, PB, 27.5/L, P, I). First stage zoeae used for additional length / weight measurements.

F, vacuum filtered seawater (Pall glass fibre filter); UF, unfiltered seawater; GB (1), 1 L glass beaker; PB, 4 L plastic bucket; #/L, density of larvae per litre; D, daily water change; 2/D, water changed every 2nd day; P, daily partial water change; ST, vessels immersed in seatray; I, vessels in incubator or fridge on 12/12 light/dark cycle.
* Female 13 was releasing larvae when captured. She also exhibited complete reversed asymmetry, with a larger left cheliped and pleopods 2-5 on only the right hand side of her abdomen (see Chapter 4).

Juvenile crabs were maintained at varying densities in containers from 100ml to 4 L with 400 µm Nitex® mesh bottoms. These containers were immersed in a seatray in the re-circulating system. In 2006, juveniles were initially fed every 48-72 hours with the encrusting bryozoan *Membranipora membranacea* and with *Artemia* nauplii which had been fed with powdered sea urchin test. This choice of a high calcium diet was based on anecdotal accounts that juvenile lithodids require a diet high in calcium salts (Jensen,

1995). In subsequent years, better growth and survival were obtained with a diet of chopped frozen krill (*Euphausia pacifica*) every 48-72 hrs, supplemented with *Membranipora* at least once per week. Juvenile crabs of the same instar were maintained together and moved to new containers upon molting. This allowed the stage of juveniles to be followed up to the fifth instar. Subsequently juveniles were maintained in bulk cultures of mixed instars.

Test for lecithotrophy and secondary lecithotrophy

Two 1 L cultures (15/L) of newly hatched zoeae were reared without food to determine whether larvae could develop lecithotrophically. To test for secondary lecithotrophy, 68 glaucothoe were collected from a 4 L mass culture of zoeae on the day of molting to stage. Five 500 ml beakers of 6 glaucothoe and one of 4 glaucothoe were provided with *Artemia* nauplii every second day. Another set of cultures was not provided with nauplii. Culture temperature was ≈ 10 ˚C and water was changed every second day. The mean number of days required to molt to the first crab instar and the arcsine transformed proportion surviving this molt were compared between treatments for the 6 replicates using Student's t-test.

Measurement

Two 1 L cultures containing 40 larvae each were reared for length and weight measurements. Measurements were made of 1st stage zoeae within 24hrs of hatching; 2nd and 3rd stage zoeae within 24 hours of molt to stage; 4th stage zoeae within 48 hours of molt to stage; glaucothoe within 72 hours of molt to stage, and 1st crab instars within 5 days post molt.

All individuals to be measured were anaesthetized with Alka Seltzer® tablets dissolved in seawater and photographed in a drop of water on a microscope slide at 25 X magnification with a Sony PowerHAD digital video camera mounted on an Olympus SZX9 stereomicroscope. Zoeae were photographed in a lateral orientation while glaucothoe and juveniles were photographed dorsally. Measurements were made using Northern Eclipse software calibrated with a slide micrometer. For zoeae, the distance from the tip of the rostrum to the posterior mid-dorsal notch of the carapace was selected as the most accurate measurement since both of these points are in the same focal plane when a zoea is viewed laterally. This measurement is the same as used by Crain and McLaughlin (2000a). No separation was made between carapace length and rostrum length as described by Epelbaum et al. (2006) for red king crab. This is because *L. foraminatus* zoeae lack the obvious notch that defines the base of the rostrum in red king crab zoeae (A. Epelbaum, pers. com., 2007). Glaucothoe and juveniles were measured from the tip of the rostrum to the mid posterior point of the carapace and across the widest point of the carapace, not including spines.

After being photographed, the wet and dry weight of each individual was determined. Larvae were passed momentarily through distilled water, blotted on lens paper, and placed on pre-weighed pieces of tinfoil. Wet weight was measured with a micro balance. Specimens were then dried for at least 24 hours in a 60 ˚C oven. At least 24 hours after removal from the oven the specimens were re-weighed on the micro balance to determine dry weight. This delay allowed specimens to equilibrate to a constant weight; weighing specimens immediately after removal from the oven was complicated by their rapid change in weight while on the balance.

Stage durations

Stage duration was determined primarily from 3 groups of 110 larvae, hatched from female 9 on Feb 22, 2008 and reared in 4 L buckets in incubators set to 8 ˚C, 12 ˚C, and 16 ˚C with a 12:12 light dark cycle. Temperature of the culture water was measured daily. Partial water changes were carried out on a daily basis and exuviae were counted (approximately) and removed. Mean date of molting to stage was calculated as: *Σ ((# of exuviae)* x *(day after hatching)) / total number of exuviae counted for that stage*. Mean stage duration was calculated as the difference between the mean date of molting to the subsequent stage and the mean date of molting to the stage in question ± the standard deviation in the date of molting to the subsequent stage. Due to slight temperature fluctuations in the incubator the mean temperature for each stage was calculated from daily temperature measurements rather than incubator presets. Stage duration data were also recorded using the same methodology for zoeae from females 2 and 13 reared in 1L glass beakers (see Table 3.1). Two beakers of 25 zoeae from female 2 were maintained in a seatray at ≈11 ˚C. Two beakers of 25 zoeae from female 13 were maintained in a seatray at ≈16 ˚C, and two beakers were maintained in a fridge at ≈8 ˚C (data for only one of these beakers is presented due to complete mortality in the other). Second order polynomial regression equations were used to describe the relationship between mean zoeal stage duration and temperature for each stage, and for all four zoeal stages combined (Paul and Paul, 1999). Regression equations were calculated using Microsoft Excel. Stage duration data for glaucothoe were recorded from beaker cultures maintained in seatrays at ≈11 ˚C and ≈16 ˚C.

Morphological description

Mortalities and healthy zoeae of each stage were fixed and preserved in 4% borate buffered formalin in seawater. Glaucothoe and juveniles were fixed for at least 24 hours in 4-5% borate buffered formalin in seawater and either preserved in the fixation solution or rinsed with 50% ethanol and preserved in 70% ethanol. Exuviae of all stages were preserved in 70% ethanol. Specimens and exuviae were dissected under a stereomicroscope and appendages were photographed in multiple focal planes using a DVC digital camera mounted on a Zeiss Axioskop compound microscope. Photographs of focal planes were imported sequentially as layers into Adobe Photoshop CS3. Drawings were made in an overlying layer by tracing structures in multiple focal planes using the pen tool. For whole animal drawings, live zoeae were held immobile on a slide using a coverslip supported by plasticine feet and photographed and drawn using the methodology described above. Using live zoeae for illustrations avoided the raised and distorted carapace typical of preserved material. Whole animal drawings of glaucothoe and drawings of juvenile crab carapaces and abdomens were produced from preserved exuviae. For clarity, illustrations of the lateral view of whole individuals show only the appendages on the near side.

All structures are illustrated and described for the first zoeal stage. For subsequent stages, only structures showing significant changes in morphology are illustrated. At least 5 specimens of each appendage for each stage were examined to confirm morphology and setal arrangement (Clark et al., 1998) (care was taken to examine both preserved healthy larvae and exuviae). Examination of exuviae allowed for observation of setae on

the opposite side of appendages and observation of preserved whole specimens allowed for confirmation of appendage shape.

Terminology

The terminology used to describe the setae of lithodid larvae and juveniles varies. Crain (1999) described 6 types of setae from the mouthparts of *Placetron wosnessenskii* following the system used by Lavalli and Factor (1992) for the mouthparts of larval lobsters. More recently, Epelbaum et al. (2006) adapted the terminology of Factor (1978) to describe 10 types of setae from *Paralithodes camtschaticus* larvae and postlarvae. Epelbaum and Borisov (2006) modified this scheme and reduced the number of setal types to 7. These systems were found to be problematic for *Lopholithodes foraminatus* due to the prevalence of setal types intermediate between described classifications. The present study uses a simplified scheme of setal terminology based on the works cited above (Figure 3.1). Setae with long setules growing primarily in 2 directions are referred to as plumose, or natatory when used for swimming. Those with sparse, long setules arranged at random are referred to as pappose. All setae bearing any combination of short stout setules, short fine setules, scales, or apparent serration are referred to as denticulate. This category includes the serrate and serrulate types of the above authors. Any denticulate setae that also bear long fine setules are referred to as plumodenticulate. Unarmed setae are referred to as simple, and spiniform processes are described individually. There is still some overlap between these setal types, partially due to the presence of minute setules at the limit of resolution with light microscopy.

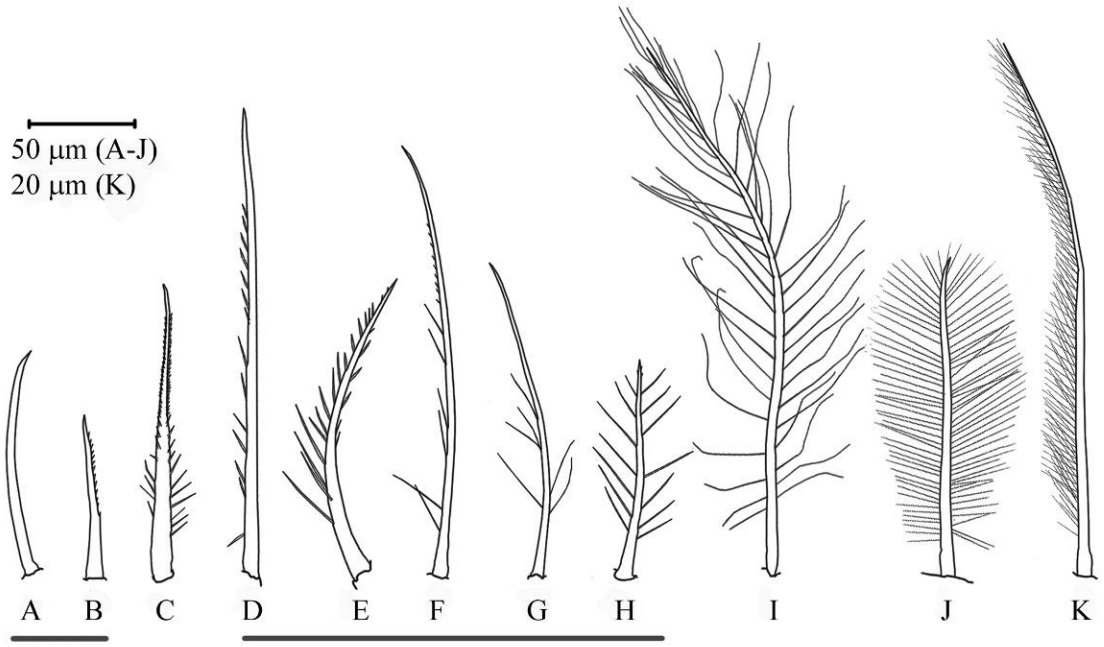


Fig. 3.1. Summary of setal types of *Lopholithodes foraminatus* larvae and post larvae: (A) simple; (B-D) denticulate; (E-F) plumodenticulate; (G) pappose; (H-J) plumose; and (K) plumose (natatory). The gray bars indicate areas of significant overlap between types.

Appendage terminology largely follows Epelbaum et al. (2006). In the case of mouthparts, external refers to the surface of the appendage facing away from the mouth, while internal refers to the surface facing towards the mouth. Unless otherwise specified, illustrations show the external surface of appendages.

Samples of zoeal instars, glaucothoe, and 1st and 2nd crab instars were deposited in the invertebrate collection of the Royal British Columbia Museum in Victoria, B.C. Canada.

RESULTS

Behaviour

A prezoeal stage was observed in hatching vessels soon after lights were turned out in the evening. Prezoeae suspended themselves by a jack knife flexion of the abdomen and thorax. The prezoeal exoskeleton was apparently shed within minutes of

hatching. At this point the first zoeal stage adopted the mode of swimming that would be used throughout zoeal development. Zoeae swam by vigorously beating the exopods of their maxillipeds while suspended in a head down orientation with the abdomen extended straight above them. Periodically they would flex the abdomen against the ventral surface of the thorax and drag the telson across the mouthparts. When not actively swimming, zoeae would sink rapidly.

Zoeae were positively phototactic and in a transparent culture vessel would tend to suspend more readily when placed on a dark coloured than a light coloured surface. Over the course of zoeal development larvae spent less time suspended. In the first days after hatching virtually all individuals would be suspended in the culture vessel while by the 4th zoeal stage less than 25% would generally be suspended at any given time.

Zoeae of all 4 stages exhibited a characteristic response to physical or chemical perturbation. When sucked into a pipette, grasped with forceps, or placed in fixative, the antennae were rapidly flexed laterally into a position where the scaphocerites were at 90˚ to the long axis of the body. This position would be maintained until the threat had passed.

Cannibalism by zoeae was almost never observed or inferred by larvae disappearing from cultures.

Swimming by glaucothoe was accomplished by beating the pleopods with the pereopods extended anteriorly and held stationary. Glaucothoe would suspend relatively frequently during the first one or two days after molting but thereafter became less active. They would seek a substrate they could grasp and in a smooth bottomed container would end up clinging to each other in a ball of many individuals. When provided with a mesh

substrate they would cling to it with their pereopods and would sometimes extend their abdomens and rhythmically beat their pleopods. Periodically the 5th pereopods could be seen through the translucent carapace being drawn back and forth across the gills. In response to stress, glaucothoe would tightly draw in their pereopods and tuck in their eyestalks in the adult manner. This response was maintained through the juvenile stages. Feeding and cannibalism by glaucothoe was not observed (see below).

Juvenile crabs no longer had the ability to swim and would walk slowly across the substrate. The first juvenile stage was relatively inactive and responded to food only if it was extremely close. Subsequent juvenile stages became more active and more proactive in walking towards a food source. Cannibalism was also more prevalent in later stages but seemed to be mostly restricted to predation on moulting or moribund individuals.

Test for lecithotrophy and secondary lecithotrophy

All first stage zoeae reared without food died by the 10th day after hatching. Some individuals shed scraps of exoskeleton or became stuck in the exuvium but none molted successfully to the second zoeal stage.

Glaucothoe were observed to grasp *Artemia* nauplii with their chelae and pull them towards the mouthparts. However, in all cases the nauplii were then pushed away again by the claws or the respiratory current. There was no significant difference in either survival to the first crab instar (Student's t-test, $t = -0.47$, $df = 10$, $P = 0.65$) or the number of days before molting (Student's t-test, $t = 0.36$, $df = 10$, $P = 0.72$) between glaucothoe that were provided with *Artemia* and those that were not.

Growth

Length measurements for all larval stages are summarized in Table 3.2. Mean dry weights (± SE) for newly hatched zoeae from females 8, 13, and 9 were 183 ± 8 µg, 179 ± 2 µg, and 199 ± 2 µg respectively. This yields an overall mean dry weight for first stage zoeae of 185 ± 3 µg (Figure 3.2A). The mean wet weights (± SE) for newly hatched zoeae from females 13, and 9 were 1.34 ± 0.06 mg, and 1.19 ± 0.02 mg respectively. This yields an overall mean wet weight for first stage zoeae of 1.26 ± 0.03 mg (Figure 3.2B).

Table 3.2. Length measurements of *Lopholithodes foraminatus* zoeae, glaucothoe, and first stage juveniles. Zoeae were measured from the tip of the rostrum to the mid dorsal notch in the posterior margin of the carapace. Carapace length of glaucothoe and juveniles was measured from the tip of the rotrum to the mid-point of the posterior carapace margin; carapace width was measured at the widest point not including spines.

♀	Stage	Carapace length ± SE (mm)	Carapace width ± SE (mm)	N
B	Z I	2.896 ± 0.018	N/A	10
C	Z I	2.888 ± 0.035	N/A	21
D	Z I	2.853 ± 0.026	N/A	10
C	Z II	3.167 ± 0.021	N/A	16
C	Z III	3.475 ± 0.050	N/A	10
C	Z IV	3.801 ± 0.017	N/A	9
C	Glaucothoe	2.419 ± 0.029	1.744 ± 0.017	17
C	Crab I	2.545 ± 0.040	1.787 ± 0.030	9
C	Crab II	2.862 ± 0.058	2.180 ± 0.029	5
C	Crab III	3.336 ± 0.066	2.543 ± 0.042	5
C	Crab IV	3.815 ±0.048	2.948 ± 0.045	5

Stage duration and survival

The relationships between stage duration and temperature for each zoeal stage and all 4 zoeal stages combined are illustrated in Figure 3.3. Initial numbers of larvae in each treatment and percent survival to the glaucothoe stage are presented in Figure 3.3E. The second order regression functions calculated for each zoeal stage, and for all 4 zoeal stages combined, level out at approximately 16 ˚C.

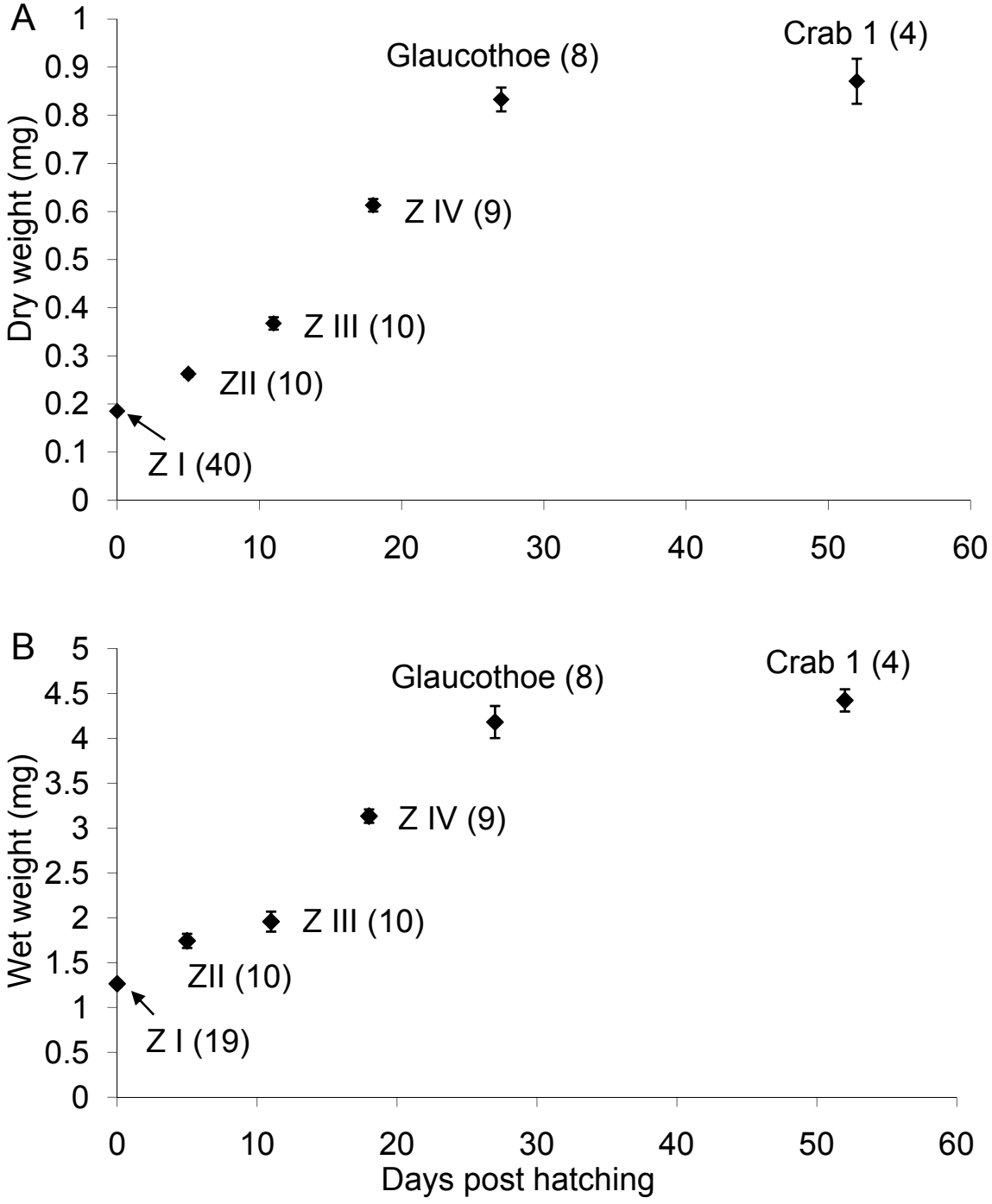
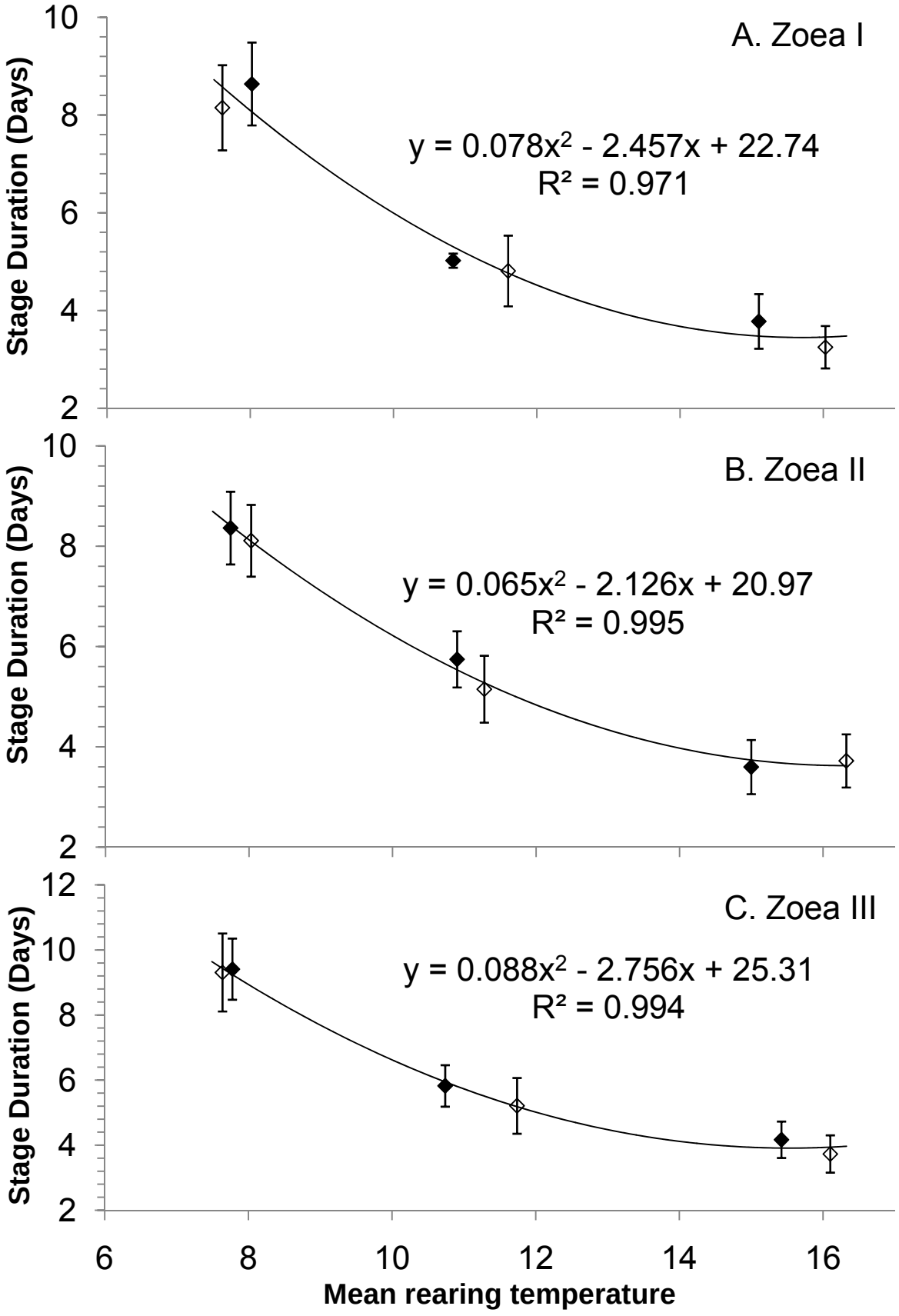


Fig. 3.2. Relationship between mean dry (A) and wet (B) weights (± SE) and days post hatching for *Lopholithodes foraminatus* zoeae, glaucothoe, and first crab instars. Stages reared in 1 L beakers at an initial density of 40/L with daily changes of filtered seawater (every second day for glaucothoe). Rearing temperature for all cultures ≈ 11 ˚C. Numbers in brackets indicate sample size.


A. Zoea I
y = 0.078x² - 2.457x + 22.74
R² = 0.971
B. Zoea II
y = 0.065x² - 2.126x + 20.97
R² = 0.995
C. Zoea III
y = 0.088x² - 2.756x + 25.31
R² = 0.994
Stage Duration (Days)
Mean rearing temperature
2
4
6
8
10
12
14
16

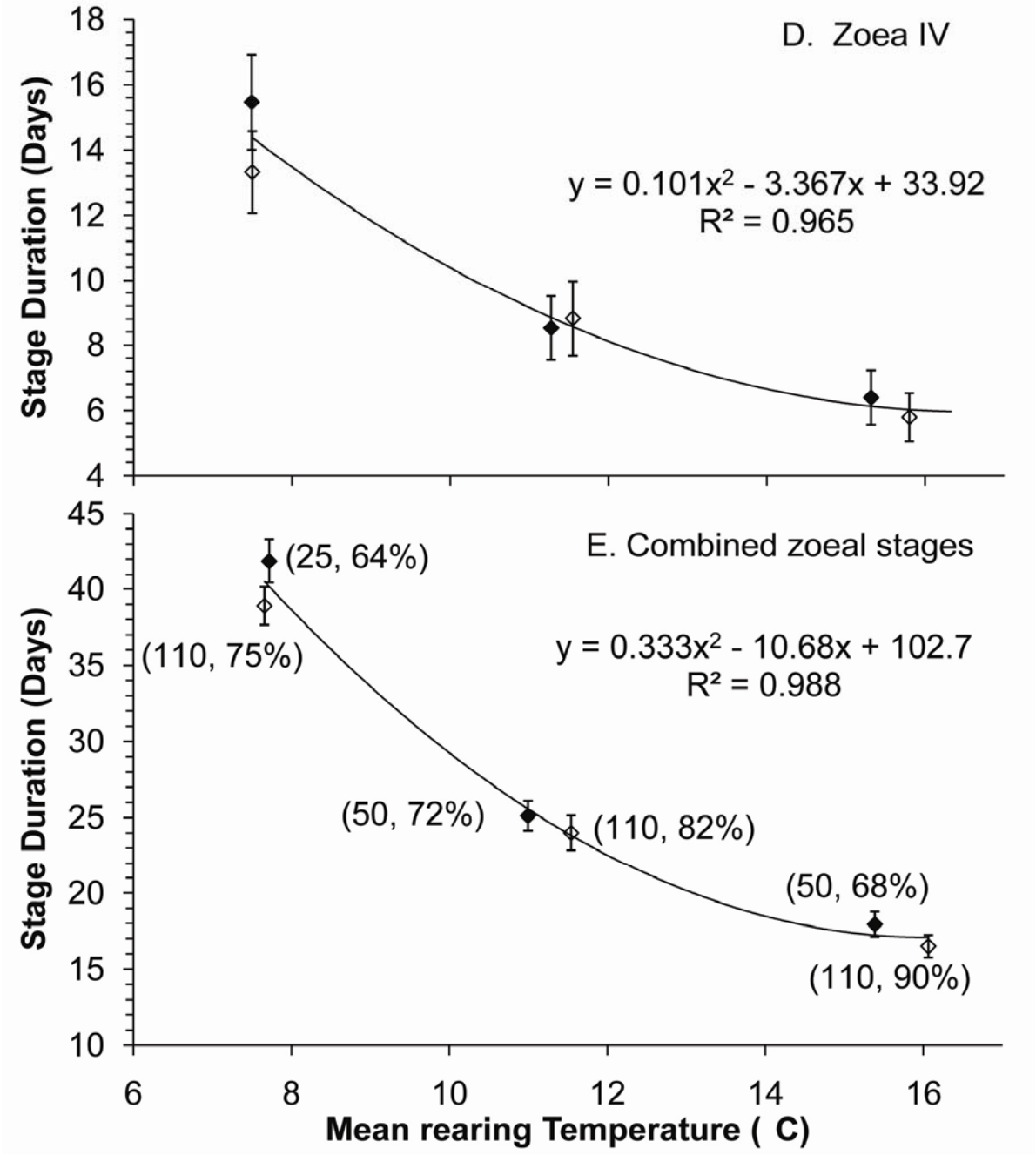


Fig. 3.3. Relationship between mean rearing temperature and mean stage duration (±SD) for *Lopholithodes foraminatus* zoeal stages (A) I, (B) II, (C) III, (D) IV; and (E) all four zoeal stages combined. Open symbols indicate mean stage duration values for larvae cultured in 4L buckets in incubators at a density of 27.5 individuals/L (50% unfiltered water changes daily); filled symbols indicate larvae cultured in beakers at a density of 25 individuals/L (complete filtered water changes daily). Beakers were immersed in flowing water (≈16 ˚C and ≈11 ˚C) or held in a fridge (≈ 8 ˚C). Parentheses in 3E indicate (Initial # of larvae, % surviving to the glaucothoe stage).

The equation for the complete zoeal period (duration = 0.333 (temp ˚C)^2 - 10.68 (temp ˚C) + 102.7) gives degree day estimates for zoeal development of 308.6 at 8 ˚C, 280.6 at 11 ˚C, and 273.1 at 16 ˚C. The duration of the glaucothoe stage (± standard deviation) was 13.7 ± 1 days at 15.5 ˚C (212 degree days, 34 glaucothoe, 85% survival to the first crab instar) and 19.0 ± 1.7 days at 11.5 ˚C (219 degree days, 36 glaucothoe, 72% survival to the first crab instar). Survival through subsequent crab stages was poor, with almost no individuals surviving for more than 1 year. When maintained in mesh containers in the sea tray at approximately 9 ˚C, the first individuals were molting to the 5th crab instar by about 28 weeks post hatching.

Morphological description

PREZOEA

Prezoeae were covered in an unarmed cuticle. The posterolateral spines were contained by this cuticle and folded down along the posterior margin of the carapace. The rostrum was folded ventrally. The prezoeal cuticle was elaborated into feathery projections where it covered the telson, antennules, and antennal exopods.

ZOEA I (Figure 3.4)

Colour:

The pale orange colour of newly hatched zoeae became more intense during the first stage. This colour was due to orange chromatosomes in the carapace, rostrum, antennae, and maxillipeds. When held in the dark, zoeae became much paler because pigment in the chromatosomes was concentrated; this remained true for subsequent zoeal stages. Chromatosomes with concentrated pigment appeared red rather than orange. There was a transparent patch dorsally in the vicinity of the heart. The abdomen and

telson were also transparent. The digestive gland was pale orange-brown. When viewed with incident light the compound eyes were golden yellow.

*Cephalothorax***:**

The rostral spine constituted approximately half of the total length of the carapace. The posterolateral spines were pronounced and the posterior margin of the carapace was deeply notched. There was a distinctly peaked middorsal carina with a generally steeper anterior than posterior slope. The lateral walls of the carapace were inflated and completely covered the coxae of the maxillipeds; the developing pereopods; and the majority of the first and second abdominal segments. The eyes were sessile.

Antennule (Figure 3.4B):

The antennule projected anteriorly and dorsally and had a slight lateral curve. It was incompletely 2 segmented with a medial unarmed endopod bud. The putative protopod had 1-2 minute dorsal setae. Distally the antennule bore 6-7 aesthetascs and 3-4 simple setae. Throughout development the exact setal arrangement of the antennule was very difficult to positively determine due to the tendency of aesthetascs to kink or break and the positioning of minute setae between the bases of the aesthetascs.

Antenna (Figure 3.4C):

Ventrally the protopod had a large unarmed spine at the base of the exopod and a spinulose spine at the base of the endopod. The endopod was not delineated from the protopod and had a bifid tip. The exopod was slightly longer than the endopod and usually reached the tip of the rostral spine. Medially the exopod had a flattened ridge bearing 5 plumose setae which became smaller and sparser distally. This ridge terminated

in a flattened lobe bearing 4 pappose and/or simple setae. The distal portion of the exopod was unarmed and acute.

Mandibles (Figure 3.4D):

The mandibles were angled slightly anteriorly and the internal surfaces were held against, and partially covered by, the labrum. This positioning was maintained throughout zoeal development and is illustrated for the third zoeal stage in Figure 3.6B. Both the left and right mandibles had a large apical tooth at their medio-ventral angle. They were distinctly asymmetrical in the balance of their denticulation. The gnathal lobe of the left mandible consisted of the apical tooth and 2 acute denticulations; the molar lobe had 3 ridges of small denticulations. The gnathal lobe of the right mandible had a series of acute denticulations which were drawn out into a convex dorsal shield. The molar lobe consisted of 2 ridges internal to this shield and 2 dorsal teeth. Although the proportions of mandibular denticulations changed, the basic left right asymmetry was maintained through subsequent zoeal stages.

Maxillule (Figure 3.4E):

The coxal endite had 1 short simple seta submarginally and 1 pappose, 1 denticulate, and 4 plumodenticulate setae marginally. The plumodenticulate setae were strongly curved anteriorly and dorsally. The basial endite had 2 submarginal simple setae and 2 or 3 large marginal spines. Each of these spines had several internally projecting denticulations. The endopod was 3 segmented. The proximal segment bore 1 or 2 minute simple setae, the second segment 1 pappose seta, and the distal segment 3 terminal pappose setae.

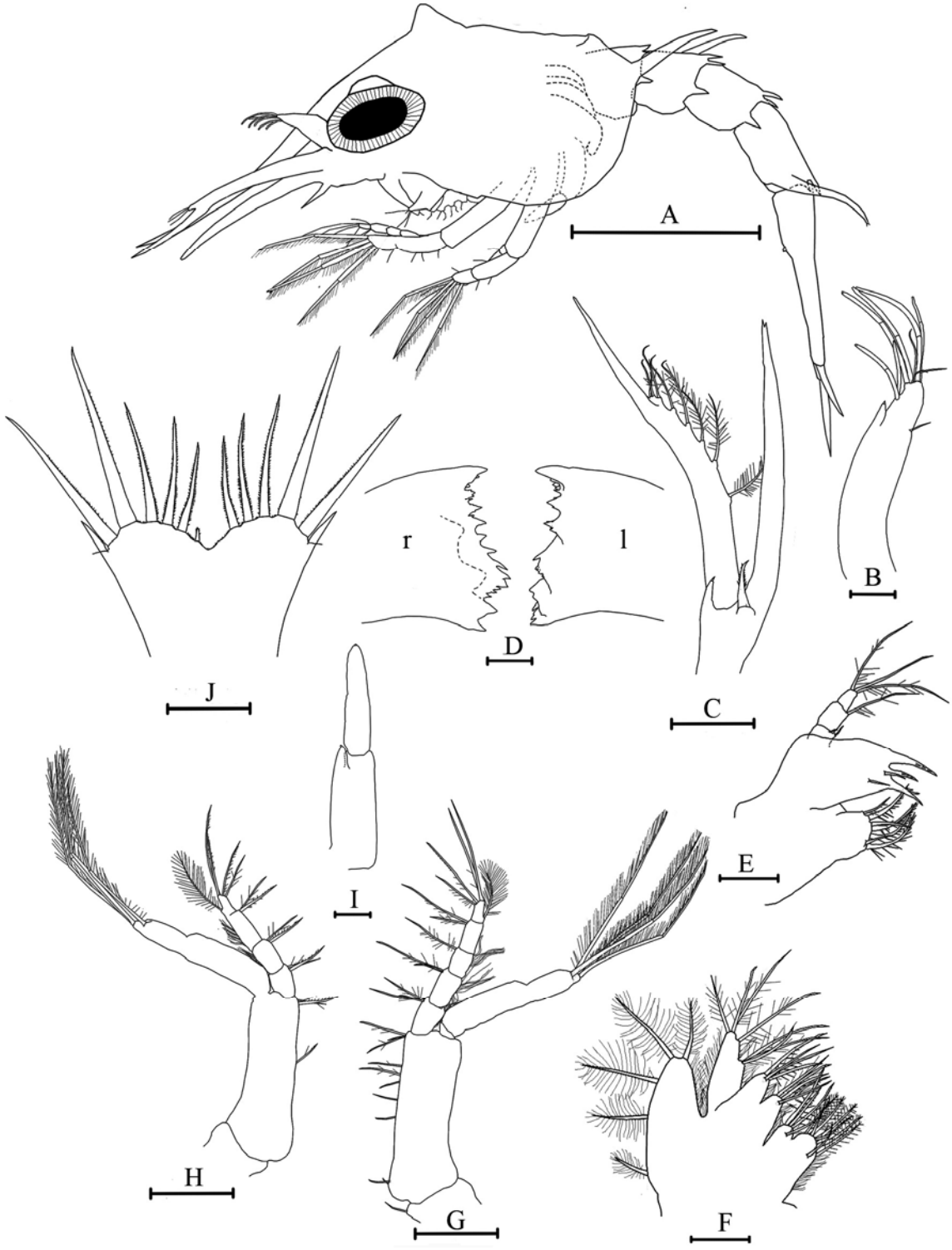


Fig. 3.4. Morphology of a representative *Lopholithodes foraminatus* A. zoea I (lateral); B. antennule (left ventrolateral); C. antenna (right ventral); D. [r]ight (dotted line indicates anterior ridges of molar lobe) and [l]eft mandibles; E. maxillule (right); F. maxilla (right) G. 1st maxilliped (left externolateral) H. 2nd maxilliped (right externolateral) I. 3rd maxilliped (right internal) J. telson (ventral) Scale bars: A = 1 mm; B, E, F, I = 100 μm; C, G, H, J = 250 μm; D = 50 μm.

Maxilla (Figure 3.4F):

Both the coxal endite and the basial endite were bilobed. The proximal lobe of the coxal endite had 1 pappose seta submarginally and 5 weakly plumose and 2 plumodenticulate setae marginally. The distal lobe of the coxal endite had 1 pappose seta submarginally and 3 plumodenticulate setae marginally. Both lobes of the basial endite had a submarginal pappose seta. The proximal and distal lobes had 3-4 and 3 marginal plumodenticulate and/or pappose setae respectively. The endopod had 2-3 pappose setae projecting from a ledge about half way along the medial margin and bore 1 subterminal and 3 terminal pappose setae. The exopod (scaphognathite) had 5 marginal plumose setae. Fine hair-like structures arose from all processes of the maxilla.

First Maxilliped (Figure 3.4G):

The coxa was usually armed with 1 small simple seta. The basis had 2 small simple setae basally; 5 simple and/or pappose setae along the medial margin; and 3 pappose and/or simple setae terminally. The endopod had 5 segments. The 3 proximal segments bore fine hair-like processes on their lateral surfaces. Progressing distally the first 4 segments of the endopod bore 3, 2, 1, and 2 pappose and/or simple setae respectively. The distal segment had 1 subterminal pappose seta, 1 subterminal plumodenticulate seta, 2 terminal plumodenticulate setae, and 1 weakly plumose seta laterally at the base of the segment. The incompletely 2 segmented exopod had 2 subterminal and 2 terminal natatory setae.

Second Maxilliped (Figure 3.4H):

The coxa was unarmed. The basis bore 1 marginally denticulate seta about halfway along the medial surface and 1 pappose and 1 denticulate seta terminally. The

endopod was 4 segmented and each segment had 1 pappose and 1 denticulate seta distally. In addition, the distal segment had a pair of long terminal denticulate setae and 1 plumose seta laterally at the base of the segment. The middle 2 segments of the endopod had fine hair-like processes on their lateral surfaces. The exopod was incompletely delineated into 2 segments and had 2 subterminal and 2 terminal natatory setae.

Third Maxilliped (Figure 3.4I):

The third maxillipeds were angled anteriorly, fitting between the bases of the second maxillipeds. The coxa was indistinct and the large basis bore an unarmed endopod bud. The exopod consisted of 1 segment and was also unarmed.

Abdomen:

The first abdominal somite was unarmed. Somites 2 through 5 had 4 posteriodorsal spines: those on somite 2 were significantly smaller than on the other 3 somites. Somites 2 through 4 bore a pair of short posterolateral spines while somite 5 bore a pair of long posterolateral spines that projected dorsally and were curved posteriorly. In some specimens, one or both of these spines had a bifid tip. The 6th abdominal somite was fused with the telson to form a single segment.

Telson (Figure 3.4J):

The telson was broad and consisted of 2 convex lobes flanking a pronounced median cleft. Laterally each lobe had a short unarmed spine that was continuous with the exoskeleton (the balance of telsonal processes were clearly delineated). Immediately medial to this process was a minute anomuran hair which projected ventrally. Progressing towards the medial cleft, each lobe bore 5 spines armed with numerous minute denticulations. The second of these processes was the longest. In many of the first stage

zoeae hatched from female 2, a small 6th spine was present adjacent to the medial cleft on 1 or both lobes. However, in first stage zoeae hatched from other females this process was almost never observed. Between spines and in the median cleft the margin of the telson bore minute denticulations.

ZOEA II (Figure 3.5)

Colour:

The orange pigmentation was considerably more intense than in the first stage. The mouthparts and bases of the antennae appeared intense red-orange. It was not clear whether this resulted from a higher density of orange chromatosomes or from the presence of chromatosomes with a darker pigment. The abdomen and telson were very slightly pigmented orange but still appeared transparent at low magnification.

Cephalothorax:

The overall shape of the carapace was unchanged from the first stage. However, the posterolateral spines were proportionally smaller. The eyes were now stalked.

Antennule (Figure 3.5B):

The antennule was now 2 segmented with a large endopod bud arising from the proximal segment. Two to 4 minute setae projected from the distal margin of the proximal segment. The distal segment had 4-6 subterminal aesthetascs, 1 to 3 terminal aesthetascs, and 2-3 simple terminal setae.

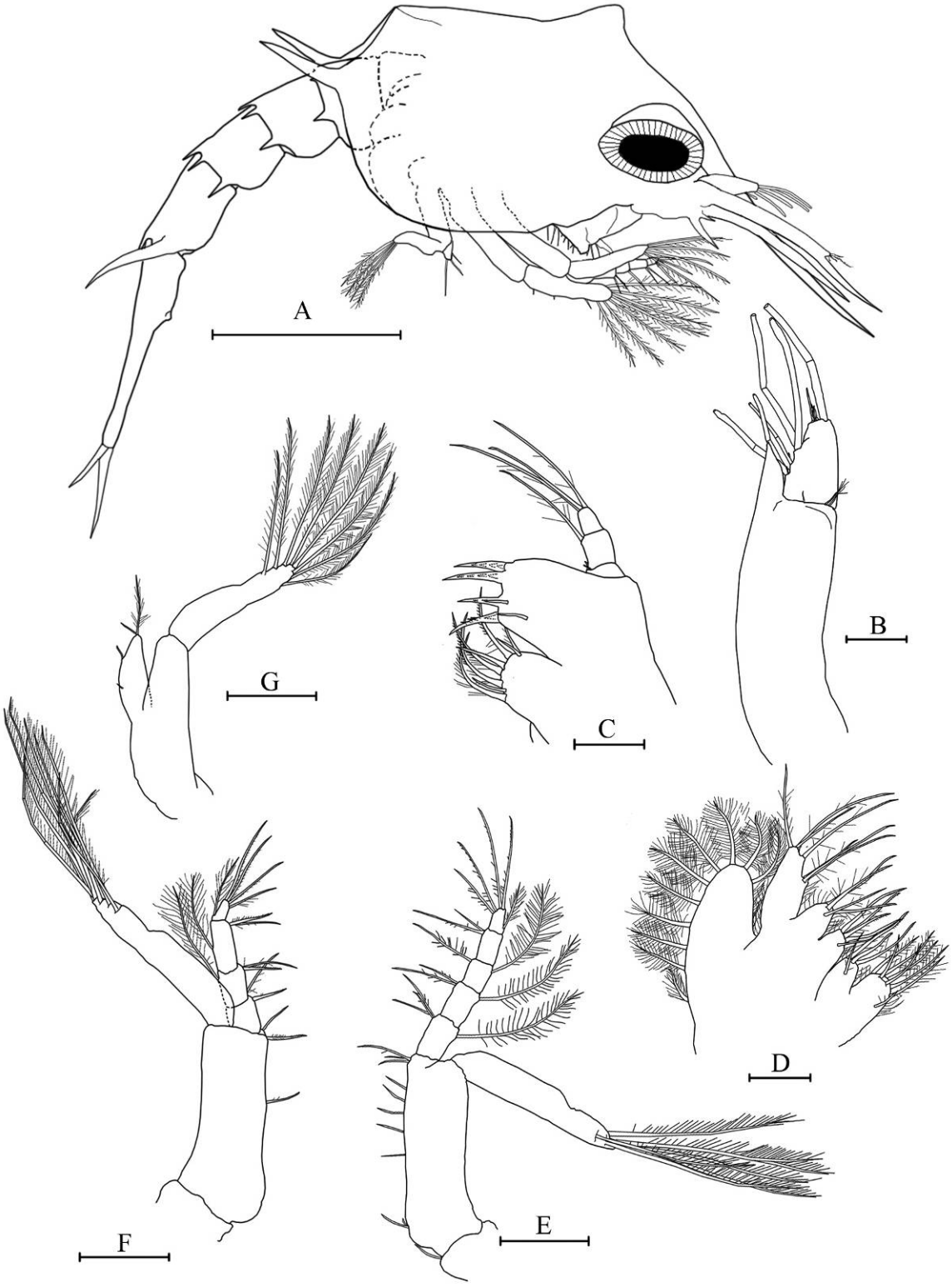


Fig. 3.5. Morphology of a representative *Lopholithodes foraminatus* A. zoea II (lateral); B. antennule (right dorsomedial); C. maxillule (left); D. maxilla (right); E. 1st maxilliped (left); F. 2nd maxilliped (right externolateral) G. 3rd maxilliped (left externolateral). Scale bars: A = 1mm; B-D = 100 μm; E-G = 250 μm.

Maxillule (Figure 3.5C):

The coxal endite now had 1 submarginal denticulate seta and 1 denticulate, 1 simple, 1 pappose, and 4 large plumodenticulate marginal setae. The basial endite now bore 2 submarginal denticulate setae and 4 marginal spines with several pronounced denticulations. In some cases an additional small simple marginal spine was present.

Maxilla (Figure 3.5D):

The scaphognathite now had 9 to 12 marginal plumose setae.

First Maxilliped (Figure 3.5E):

All segments of the endopod now had a plumose seta on their lateral surface; the hair-like processes present in corresponding locations in the first stage were absent. The exopod now bore 7 or 8 natatory setae.

Second Maxilliped (Figure 3.5F):

The 2nd and 3rd segments of the endopod now each had a plumose seta on their outer surface. The hair-like processes present in corresponding locations in the first stage were absent. The exopod now bore 7 or 8 natatory setae.

Third Maxilliped (Figure 3.5G):

The endopod was now proportionally larger than in the first stage but was still unsegmented and apparently did not articulate with the basis. It bore 2 denticulate and 1 plumose setae. The exopod now bore 7or 8 natatory setae.

Telson:

The telson was largely unchanged from the first stage. However, the small spiniform process present or absent from one or both sides of the median cleft in the first stage was now present on both sides.

ZOEA III (Figure 3.6)

Colour:

The orange colour of the carapace and thoracic appendages was very intense. The abdomen now had a diffuse orange pigmentation that was visible at low magnification; this pigment did not appear to be organized in chromatosomes.

Mandibles (Figure 3.6B-C):

The mandibles now had small palp buds anteriorly. The asymmetry in dentition of the left and right mandibles agreed with that in the previous 2 stages.

Maxillule (Figure 3.6D):

The coxal endite now had 2 denticulate, 1 small plumodenticulate, and 4 large plumodenticulate marginal setae. The basial endite now always bore 5 denticulate spines.

Maxilla (Figure 3.6E):

The scaphognathite now bore 10 to 13 plumose marginal setae interspersed with up to 3 minute buds.

Abdomen (Figure 3.6F):

Somites 2-5 now bore minute pleopod buds and uropods were present on somite 6. The uropods consisted of a single segment with one or two simple terminal setae.

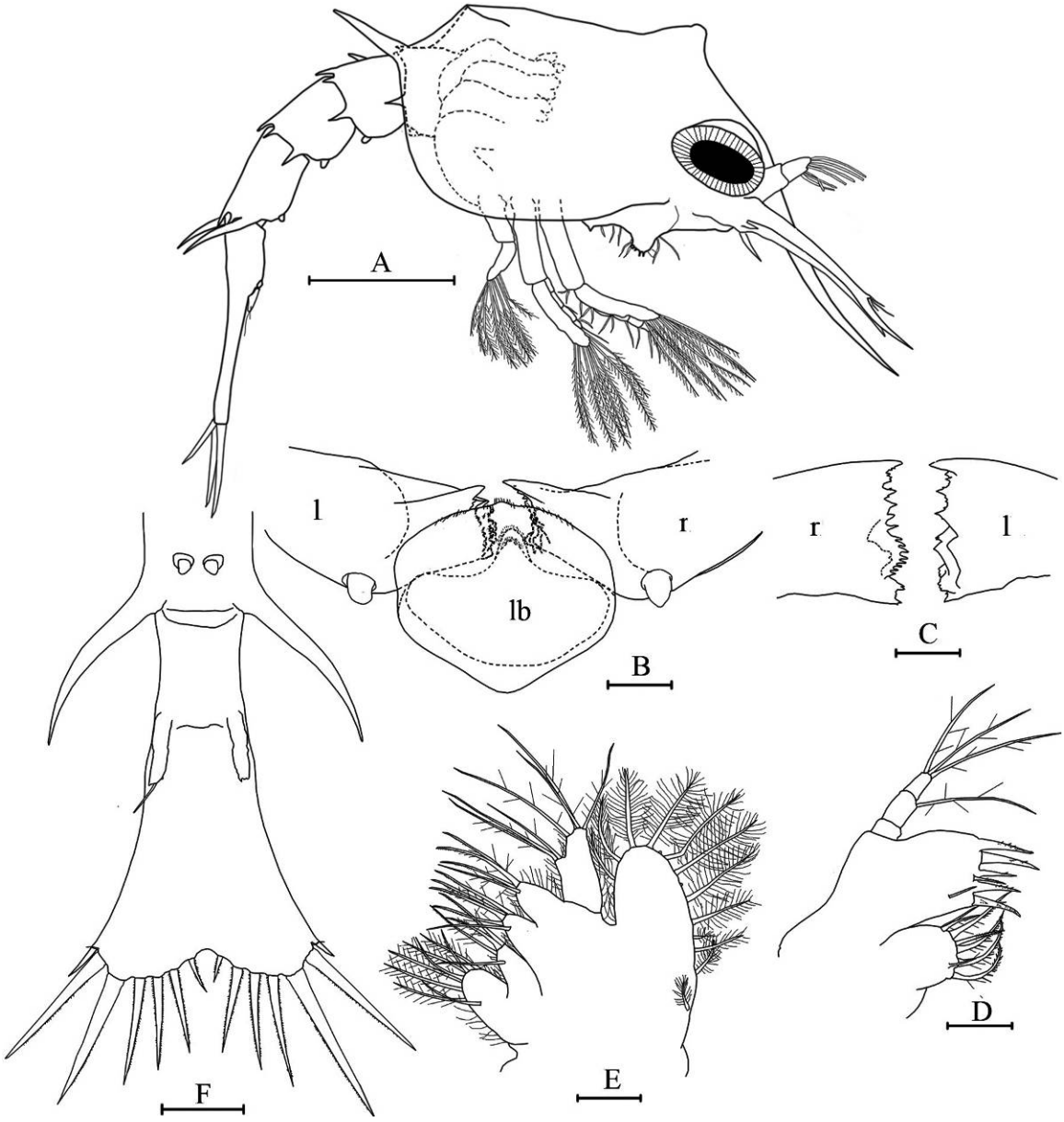


Fig. 3.6. Morphology of a representative *Lopholithodes foraminatus* A. zoea III (lateral); B. [l]eft and [r]ight mandibles and labrum (lb) (ventral, in situ); C. mandibles ([r]ight and [l]eft); D. maxillule (right); E. maxilla (left); F. telson and abdominal segments 5 and 6 (ventral). Scale bars: A = 1mm; B-E = 100 μm; F = 250 μm.

ZOEA IV (Figure 3.7)

Colour:

The thoracic appendages and carapace were still bright orange due to the presence of chromatosomes. The diffuse orange colour of the abdomen extended into the

developing pleopods. In exuviae the collapsed exoskeleton of the pereopods and pleopods and the exoskeleton of the mouthparts were visibly orange.

Cephalothorax:

The carapace was markedly more globular than in the preceding stages. Large lipid droplets were now visible in the vicinity of the digestive gland; directly posterior to the eye and ventral to the heart. The pereopods (Figure 3.7F) were much larger than in previous stages and the developing chelae projected from under the edge of the carapace. The developing 5th pereopod lay under the other pereopods. Pairs of developing gills at the base of the pereopods were proportionally larger than in the third zoeal stage.

Antennule:

The exopod now had 6-7 subterminal aesthetascs, up to 1 subterminal seta, 2-3 terminal aesthetascs, and 1 to 6 terminal setae.

Antenna: (Figure 3.7B):

The exopod now had 6-7 plumose setae and 2-3 minute seta. The endopod had a scattering of minute denticulations and in some cases a minute subterminal seta. In some instances these endopod characters were partially developed in the previous 2 stages.

Mandibles (Figure 3.7C):

The mandibular palp buds were slightly larger than in the third zoeal stage but still unarmed.

Maxillule:

The basial endite (Figure 3.7D) now had an additional 2 small marginal denticulations: one at the distal angle of the endite and one between the 2nd and 3rd most distal spines. The setation was otherwise unchanged.

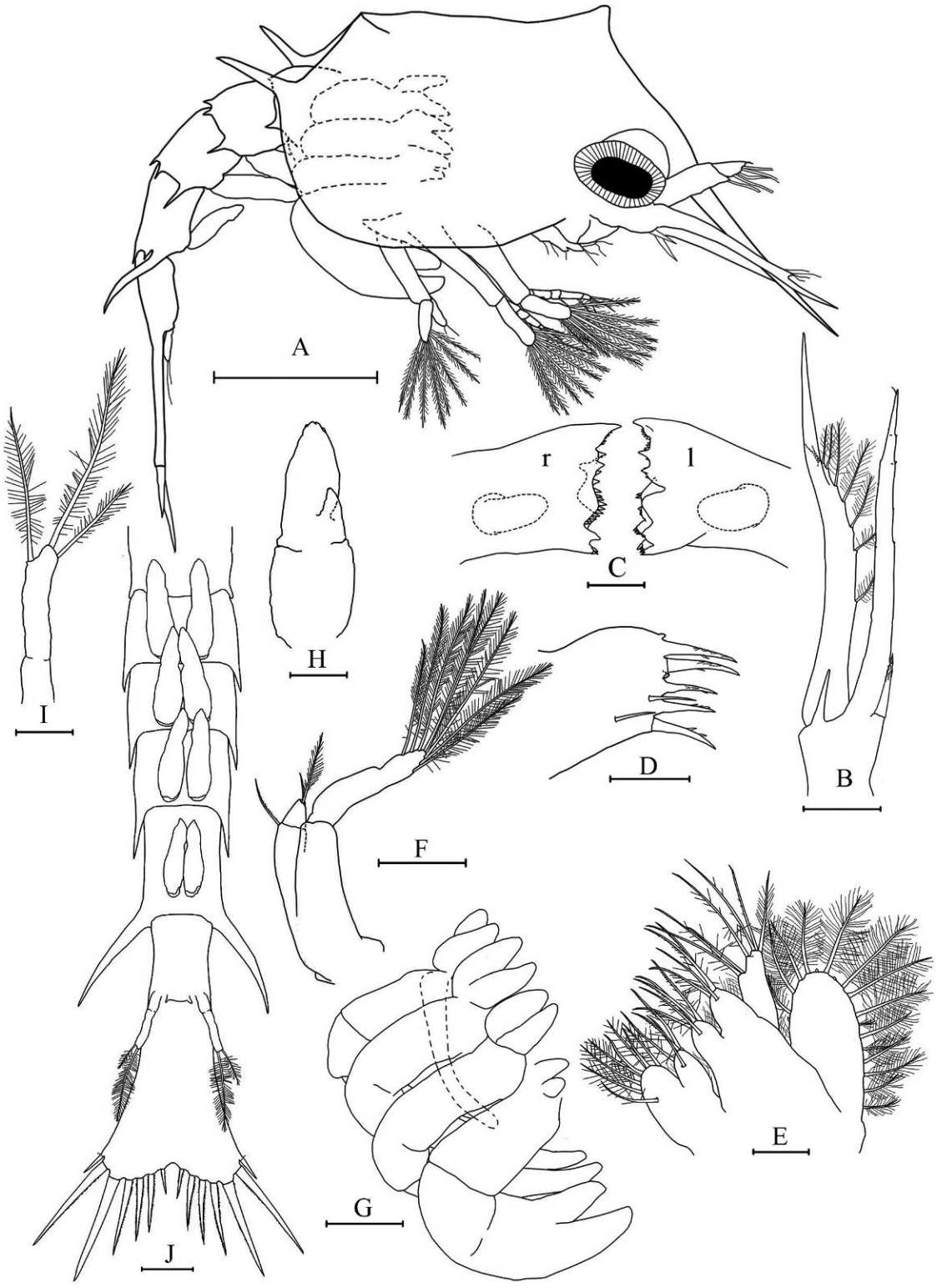


Fig. 3.7. Morphology of a representative *Lopholithodes foraminatus* A. zoea IV (lateral); B. antenna (right ventral); C. mandibles ([r]ight and [l]eft); D. basial endite of maxillule (right); E. maxilla (left); F. 3rd maxilliped (left externolateral) G. pereopods (right lateral); H. 2nd pleopod (left anterior); I. uropod (right ventral); J. abdomen (ventral). Scale bars: A = 1 mm; B, F-G, J = 250 μm; C-E, H-I = 100 μm.

Maxilla (Figure 3.7E):

One of the 3 terminal setae on the endopod was now weakly plumose, the other two remained pappose. The scaphognathite now bore 14 to 18 plumose marginal setae interspersed with 1 to 5 minute buds.

Third Maxilliped (Figure 3.7F):

The endopod now appeared 2 segmented. The proximal segment had 1 denticulate seta. The distal segment bore 1 plumose and 1 denticulate seta.

Abdomen (Figure 3.7J):

Somites 2-5 now bore pairs of incompletely 2 segmented, bilobed, unarmed pleopods (Figure 3.7H). The uropods on somite 6 were now marginally 2 segmented and had 2 or 3 plumose setae (Figure 3.7I). While the telson and 6th somite were now partially delineated they did not appear capable of flexion.

GLAUCOTHOE (Figures 3.8 and 3.9)

Colour:

Newly molted glaucothoe were bright orange due to uniform pigmentation of the exoskeleton and the presence of numerous red/orange chromatosomes. This orange pigmentation became less intense towards the end of the stage and an area of darker pigmentation developed in the center of the carapace in the region of the digestive gland. At this point the legs also developed light and dark banding.

Cephalothorax (Figure 3.8A-C):

The carapace was approximately 1.4 times as long (including the rostrum) as it was wide (not including spines). The branchial regions were inflated and the posterior margin was broadly concave. Beginning with a pair of bifid or trifid spines at the base of

the rostrum and moving posteriorly there were 5 pairs of mediodorsal spines. Like all other spines on the glaucothoe, they bore a single seta. The second and third most anterior of these pairs were at the four corners of a distinct gastric lobe. A simple blunt spine was located mid dorsally on the posterior part of this lobe; it lacked an accessory seta. Posterior to the last pair of mediodorsal spines was a short but pronounced midline ridge. At least another 16 pairs of lateral and marginal spines were arranged symmetrically on opposite sides of the carapace; these were either simple, bifid, or trifid. In some cases an additional small spine was present on one or both sides near the anterioventral angle of the pterygostomial region. Several additional spines were often present asymmetrically in the branchial region.

Antennule (Figure 3.8D):

The protopod was three segmented with the proximal segment projecting medially and the distal two segments projecting dorsally. Each of these segments was armed with 1-6 minute setae. The proximal segment had a distinct dorsal invagination with a distal opening. The endopod was two segmented with 2 simple setae on the proximal segment and 7-8 simple setae on the distal segment. The exopod was 3 or 4 segmented with 9-11 aesthetascs and 4-5 simple setae on the middle segment(s) and 2-3 subterminal aesthetascs and 3-6 simple setae on the terminal segment.

Antenna (Figure 3.8E):

The peduncle was indistinctly 2 segmented and bore a pronounced ventral spine on its distal margin. The endopod was 9 segmented and progressing distally bore 0, (1-2), 2, 0, (2-4), (0-2), (5-6), (1-4), and (7-8) simple setae. The exopod was a blunt process which which in some specimens bore a single seta and/or spine.

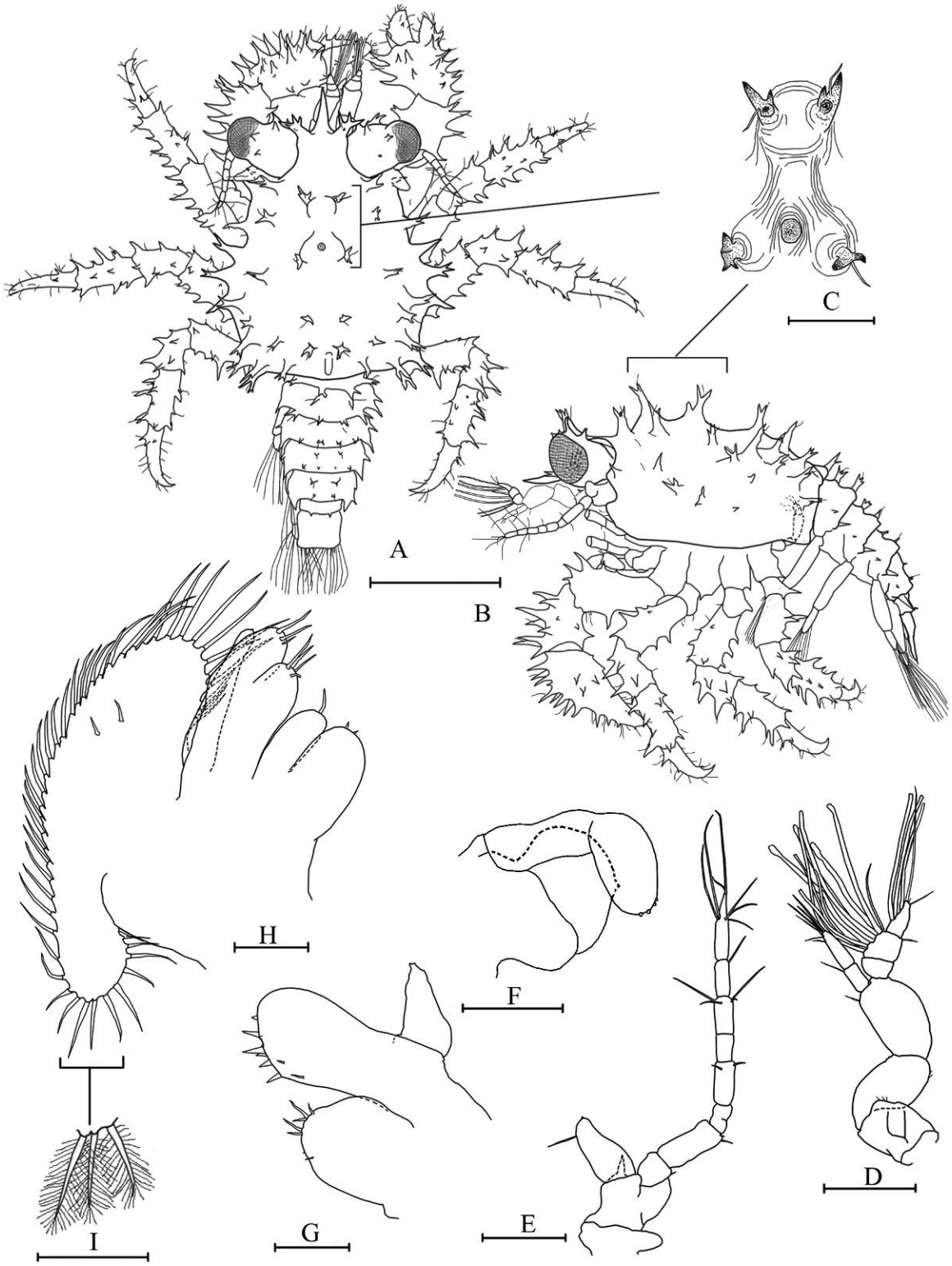


Fig. 3.8. Morphology of a representative *Lopholithodes foraminatus* A. glaucothoe (dorsal); B. (lateral); C. mid dorsal lobe of carapace; D. antennule (right medial); E. antenna (left dorsal); F. mandible (left internal); G. maxillule (left); H. maxilla (right); I. plumose setae of scaphognathite. Scale bars: A,B = 1 mm; C-E = 250 μm; F-I = 100 μm.

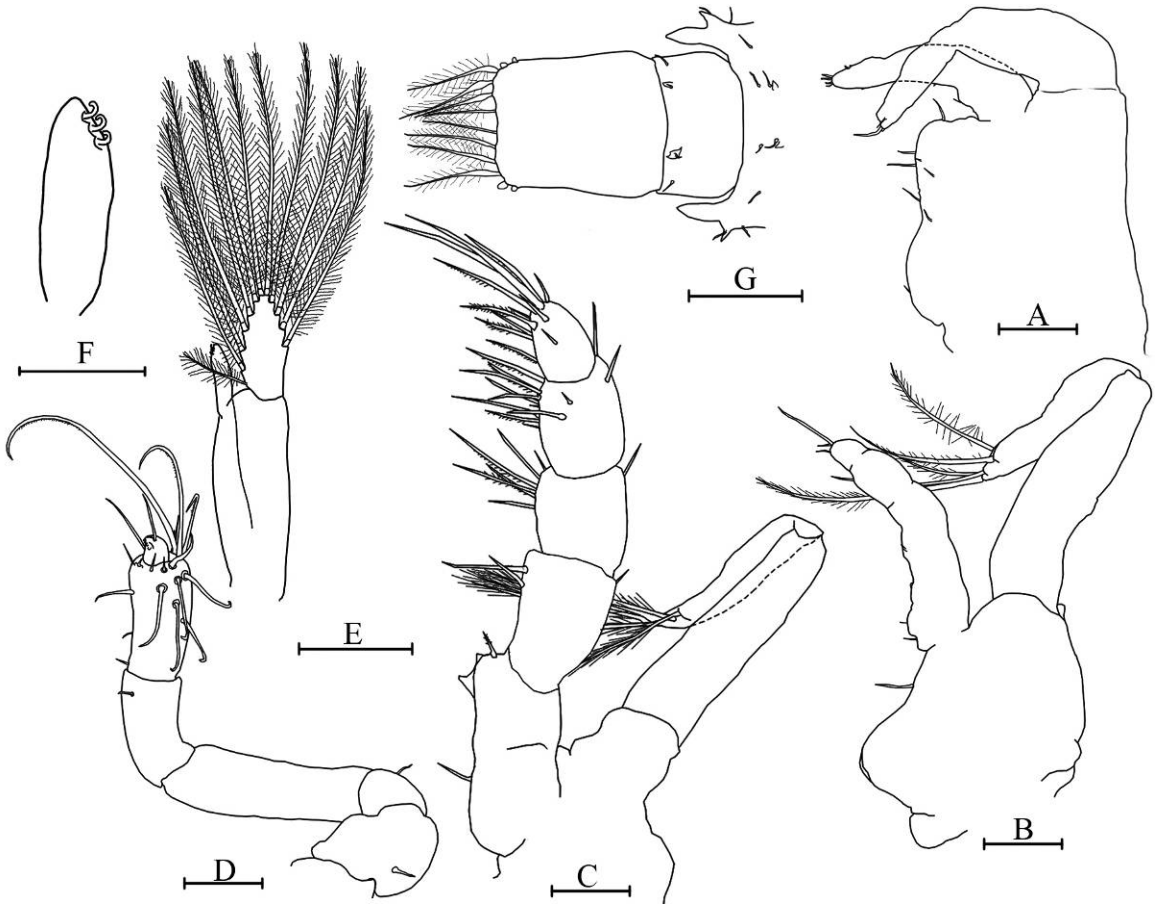


Fig. 3.9. Morphology of a representative *Lopholithodes foraminatus* glaucothoe A. first maxilliped (left); B.second maxilliped (left); C. third maxilliped (left) D. fifth pereopod (left dorsal); E. second pleopod (right anterior); F. endopod of pleopod; G. telson, abdominal somite 6 and part of 7 (dorsal). Scale bars: A-D = 100 μm; E, G = 250 μm; F = 50 μm.

Mandible (Figure 3.8F):

The mandibles were uncalcified and proportionally much smaller than in the zoeal stages. The denticulations present in the zoeal stages were also absent. The palp was large and incompletely 2 segmented; it was held against the internal surface of the mandible.

Maxillule (Figure 3.8G)*:*

The coxal endite now bore 4-8 simple and minute marginal setae. The basial endite had 2 simple submarginal setae, 5-6 small simple spines and 2-4 small marginal denticulations. The endopod lacked setae or clear segmentation.

Maxilla (Figure 3.8H, I)*:*

The proximal and distal lobes of the coxal endite bore 0-2 minute marginal setae and 1 simple seta respectively. Both lobes of the basial endite had 3-4 pappose and/or simple setae. The endopod was reduced and and in some cases bore 2 minute setae. The scaphognathite now had an elongated posterior lobe and 42-49 marginal plumose setae. There were 1 or 2 simple setae on the internal and external surfaces of the scaphognathite.

First Maxilliped (Figure 3.9A)*:*

The coxa and basis were incompletely delineated with 1 and 6 to 11 simple and/or pappose setae respectively. The endopod was not clearly segmented and had 3 to 6 minute terminal processes. In some specimens the exopod was indistinctly two segmented with several minute terminal processes and/or a single terminal plumose seta.

Second Maxilliped (Figure 3.9B)*:*

The protopod was indistinctly delineated into coxa and basis and bore 1 or 2 simple setae. The endopod was indistinctly segmented and had several minute setae and terminal exoskeletal projections. The exopod was two segmented and bore 1 simple seta and 5 plumose setae on the terminal segment.

Third Maxilliped (Figure 3.9C):

The segmentation of the coxa and basis was indistinct. The coxa in some cases bore a small simple seta. The endopod was 5 segmented. The proximal segment had 0-2 proximal simple setae, one distal denticulate seta, and 2 to 5 small denticulations of the cristae dentata along the medial margin. Moving distally the 4 remaining segments of the endopod had 3-5, 7-9, 14-18, and 14-17 setae of various types. The exopod was two segmented with one simple or denticulate seta on the proximal segment, and 1 simple and 5 plumose setae on the terminal segment.

Pereopods:

The pereopods now resembled those of the adult in proportion and segmentation. The chelae were large and obviously asymmetrical, with the right being larger in approximately 70% of individuals (a manuscript discussing the 30% incidence of reversed asymmetry is in preparation). The chelae and walking legs were armed with numerous simple spines, each bearing a single seta. The terminal articles of the pereopods bore numerous additional setae. The fifth pereopods (Fig 3.9D) were folded back on themselves and held in the branchial chamber. The propodus bore several long hooked setae and had small terminal denticulations. These denticulations appeared to interdigitate in a sub chelate manner with similar structures on the minute dactylus.

Abdomen:

Somites 1-6 bore 4, 8, 6, 6, 4, and 2 dorsal spines respectively. The spines on the anterior segments were mostly bifid or trifid and became smaller and were mostly simple on the posterior segments; each bore a single seta. Somites 1 and 6 had 2 additional setae while the remaining somites had 6 each. Somites 2-5 were each armed with a pair of

simple lateral spines and a pair of marginally bifid posterolateral spines. Somites 2-5 bore pairs of two segmented pleopods, each with an endopod bud projecting from the proximal segment (Figure 3.9E). The endopod bud was armed with three minute hooked setae mediodistally (Figure 3.9F). In intact specimens these hooked setae were occasionally observed to be linked with those on the opposite pleopod. The exopod had 11-12 plumose natatory setae. The uropods were more obviously two segmented than in Zoea IV but were otherwise unchanged.

Telson (Figure 3.9G)*:*

The telson was now rectangular. Each side had 2 small bulbous processes laterally and 4 plumose setae along the posterior margin. The three most lateral of these setae had distinctly bulbous bases. In many specimens several telsonal processes were missing.

FIRST CRAB INSTAR (Figures 3.10 and 3.11)

Colour:

The exoskeleton of the carapace was either completely white or had small orange patches. There were scattered red chromatosomes in the antennae and mouthparts and in the carapace, primarily under the ventral and lateral margins. The walking legs were distinctly banded. The exoskeleton of the dactyl was orange with a proximal concentration of red chromatosomes forming a distinct dark band. The propodus was bright white distally and red orange proximally.

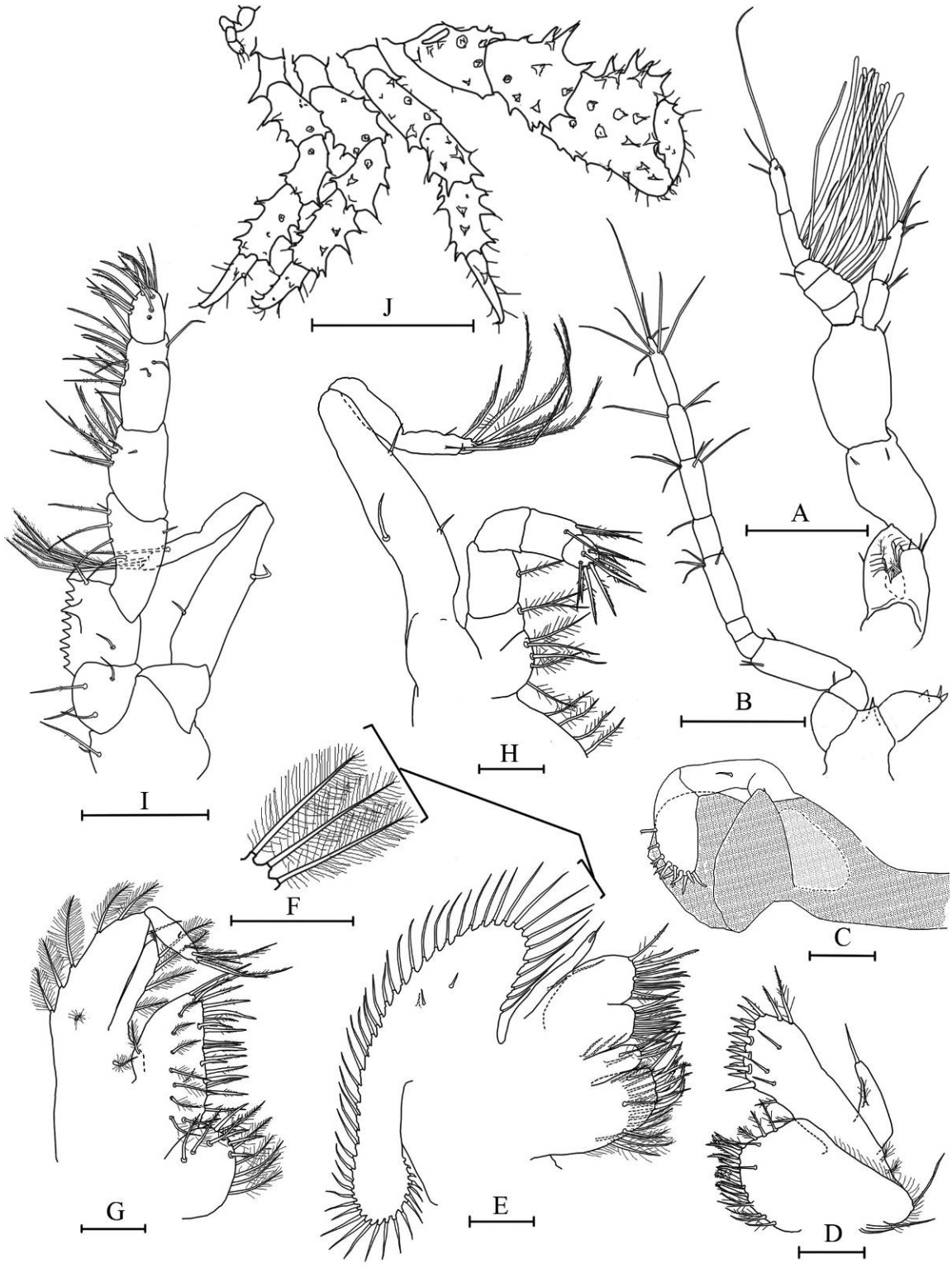


Fig. 3.10. Morphology of a representative *Lopholithodes foraminatus* first crab instar A. antennule (left medial); B. antenna (right dorsal); C. mandible (right internal; stippling indicates calcification); D. maxillule (left); E. maxilla (right); F. plumose setae of scaphognathite; G. first maxilliped (right); H. second maxilliped (right); I. third maxilliped (left); J. pereopods (right dorsal, in situ). Scale bars: A, B, I = 250 μm; C-E, G, H = 100 μm; F = 50 μm; J = 1 mm.

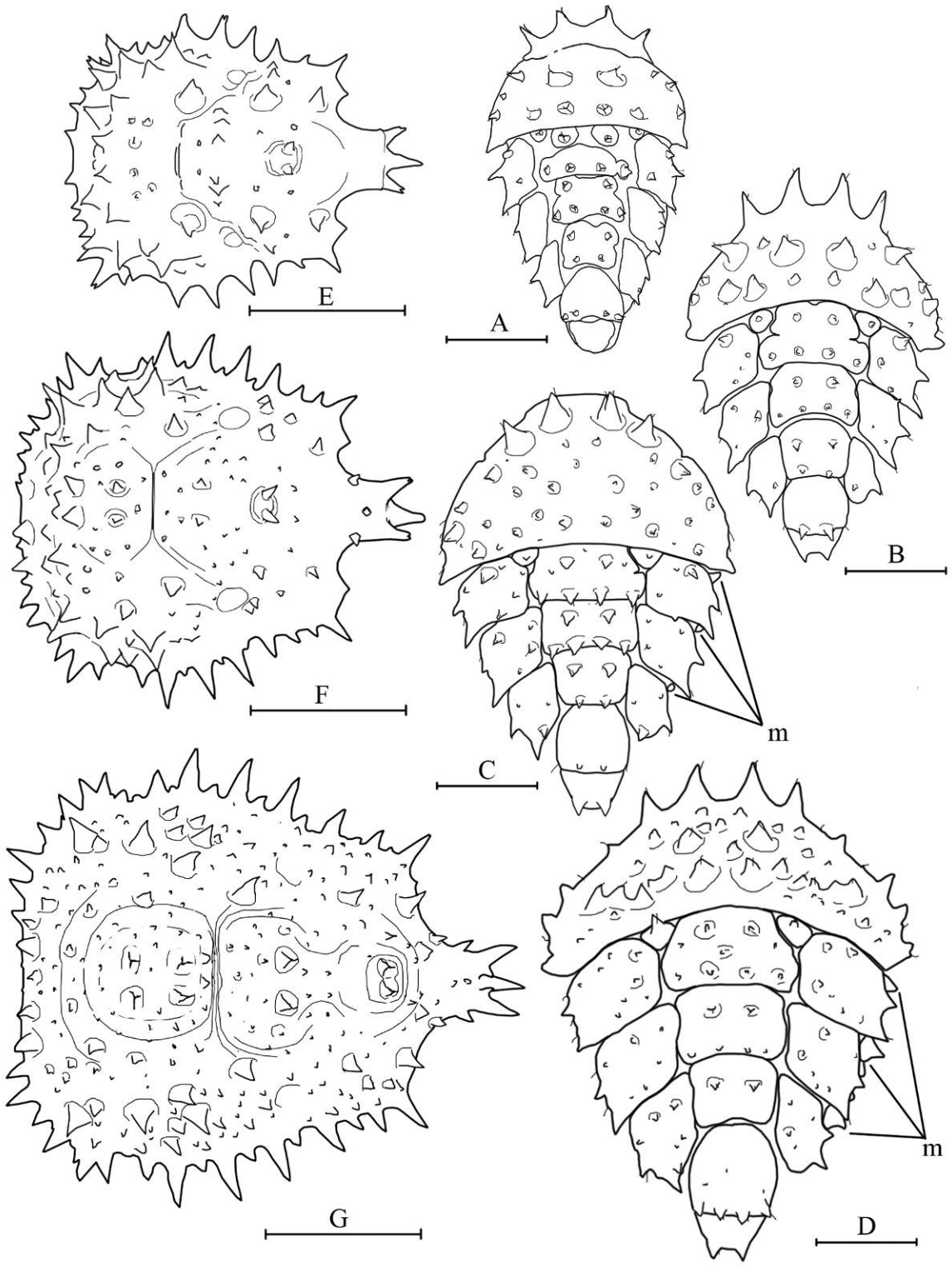


Fig. 3.11. . Morphology of a representative *Lopholithodes foraminatus* A. abdomen (1st crab instar); B. abdomen (2nd crab instar); C. abdomen (female 3rd crab instar), (m)arginal plates; D. abdomen (female 4th crab instar), (m)arginal plates; E. carapace (1st crab instar); F. carapace (2nd crab instar); G. carapace (3rd crab instar). Scale bars: A-D = 500 μm; E-G = 1 mm.

Carapace (Figure 3.11E):

The overall shape of the carapace was unchanged from the glaucothoe. The spines at the base of the rostrum now had marginally bifid tips. The cervical groove was now clearly defined and separated the gastric and cardiac regions of the carapace. The distinct gastric lobe of the glaucothoe was no longer evident. The anterolateral carapace margin was armed with 2 large spines flanking a single small spine. Just posterior to these were three very large marginal spines. The majority of spines on the first crab stage were simple and bore a single seta.

Antennule (Figure 3.10A):

The invagination in the proximal segment of the peduncle now had 5-6 stout setae projecting distally across the opening; at least two of which were plumose. About 6 setae were completely contained in the proximally projecting lumen of the pit. This segment also bore 5-7 pappose and minute setae. In some specimens the endopod was now 3 segmented. When present, the second segment was unarmed. The setation of the endopod was otherwise unchanged. The exopod was 4 or 5 segmented. The first segment was unarmed. The 2nd segment had 6-10 aesthetascs and the 3rd had 2-5 aesthetascs and 5 setae. The 4th segment had 2-4 aesthetascs near the base and was in some cases delineated from the terminal segment which had 3-5 simple setae.

Antenna (Figure 3.10B):

The peduncle had up to 2 simple setae. The endopod was 9 or 10 segmented with the 4th most proximal segment usually subdivided into 2 sections. Progressing distally the endopod bore (0-1), (1-2), (2-3), 0, (2-4), (0-1), 6, (3-4), and (7-8) simple setae. The exopod had 1 or 2 spiniform projections and in some cases a single seta.

Mandible (Figure 3.10C):

The mandible was now proportionally much larger than in the glaucothoe and with the exception of the mandibular palp was completely calcified. The mandibular palp was incompletely 3 segmented with one simple seta on both the 1st and 2nd segments and 11-14 stout denticulate setae on the terminal segment.

Maxillule (Figure 3.10D):

The protopod had 3-4 long, simple or marginally denticulate setae at its base and 1-2 plumose setae laterally, posterior to the base of the endopod. The coxal endite had fine hairs along the anterolateral margin. It was armed with 1 or 2 external, submarginal, simple setae and 20-22 plumodenticulate, pappose, and simple marginal setae. The basial endite had 3 submarginal setae; 2 or 3 pappose setae along the proximal margin; 2 or 3 distal plumodenticulate and denticulate setae; and 16-17 stout marginal spines, some of which were denticulate. The endopod was armed with 2-3 setae.

Maxilla (Figure 3.10E-F):

The proximal lobe of the coxal endite now had 9-10 stout plumodenticulate setae arranged in loose pairs along the margin. One seta in each pair was strongly internally curved while the other was not. There was also a distinct submarginal row of 14 -16 internally curved plumose setae. Both the internal and external surfaces of the coxal endite had several additional submarginal setae. The distal lobe of the coxal endite had 1-2 external, submarginal, plumose setae; and 1denticulate and 3-4 plumose internal submarginal setae. It also had 6 plumodenticulate, denticulate and simple marginal setae. The basial endite had two small simple setae externally near the junction of the proximal and distal lobes. These lobes bore 10-15 and 12-15 marginal and slightly submarginal

setae respectively (denticulate, plumodenticulate, and simple). The endopod was reduced and bore 1-3 setae. The scaphognathite had 2 minute simple seta on the middle of the external surface, 1 on the internal surface and 49-53 marginal plumose setae.

First maxilliped (Figure 3.10G):

The protopod generally had 2 external plumose setae, one at the base of the endopod and one at the base of the exopod. The coxal endite was armed with 10-13 denticulate, plumodenticulate, and plumose setae. The basial endite had a submarginal row of 7-8 externally projecting, weakly plumose setae; 3-5 internal submarginal simple setae; and 19-21 marginal denticulate and plumodenticulate setae. There was often one plumose seta at the base of the lateral margin. The endopod was unsegmented and bore 5-6 plumose setae along its medial margin. In some specimens an apical seta and/or 1-3 additional setae were present. The exopod was 2 segmented with 3 to 5 plumose setae along the lateral margin of the proximal segment and 1 or 2 simple setae on the medial margin. The distal segment bore 4-7 plumose, and sometimes 1-2 simple setae.

Second Maxilliped (Figure 3.10H):

The coxa was armed with 3-4 marginal weakly plumose setae and 1 small external plumose seta. The basis and ischium were incompletely delineated. The basis bore 5 pappose setae and a small simple seta. The ischium had 1-2 pappose setae. Progressing distal to proximal the remaining segments of the endopod bore 1-3, 1, 5-6, and 7-9 setae. The exopod was 2 segmented with the proximal segment bearing 1-3 simple setae and the distal segment generally bearing 1-2 simple setae and 6 plumose setae.

Third Maxilliped (Figure 3.10I):

The basal articles of the third maxilliped were now well calcified. The coxa bore 1-3 pappose and several weakly plumose setae. The basis of the endopod had 4-6 setae and the ischium had 2-3 subterminal setae and one denticulate seta at the mediodistal angle. The cristae dentata consisted of 7-11 teeth along the medial margin of the ischium and one larger external tooth. The merus of the endopod had 5-7 setae and the distal 3 articles all bore dense clumps of long, primarily denticulate setae. The first segment of the exopod was armed with 2-4 simple setae while the terminal segment generally had 6 plumose and 2 simple setae.

Pereopods: (Figure 3.10J):

The peropods, in particular the chelae, were somewhat more bulky than in the glaucothoe. However they were otherwise largely unchanged.

Abdomen (Figure 3.11A):

The abdomen was now flexed against the underside of the cephalothorax with the dorsal surface directed ventrally. The first and second somites were covered by calcified tergal plates which were partially fused to each other. The tergite of the second somite was considerably larger. The 4th and in most cases the 5th abdominal tergites were divided into a pair of lateral plates and a medial plate. On the third somite the lateral plates were frequently subdivided on one or both sides, with the anteromedial spines forming small separate plates. Similarly the portions of the medial plate bearing the anterior 2 spines were usually partially or completely separated. The 6th abdominal tergite and telson consisted of single plates and the telson lacked spines. The exoskeleton between tergites

was thin and membranous. Pairs of degenerate pleopods lacking setae were often present on the ventral surface of somites 2 through 5.

SUBSEQUENT INSTARS (Figure 3.11):

Colour:

Several distinct colour morphs were observed in juvenile crabs. By the third crab instar most individuals had developed brilliant orange chelae, with the dactyl and tip of the propodus normally remaining white. In some individuals the carapace remained white while in others it became dull orange, brilliant orange, dull red, or in several cases white anteriorly and brilliant orange posteriorly. Once a colour pattern had been established it was maintained through subsequent molts. The banding pattern of the walking legs was maintained in all individuals. A distinctly lighter distal band on the propodus is evident even in adult *L. foraminatus*.

Cephalothorax:

The carapace of the second crab instar (Figure 3.11F) was somewhat less elongate than that of the first instar: approximately 1.3 times as long (including the rostrum) as it was wide (not including spines). This ratio was maintained through the subsequent 2 stages. The anterolateral margin of the carapace was now armed with 2 small spines flanked by two large spines. Posterior to this was an alternating sequence of 3 small and 3 large spines. This basic pattern was maintained through subsequent instars, but generally became less regular and more complex. The carapace of the third crab instar (Figure 3.11G) was more dorsally peaked than in previous stages. There were two distinct peaks in the gastric region and one in the cardiac region. The pair of spines at the base of the

rostrum now bore small, medial, accessory projections. In subsequent instars the carapace became broader relative to its length (in adult specimens it is wider than it is long).

Pereopods:

The distinct interdigitating invaginations of the carpi of the chelae and first walking legs, characteristic of adult *L. foraminatus,* were not evident by the fifth crab instar. However, these structures were almost completely developed in 1 year old juveniles, likely in the 7th or 8th instar.

Abdomen:

The abdomen of the 2nd crab instar (Figure 3.11B) was broader and more triangular than that of the first instar. The tergites of the 1st and 2nd segments were now completely fused. In most individuals the medial tergite of the third segment now consisted of a single fused plate. In some specimens the telson bore small terminal spines. Pleopods were totally absent. In the third crab instar (Figure 3.11C) minute marginal plates were present on the right hand side of the 3rd through 5th somites in some females and on both sides in males. Minute, membranous pleopod buds also appeared on the left side of the ventral surface of the abdomen of some females. By the 4th instar (Figure 3.11D) marginal plates were always present and sex could be clearly determined. In females with a larger left cheliped, abdominal asymmetry was also reversed.

DISCUSSION

Behaviour

Lopholithodes foraminatus zoeae were apparently positively phototactic, although this was not confirmed experimentally. Positive phototaxis at high light intensities, and negative phototaxis at low light intensities, has been demonstrated for *Paralithodes*

camtschaticus (Shirley & Shirley, 1988). This response likely facilitates the reverse diel vertical migration of *P. camtschaticus*, which consists of rising to the surface at sunrise and descending below 30 m during the night (Shirley & Shirley, 1987).

It is difficult to determine whether the observed decline in swimming activity by *L. foraminatus* zoeae over the course of development reflects a naturally occurring ontogenetic shift in behaviour or is an artefact of laboratory culture. If reduced swimming indeed reflects a natural ontogenetic change in behaviour, it could indicate transition to a demersal habit late in development. An almost complete lack of swimming activity in the lecithotrophic zoeae of *Lithodes aequispinus* was interpreted by Shirley and Zhou (1997) as evidence of a benthic habit. However, there is also a tendency for lab cultured decapod larvae to be less active and robust than those observed in the field (G. Jensen, pers. com., 2006). Given the planktotrophic development of *L. foraminatus* zoeae and their apparent positive phototaxis, it seems likely that they spend considerable time in the photic zone. Behavioural experiments in the lab combined with plankton sampling in the field will be necessary to obtain a complete understanding of the movements of *L. foraminatus* zoeae in the water column.

Flaring of the antennae by *L. foraminatus* zoeae in response to chemical or physical trauma is likely a defence mechanism against gape-limited predators. Flaring of the antennal spines of the zoeae of the brachyuran *Rhithropanopeus harrisii* is part of a spine based defense that is effective against predation by small fish (Morgan, 1989). Morgan (1987) suggested that the degree of spination among brachyuran zoeae may be correlated with the zoeal habitat. Species that undergo larval development in estuarine environments heavily populated by fish exhibit more pronounced spines than those from

non-estuarine coastal waters. Some lithodid crabs have much smaller antennal scaphocerites than *L. foraminatus,* e.g. *Cryptolithodes expansus* (Kim & Hong, 2000). It would be interesting to know if species such as *C. expanses* also exhibit a spine flaring defense, or if some aspect of their life history provides an alternative protection against small gape limited predators.

The almost complete absence of cannibalistic behavior by zoeae in this study is surprising given the reports of frequent cannibalism by *Paralithodes camtschaticus* (Epelbaum et al., 2006) and the very similar size (see below: growth and stage duration) and mouthpart morphology of these larvae. It is possible that this behavioural difference is related to differences in trophic ecology between these two species.

Lecithotrophy and secondary lecithotrophy

Lopholithodes foraminatus passed through 4 planktotrophic zoeal stages and died without molting to the second zoeal stage when not provided with food. Development through 4 feeding zoeal stages is likely a primitive character in the Lithodidae (Konishi, 1986; Zaklan, 2002).

The term "secondary lecithotrophy" was suggested by Anger (1989) to describe the situation where a larval stage does not feed and instead relies upon energy reserves accumulated by preceding stages. *L. foraminatus* glaucothoe were not observed to feed; they molted to the first crab stage after the same period and with the same survivorship whether or not provided with food; and they exhibited reduced mouthparts (Figures 3.8 & 3.9) that were less well armed than those of the 4th zoeal (Figure 3.7) and first juvenile (Figure 3.10) stages. The stored reserves required for this secondary lecithotrophic

development were visible as lipid droplets in the digestive gland of both the late zoeal stages and the early glaucothoe.

Evidence for secondary lecithotrophy in the lithodids *Paralithodes camtschaticus, P. brevipes*, and *P. platypus* is provided by a reduction in function of the mouthparts and foregut of the glaucothoe relative to both the zoeal and juvenile crab stages (Abrunhosa and Kittaka, 1997). Descriptions of a feeding glaucothoe stage in *Paralithodes* species by previous authors may have been based on disappearance of food items due to feeding by 4^{th} zoeae or first stage juveniles present in the culture vessels (Epelbaum et al., 2006). The glaucothoe of *Hapalogaster mertensii* Brandt, 1850 was also described as a non-feeding stage by Miller & Coffin (1961) (confirmed by Duguid pers. obs.). Descriptions of development for *Acantholithodes hispidus* (by Hong et al., 2005), *Lopholithodes mandtii* (by Crain & McLaughlin, 2000a), *Hapalogaster dentata* (by Konishi, 1986), and *Cryptolithodes expansus* (by Kim & Hong, 2000) all illustrate a reduction of mouthpart armature in the glaucothoe similar to that described by Abrunhosa and Kittaka (1997) and observed in the present study. A less dramatic reduction in mouthparts is also evident in illustrations of the glaucothoe of *Placetron wosnessenskii* (Crain & McLauglin, 2000b). The glaucothoe has also been demonstrated to be a non-feeding stage in lithodids with lecithotrophic zoeal development: *Lithodes aequispinus* (Shirley & Zhou, 1997); *Lithodes maja* (Anger, 1996); *Lithodes santolla* and *Paralomis granulosa* (Calcagno et al., 2004); and *Paralomis spinosissima* (Watts et al., 2006). In the absence of experimental evidence of feeding, it seems possible that all lithodid glaucothoe are lecithotrophic.

Secondary lecithotrophy also occurs in other crustaceans, including the cypris larvae of barnacles, the puerulus stage of rock lobsters, and the glaucothoe stage of

pagurid crabs (reviewed in Anger, 2001). Secondary lecithotrophy could facilitate location of a suitable substrate for settlement and might be associated with a high degree of juvenile habitat specialization (Anger, 1989; Epelbaum et al., 2006). Lithodids occupy diverse habitats and it would be surprising if they were to have settlement requirements more specific than other decapods such as brachyuran crabs. On the other hand, in pagurid hermit crabs, a requirement for gastropod shells at settlement provides a potential explanation for secondary lecithotrophy (Anger, 1989). An alternative explanation is the massive reorganization of the digestive system that occurs between the last zoeal and first juvenile stage of pagurids, including the movement of the midgut gland from the cephalothorax to the abdomen (Thompson, 1903). This reorganization may preclude feeding (Harvey & Colasurdo, 1993).

Regardless of the underlying cause, secondary lecithotrophy in pagurids provides an intriguing alternative explanation for the non-feeding glaucothoe of lithodid crabs. Recent molecular evidence (Cunningham et al., 1992; Morrison et al., 2002; Zaklan, 2002; Tsang et al., 2008) unanimously nests the lithodids within the hermit crab family Paguridae (but see review and criticism of this hypothesis in McLaughlin et al., (2007)). Therefore, if secondary lecithotrophy is indeed a universal character of lithodids, it may be the result of ancestry rather than adaptation. The atavistic reacquistion of structures required for feeding by planktonic larvae is less likely than their original loss (Strathmann, 1978). This could provide an explanation for why secondary lecithotrophy persists in lithodids despite the lack of an apparent life history advantage. Should future research support universal secondary lecithotrophy in lithodids, this could provide further support for a pagurid ancestry of the Lithodidae.

Growth

The carapace length of *Lopholithodes foraminatus* larvae and post larvae was very slightly longer at each stage than that described for both *L. mandtii* (Crain & McLaughlin, 2000a) and *Paralithodes camtschaticus* (Epelbaum et al., 2006 carapace length and rostrum length reported separately). Similarly the dry and wet weights of *L. foraminatus* larvae and postlarvae were higher at each stage than those of *Paralithodes camtschaticus,* with the exception of the dry weight of the first crab instar and the wet weight of the third zoeal stage (Epelbaum et al., 2006). Overall the larval size of these planktotrophic Northeast Pacific lithodids is apparently very similar.

The first crab instars were also only marginally larger than glaucothoe in carapace dimensions and wet and dry weight. These results are not surprising considering the non-feeding habit of the glaucothoe stage. The slight increases in wet and dry weight could reflect the deposition of calcium sequestered from seawater in the shell of the first crab instar.

Stage duration

Mean surface water temperature in the Northern Strait of Georgia increases from approximately 8 ˚C in the winter (Dec 1- Feb 28) to almost 9.5 ˚C in the spring (March 1 - May 31) and 16 ˚C in the summer (June 1 – Aug 31). At 120 dbar (approximately 120 m) the mean temperature ranges between 8 ˚C and 9 ˚C over the same period (Fisheries & Oceans, 2008). Given an extended hatching period in the late winter and spring (Table 3.1; Chapter 2, Figures 2.2, 2.5, & 2.6) it seems likely that *L. foraminatus* in this region would reach the glaucothoe stage within 22-39 days of hatching (based on durations of development at 8 ˚C and 12 ˚C calculated using the relationship in Figure 3.3E). The

duration of the glaucothoe stage at temperatures below 11.5 ˚C was not determined. However, based on a requirement of 219 degree days at 11.5 ˚C, and assuming ambient temperatures of 9 ˚C or above, development of this stage would be unlikely to require more than 25 days in the field.

The 39 days required for zoeal development of *L. foraminatus* at 8 ˚C agrees well with 39 days for red king crab reared at 7 - 8 ˚C (Epelbaum et al., 2006) and 44.9 for blue king crab reared at 9 ˚C (Stevens et al., 2008). Zoeal development of lithodids with lecithotrophic larvae is much more rapid; for example, 9.6 days at 9 ˚C for *Paralomis granulosa,* (Anger et al., 2003) and 23.9 days at 9 ˚C for *Lithodes aequispinus* (Paul & Paul, 1999). This reflects both a reduction in the number of zoeal stages and the provisioning of those stages with maternal reserves for development.

For all larval stages investigated in the present study, an increase in temperature from 8 ˚C to 12 ˚C led to a greater reduction in stage duration than an increase from 12 ˚C to 16 ˚C (Figure 3.2). A similar levelling out of the relationship between stage duration and temperature has been observed for other lithodids including *Lithodes aequispinus* (Paul & Paul, 1999), *Paralomis granulosa* (Anger et al., 2003) and *Paralithodes platypus* (Stevens et al., 2008). However, in all of these species, decrease in the sensitivity of stage duration to temperature occurred over a lower temperature range than observed in the present study. For example, the duration of the combined zoeal stages of *Paralithodes platypus* decreased from 71.8 days at 3 ˚C to 44.7 days at 6 ˚C, but was not lower at 9 ˚C (44.9 days) (Stevens et al., 2008). The sensitivity of stage duration over a higher temperature range in the present study may reflect adaptation to the relatively warm waters of Southern British Columbia. *Lopholithodes foraminatus* occurs

from California to Alaska (Jensen, 1995), a much greater latitudinal range than other large coastal lithodids. It would be interesting to know if the relationship between larval development rate and temperature is constant throughout this range.

Colour

The brilliant orange colour of *Lopholithodes foraminatus* zoeae is similar to that described for *L. mandtii* (Crain & McLaughlin, 2000a) and *Placetron wossenenski* (Crain & McLaughlin, 2000b). However it differs from the largely transparent appearance of some other lithodid zoeae, such as *Hapalogaster mertensii* (Duguid, pers. obs.) and *Paralithodes camtschaticus* (colour photographs in Kovatcheva et al., 2006). It seems likely that this distinct difference in appearance is related to differences in planktonic ecology between lithodid species.

Juvenile colour polymorphism in decapods has been demonstrated to provide protection from small visual predators in the recruitment habitat (Palma & Steneck, 2001). Early juvenile stages of blue king crab (*Paralithodes platypus*) occur in multiple colour morphs (Jensen, G., pers. com., 2007). Armstrong et al. (1985) found that juvenile *P. platypus* abundance around the Pribilof Islands was strongly correlated with a heterogeneous substrate composed of broken mollusc shells. The authors concluded that the availability of this shellhash substrate could be a limiting factor in blue king crab distribution. The habitat and ecology of early ontogenetic stages of *Lopholithodes foraminatus* are completely unknown. The colour polymorphism observed in juveniles suggests a habitat with considerable small scale visual heterogeneity. The lack of such heterogeneity on a mud bottom could explain the tendency of adult *L. foraminatus* to be

distributed on soft substrates in the immediate vicinity of rock features (Zhang et al., 1999).

Identification

To the our knowledge, the only prior account of *Lopholithodes foraminatus* larvae is a series of figures of putative *Lopholithodes* spp. C, D, and E zoeae and glaucothoe (chelae only) in Lough's 1975 PhD thesis. Lough's species C is almost certainly *Acantholithodes hispidus,* described by Hong et al. (2005). The identity of species D is less clear, however; the proportions of the rostrum and posterolateral spines preclude both species of *Lopholithodes.* The gross morphology of Lough's species E agrees very well with the description of *L. foraminatus* in the present study. Two specimens of this species (zoea III) were collected on April 22, 1971 in a plankton tow approximately 18 km off the Oregon Coast, in water 85 m deep. The peaked mid dorsal carina observed in Lough's species E and in all zoeal stages in this study is a feature that is not shared with any other Northeast Pacific lithodid for which development has been described. We suggest this feature as diagnostic of *L. foraminatus* zoeae.

Lopholithodes foraminatus glaucothoe are very similar in morphology to *L. mandtii* glaucothoe as described by Crain and McLaughlin (2000a). Abdominal somites 2-5 of *L. foraminatus* bear 8, 6, 6, and 4 dorsal spines whereas those of *L. mandtii* bear 8 dorsal spines each. The carapace spination of *L. foraminatus* glaucothoe is apparently different from that illustrated for *L. mandtii*, but this may be due to differing levels of detail between illustrations. The distinct mid dorsal lobe of *L. foraminatus* (Figure 3.8C) is unique among described lithodid glaucothoe and could be useful for identification.

There are number of differences between the juvenile crab stages of *Lopholithodes foraminatus* and those of *L. mandtii* as described by Crain and McLaughlin (2000a). The lateral carapace spination of the first 2 crab stages of *Lopholithodes foraminatus* (Figure 3.11E, F) is distinctive and differs from that illustrated for *L. mandtii* and from other described lithodid juveniles. Also, unlike in *L. mandtii,* the lateral and medial plates of the third abdominal somite are generally not entire in the first crab instar of *L. foraminatus.* The overall white appearance of the first crab stage is different from the bright orange carapace of *L. mandtti*. However, subsequent crab stages sometimes develop into a bright orange colour morph, and the pigmentation of *L. mandtii* juveniles past the first crab stage has not been documented. The distinct red proximal bands on the propodi of the walking legs also differ from the solid white propodi described for *L. mandtii.* The distinctive smooth invaginations in the carpi of the chelae and first walking legs apparently cannot be relied on as a diagnostic feature until crabs are in their second year.

REFERENCES

Abrunhosa F.A. and Kittaka J. (1997) Functional morphology of mouthparts and foregut of the last zoea, glaucothoe, and first juvenile of king crabs *Paralithodes camtschaticus, P. brevipes*, and *P. platypus*. *Fisheries Science*, 63, 923-930.

Anger K. (1989) Growth and exuvial loss during larval and early juvenile development of the hermit crab *Pagurus bernhardus* reared in the laboratory. *Marine Biology*, 103, 503-511.

Anger K. (1996) Physiological and biochemical changes during lecithotrophic development and early juvenile growth in the northern stone crab, *Lithodes maja* (Decopoda: Anomura). *Marine Biology*, 126, 283-296.

Anger K. (2001) Nutrition: secondary lecithotrophy. In Vonk R. (ed.) *Biology of Decapod Crustacean Larvae*. Lisse: A. A. Balkema Publishers, pp. 112-3.

Anger K., Thatje S., Lovrich G. and Calcagno J. (2003) Larval and early juvenile development of *Paralomis granulosa* reared at different temperatures: tolerance of cold and food limitation in a lithodid crab from high latitudes. *Marine Ecology Progress Series*, 253, 243-251.

Armstrong D.A., Palacios R., Williams G., Jensen G.C. and Pearson W. (1985). Early life history of juvenile blue king crab, *Paralithodes platypus*, around the Pribilof Islands. In Melteff B. (ed.) *Proceedings of the International King Crab Symposium*, Anchorage, AK: University of Alaska Sea Grant, pp. 211–230.

Calcagno J.A., Anger K., Lovrich G.A., Thatje S. and Kaffenberger A. (2004) Larval development of the subantarctic king crabs *Lithodes santolla* and *Paralomis granulosa* reared in the laboratory. *Helgoland Marine Research*, 58, 11-14.

Calcagno J.A., Lovrich G.A., Thatje S., Nettelmann U. and Anger K. (2005) First year growth in the lithodids *Lithodes santolla* and *Paralomis granulosa* reared at different temperatures. *Journal of Sea Research*, 54, 221-230.

Clark P.F., Calazans D.K. and Pohle G.W. (1998) Accuracy and standardization of brachyuran larval descriptions. *Invertebrate Reproduction and Development*, 33, 127-144.

Crain J.A. (1999) Functional morphology of prey ingestion by *Placetron wosnessenskii* Schalfeew zoeae (Crustacea: Anomura: Lithodidae). *Biological Bulletin*, 197, 1899-1999.

Crain J.A. and McLaughlin P.A. (2000a) Larval and early juvenile development in the Lithodidae (Decapoda: Anomura: Paguroidea) reared under laboratory conditions. 1. subfamily Lithodinae: *Lopholithodes mandtii* Brandt, 1848. *Invertebrate Reproduction and Development*, 37, 43-59.

Crain J.A. and McLaughlin P.A. (2000b) Larval and early juvenile development in the Lithodidae (Decapoda: Anomura: Paguroidea) reared under laboratory conditions. 2. subfamily Hapalogastrinae: *Placetron wosnessenskii* Schalfeew, 1892 with notes on comparative development within the subfamilies of the Lithodidae. *Invertebrate Reproduction and Development*, 72, 113-127.

Cunningham C.W., Blackstone N.W. and Buss L.W. (1992) Evolution of king crabs from hermit crab ancestors. *Nature*, 335, 539-542.

Dew C.B. and McConnaughey R.A. (2005) Did trawling on the broodstock contribute to the collapse of Alaska's king crab? *Ecological Applications*, 15, 919-941.

Epelbaum A. and Borisov R. (2006) Feeding behaviour and functional morphology of the feeding appendages of red king crab *Paralithodes camtschaticus* larvae. *Marine Biology Research*, 2, 77-88.

Epelbaum A.B., Borisov R.R. and Kovatcheva N.P. (2006) Early development of the red king crab *Paralithodes camtschaticus* from the Barents Sea reared under laboratory conditions: morphology and behaviour. *Journal of the Marine Biological Association of the United Kingdom,* 86, 317-333.

Factor J.R. (1978) Morphology of the mouthparts of larval lobsters, *Homarus americanus* (Decapoda: Nephropidae), with special emphasis on their setae. *Biological Bulletin*, 154, 383-408.

Fisheries and Oceans Canada (2008) Inside Passage: Northern Strait of Georgia: winter, spring, and summer. In *Range Profiles Data Page*. Retrieved Jan 22, 2009, from http://www-sci.pac.dfo-mpo.gc.ca/osap/data/SearchTools/rangeprofiles_e.htm

Gould S.J. (1992) We are all monkeys' uncles. *Natural History*, 6, 14–21.

Harvey A.W. and Colasurdo E.A. (1993) Effect of shell and food availability on metamorphosis in the hermit crabs *Pagurus hirsutiusculus* (Dana) and *Pagurus granosimanus* (Stimpson). *Journal of Experimental Marine Biology and Ecology*, 165, 237-249.

Haynes E.B. (1993) Stage-I zoeae of laboratory-hatched *Lopholithodes mandtii* (Decapoda, Anomura, Lithodidae). *Fishery Bulletin*, 91, 379-381.

Hong S.Y., Perry R.I., Boutillier J.A. and Kim M.H. (2005) Larval development of *Acantholithodes hispidus* (Stimpson) (Decapoda : Anomura : Lithodidae) reared in the laboratory. *Invertebrate Reproduction and Development*, 47, 101-110.

Jensen G.C. (1995) *Pacific Coast crabs and shrimps*. Monterey, California: Sea Challengers.

Kim M.H. and Hong S.Y. (2000) Larval development of *Cryptolithodes expansus* Miers (Decapoda: Anomura: Lithodidae) reared in the laboratory. *Proceedings of the Biological Society of Washington*, 113, 54-65.

Konishi K. (1986) Larval development of the stone crab, *Hapalogaster dentata* (De Haan, 1844) (Crustacea: Anomura: Lithodidae) reared in the laboratory. *Journal of the Faculty of Science of Hokkaido University Series VI, Zoology*, 24, 155-172.

Kovatcheva N., Epelbaum E., Kalinin A., Borisov R. and Levdev R. (2006) *Early life history stages of the red king crab* Paralithodes camtschaticus *(Tilesius, 1815) biology and culture*. Moscow: Russian Federal Research Institute of Fisheries and Oceanography.

Lavalli K.L. and Factor J.R. (1992) Functional morphology of the mouthparts of juvenile lobsters, *Homarus americanus* (Decapoda: Nephropidae), and comparison with the larval stages. *Journal of Crustacean Biology*, 12, 467-510.

Lough R.G. (1975) *Dynamics of Crab Larvae (Anomura, Brachyura) off the Central Oregon Coast*. PhD Dissertation, Oregon State University, Corvallis, Oregon.

MacDonald J.D., Pike R.B. and Williamson D. (1957) Larvae of the British species of *Diogenes*, *Pagurus*, *Anapagurus*, and *Lithodes* (Crustacea, Decapoda). *Proceedings of the Zoological Society of London*, 128, 209-257.

McLaughlin P.A. and Lemaitre R. (1997) Carcinization in the Anomura – fact or fiction? I. Evidence from adult morphology. *Contributions to Zoology*, 67, 79-123.

McLaughlin P.A. and Paul A.J. (2002) Abdominal and pleopod changes in *Lithodes aequispinus* Benedict, 1895 (Crustacea: Decapoda: Anomura: Lithodidae) from megalopa to juvenile. *Proceedings of the Biological Society of Washington*, 115, 138-147.

McLaughlin P.A., Anger K., Kaffenberger A. and Lovrich G.A. (2003) Larval and early juvenile development in *Paralomis granulosa* (Jacquinot) (Decapoda: Anomura: Paguroidea: Lithodidae), with emphasis on abdominal changes in megalopal and crab stages. *Journal of Natural History*, 37, 1433-1452.

McLaughlin P.A., Lemaitre R. and Tudge C.C. (2004) Carcinization in the Anomura - fact or fiction? II. Evidence from larval, megalopal and early juvenile morphology. *Contributions to Zoology*, 73, 165-205.

McLaughlin P.A., Lemaitre R. and Sorhannus U. (2007) Hermit crab phylogeny: a reappraisal and its “fall-out”. *Journal of Crustacean Biology*, 27, 97-115.

Miller P. and Coffin H. (1961) A laboratory study of the developmental stages of *Hapalogaster mertensii* (Brandt), (Crustacea, Decapoda). *Publications, Department of Biologcal Sciences*, *Walla Walla College,* 30, 1–18.

Morgan S.G. (1987) Morphological and behavioral antipredatory adaptations of decapod zoeae. *Oecologia (Berlin)*, 73, 393- 400.

Morgan S.G. (1989) Adaptive significance of spination in estuarine crab zoeae. *Ecology*, 70, 464-482.

Morrison C.L., Harvey A.W., Lavery S., Tieu K., Huang Y. and Cunningham C.W. (2002) Mitochondrial gene rearrangements confirm the parallel evolution of the crab-like form. *Proceedings of the Royal Society Series B*, 269, 345-350.

Palma A.T. and Steneck R.S. (2001) Does variable coloration in juvenile marine crabs reduce risk of visual predation? *Ecology*, 82, 2961-2967.

Paul A.J. and Paul J.M. (1999) Development of larvae of the golden king crab *Lithodes aequispinus* (Anomura: Lithodidae) reared at different temperatures. *Journal of Crustacean Biology*, 19, 42-45.

Richter S. and Scholtz G. (1994) Morphological evidence for a hermit crab ancestry of lithodids (Crustacea, Decapoda, Anomala, Paguroidea). *Zoologischer Anzeiger*, 223, 187-219.

Shirley T.C. and Shirley S.M. (1987) Diel vertical movements of Alaskan red king crab zoeae. *American Zoologist*, 27, 103A.

Shirley T.C. and Shirley S.M. (1988) Behavior of red king crab larvae: phototaxis, geotaxis, and rheotaxis. *Marine Behaviour and Physiology*, 13, 369-388.

Shirley T.C. and Zhou S. (1997) Lecithotrophic development of the golden king crab *Lithodes aequispinus* (Anomura: Lithodidae). *Journal of Crustacean Biology*, 17, 207-216.

Stevens B.G. (2006) Timing and duration of larval hatching for blue king crab *Paralithodes platypus* (Brandt, 1850) held in the laboratory. *Journal of Crustacean Biology*, 26, 495-502.

Stevens B.G., Persselin S. and Matweyou J. (2008) Survival of blue king crab *Paralithodes platypus* Brandt, 1850, larvae in cultivation: effects of diet, temperature and rearing density. *Aquaculture Research*, 39, 390-397.

Strathmann R.R. (1978) The evolution and loss of feeding larval stages of marine invertebrates. *Evolution*, 32, 894-906.

Thompson M.T. (1903) The metamorphosis of the hermit crab. *Proceedings of the Boston Society of Natural History,* 31, 147-209.

Tsang L.M., Ma K.Y., Ahyong S.T., Chan T.-Y. and Chu K.H. (2008) Phylogeny of Decapoda using two nuclear protein-coding genes: Origin and evolution of the Reptantia. *Molecular Phylogenetics and Evolution*, 48, 359-368.

Watts J., Thatje S., Clarke S. and Belchier M. (2006) A description of larval and early juvenile development in *Paralomis spinosissima* (Decapoda: Anomura: Paguroidea: Lithodidae) from South Georgia waters (Southern Ocean). *Polar Biology*, 29, 1028-1038.

Zaklan S.D. (2002) Review of the family Lithodidae (Crustacea: Anomura: Paguroidea): Distribution, biology, and fisheries. In Paul A.J., Dawe E.G., Elner R., Jamieson G.S., Kruse G.H., Otto R.S., Sainte-Marie B., Shirley T.C. and Woodby D. (eds), *Crabs in Cold Water Regions: Biology, Management, and Economics*. Fairbanks: University of Alaska Sea Grant College Program, pp. 751-845.

Zhang Z.Y. (2001) Evaluation of a box crab (*Lopholithodes foraminatus*) trap test in British Columbia. *Canadian Science Advisory Secretariat Research Document* 2001/158. Ottawa: Fisheries and Oceans Canada, 41 pp.

Zhang Z.Y., Workman G. and Phillips A.C. (1999) A review of the biology and fisheries of the box crab (*Lopholithodes foraminatus* Stimpson) in British Columbia. *Canadian Stock Assessment Secretariat Research Document* 99/131. Ottawa: Fisheries and Oceans Canada, 38 pp.

Chapter 4. An elevated incidence of reversed asymmetry in the offspring of both normal and reversed female *Lopholithodes foraminatus*

ABSTRACT

*Mutations or environmental factors that result in reversal of conspicuous left-right asymmetries provide an opportunity to study developmental mechanisms. They may also provide insight into evolutionary change in asymmetry states within and between species. Representatives of the family Lithodidae (king crabs) have a larger (major) right chela and females exhibit a dextrally offset abdomen. The first reported capture of a reversed female lithodid (*Lopholithodes foraminatus*) brooding eggs provided an opportunity to characterize the reversed asymmetry phenotype and to compare its incidence in the offspring of reversed and normal females. Asymmetry of the chelae became apparent in the first post-zoeal stage (glaucothoe). The ratio of right claw length to left claw length had a bimodal distribution, indicating distinct left-handed and right-handed phenotypes. The left handed phenotype was maintained through subsequent instars, and females with larger left claws developed reversed (sinistrally offset) abdominal asymmetry by the 4th crab stage. Removal of the right cheliped of 4th stage zoeae, and the major cheliped of glaucothoe, did not reverse the direction of asymmetry. Elevated larval rearing temperature also did not affect the frequency of reversed individuals. The incidence of reversed asymmetry was quantified for glaucothoe reared from 1 reversed and 3 normal females. The proportion of these glaucothoe with a larger left claw was high (between 20% and 30%), and apparently independent of maternity (P = 0.67). This elevated incidence of reversed asymmetry is surprising considering the infrequency of this phenotype in field collected adults of* L. foraminatus *(1 out of 111 individuals observed) and of other lithodids. Further investigation of reversed asymmetry in lithodid crabs may provide valuable insights into the development and evolution of left-right asymmetries.*

INTRODUCTION

The evolution and development of conspicuous left-right asymmetries in otherwise bilaterally symmetrical metazoans has long been the focus of extensive research (e.g. Sumner & Huestis, 1921). Recent work has investigated mechanisms controlling the development of asymmetry (reviewed by Spéder et al., 2007; Levin & Palmer, 2007; Levin & Aw, 2008; Okumura et al., 2008) and the evolutionary significance of differences in asymmetry states within and between species (Palmer, 1996; 2004; 2005; Sutcharit et al., 2007). Conspicuous asymmetry states can be defined as directional asymmetry, when most individuals in a population exhibit the same direction of asymmetry (handedness); and antisymmetry, where individuals may exhibit either handedness (Van Valen, 1962). Antisymmetry may be further subdivided into true antisymmetry (50:50 mix of left and right handed individuals) and biased antisymmetry, where a majority of individuals exhibit the dominant handedness (Palmer, 2005). Handedness is generally under environmental control in antisymmetrical species, but is determined genetically in species exhibiting biased antisymmetry or directional asymmetry (Palmer, 1996).

The crustacean order Decapoda includes many taxa exhibiting either antisymmetry or directional asymmetry in chela size and morphology. Research on these groups has been valuable in understanding how the direction of asymmetry is determined and maintained. In American lobsters (Govind & Pearce, 1986, 1989a, 1989b) and some fiddler crabs (Yamaguchi, 1977) handedness is random, but becomes fixed by environmental factors during ontogeny and cannot be reversed subsequently. In antisymmetrical alphaeid shrimp, handedness can be reversed throughout life by damage

to, or loss of, the major claw (Read & Govind, 1997). Slight asymmetry of the chelae in cancrid crabs apparently results from differential usage (based on *Cancer productus,* Smith & Palmer, 1994). However, in many other families of predatory brachyurans, the right claw is almost universally larger (Vermeij, 1977; Ng & Tan, 1985; Mariappan et al., 2000), suggesting a genetic control of handedness. Similarly, in some fiddler crabs the direction of handedness is apparently under genetic control, with some species having a larger right claw (Jones & George, 1982) and others a larger left claw (Trott, 1987).

Several families within the superfamily Paguroidea (Decapoda: Anomura) exhibit conspicuous asymmetry involving more than the chelae. The Diogenidae, Parapaguridae, Coenobitidae, Paguridae and Lithodidae all exhibit abdominal asymmetry; often accompanied by loss of the pleopods on one side (Richter & Scholtz, 1994). The first 4 of these families are hermit crabs. Their asymmetry can be explained by occupation of gastropod shells, the vast majority of which are dextrally coiled in the marine environment. However, representatives of the family Lithodidae (king crabs) do not occupy gastropod shells, and closely resemble brachyuran crabs in general morphology. Since the 19th century, many authors have attributed lithodid asymmetry to evolution from an ancestor in the right handed hermit crab family Paguridae (see historical reviews in McLaughlin & Lemaitre, 1997 & McLaughlin et al., 2007). This hypothesis has been supported by developmental (Macdonald et al., 1957; Gould, 1992), morphological (Richter & Scholtz, 1994), and more recently molecular data (Cunningham et al., 1992; Morrison et al., 2002; Zaklan, 2002; Tsang et al., 2008). However, a pagurid ancestry for lithodids is still not universally accepted (McLaughlin & Lemaitre, 1997; McLaughlin et al., 2004; McLaughlin et al., 2007).

Lithodid asymmetry differs between the sexes (Zaklan, 2002). Both males and females have a larger right chela. The left and right chelae generally differ in morphology as well as size: the right chela has large molars, while the propodus and dactylus of the left chela have concave surfaces and fit tightly together when closed. Male lithodids have a symmetrical abdomen which lacks appendages, while the abdomen of females is offset, with the telson oriented to the right of the midline. In females of species with calcified abdominal plates, the lateral plates of somites 3 to 5 are larger on the left side than the right. Small marginal plates are found only on the right, associated with the smaller lateral plates. The first abdominal segment generally has paired pleopods, while segments 2-5 have pleopods only on the left.

Cases of reversed asymmetry in adult lithodids are sufficiently unusual to warrant published notes (female *Paralomis granulosa*, Campodonico, 1978; female *Lithodes maja*, Zaklan, 2000; male *Paralomis hystrix,* Motoh & Toyota, 2006). However, Campodonico (1978) noted personal communications with 3 other researchers who had encountered lithodid crabs with reversed asymmetry. Sandberg and McLaughlin (1998) also reported a case of reversed asymmetry in a female specimen of *Lithodes maja,* and Stevens and Munk (1991) stated that reversed abdominal asymmetry is an occasional abnormality of red king crab (*Paralithodes camtschaticus*), but did not provide a reference. I have also personally examined a badly damaged female lithodid with reversed abdominal asymmetry in the collections of the Royal BC Museum (likely *Lithodes couesi,* specimen 999-75-3, captured by trawl March 11, 1999 between 441 and 509 fathoms, (49˚ N, 127˚ W). Sandberg and McLaughlin (1998), point out that as female lithodids are actively avoided by commercial fisheries, and males lack an asymmetrical

abdomen, complete reversed asymmetry in lithodid crabs may be more frequent than previously recognized.

Reversed asymmetry has also been described for juvenile lithodids reared in the laboratory. McLaughlin and Paul (2002) identified 2 cases of abdominal reversal in a sample of < 30 laboratory reared female *Lithodes aequispinus*. Crain and McLaughlin (2000) also illustrated reversal in asymmetry of the chelae in a laboratory reared first crab instar of *Lopholithodes mandtii* (Figure 14, Crain & McLaughlin, 2000), although they made no mention of it in the text. A high incidence of reversed asymmetry of the chelae (approximately 20%) has been observed in glaucothoe of *P. camtschaticus* reared at 7-8 ˚C, a higher temperature than normally experienced in the field (< 5˚C) (A. Epelbaum, pers. com., 2007).

Mutations that reverse the direction of left-right asymmetries have been central to studies of the mechanisms controlling the direction of lateral asymmetries (e.g. Brown & Wolpert, 1990; Spéder et al., 2006), and their inheritance (e.g. Freeman & Lundelius, 1982). Where a mutation occurs to reverse the handedness of a directionally asymmetrical character, the reversal is generally heritable (Palmer, 2004; 2005). To date, no research has investigated the heritability of reversed asymmetry in directionally asymmetrical decapod taxa.

In the course of rearing work to describe the development of *Lopholithodes foraminatus* (Stimpson 1859) (brown box crab), I observed that a significant proportion of glaucothoe and early juvenile instars had a larger left claw (Chapter 3). I also captured a brooding female of *L. foraminatus* exhibiting reversed asymmetry of both the abdomen and chelae. These circumstances presented a unique opportunity to investigate the

phenomenon of reversed asymmetry in lithodid crabs. The present study seeks to characterize reversed asymmetry in post-larvae of *Lopholithodes foraminatus* and to provide a foundation for further research by addressing a series of questions:

Is the frequency of reversed asymmetry in Lopholithodes foraminatus *juveniles related to the direction of maternal asymmetry?*

Does reversed asymmetry of the chelae in glaucothoe represent one end of a continuously distributed asymmetry phenotype, or do individuals with larger left claws represent a reversal of the normal phenotype?

Is reversed asymmetry of the chelae in glaucothoe maintained through development and do individuals with larger left claws also develop reversed abdominal asymmetry (if female)?

Is the frequency of reversed asymmetry increased by rearing larvae at temperatures above those experienced in the field?

Can the direction of asymmetry be reversed by cheliped removal at either the 4th zoeal or glaucothoe stage?

Is reversed asymmetry of glaucothoe correlated with evidence of deleterious phenotypic characters (reduced size)?

METHODS

Overview

Box crabs were captured in the Northern Strait of Georgia in winter and spring of 2006 and 2007 (n = 39). All crabs were scored for direction of asymmetry of the chelae and females were scored for direction of abdominal asymmetry. For the one female exhibiting reversed asymmetry (female 13, Table 2.1), carapace length, major propodus length and minor propodus length were measured with vernier calipers (Goddard, 1997). Additional crabs from several sources were also examined and scored for direction of asymmetry. These crabs were obtained from: the invertebrate collection of the Royal BC Museum (n = 42); collections by researchers at the Pacific Biological Station

(Department of Fisheries and Oceans) (n = 25); by-catch in the shrimp trawl fishery in Barkley Sound (n = 2); and by-catch in the groundfish trawl fishery off the West Coast of Vancouver Island (n = 3).

Larvae released by females 2, 8, 13, and 9 (Table 2.1) were utilized for rearing experiments (Table 4.1).

Table 4.1. Use of larvae released by four female *Lopholithodes foraminatus* in characterizing the reversed asymmetry phenotype.

♀*	**Capture date**	**Asymmetry**	**Rearing season**	**Utilization in the present study.**
2	Mar 12, 2006	Normal	2006	157 1st, 2nd, and 3rd crab instars scored for asymmetry of the chelae in July of 2006.
8	Jan 13, 2007	Normal	2007	97 glaucothoe scored for asymmetry of the chelae (propodi measured for 94); right cheliped bud removed from19 4th stage zoeae; 19 left handed glaucothoe reared to determine ontogeny of asymmetry.
13	Mar 4, 2007	Reversed	2007	291 glaucothoe scored for asymmetry of the chelae (propodi measured for 158); right cheliped bud removed from seven 4th stage zoeae and the major cheliped removed from 21 right and 9 left handed glaucothoe; 42 left handed glaucothoe reared to determine ontogeny of asymmetry.
9	Jan 13, 2007	Normal	2008	3 replicates of 110 larvae reared at approximately 8 ˚C, 12˚C, and 16˚C (total 660) to determine the effect of temperature on incidence of reversed asymmetry; the incidence of reversal in 250 individuals successfully molting to glaucothoe at 12 ˚C was compared to that for glaucothoe from the other 3 females. Dry weight was also compared between 10 left handed and 10 right handed glaucothoe reared at each temperature.

*The number designating each female corresponds to numbers used to identify the same females in Tables 2.1 and 3.1).

Methods for collecting and rearing zoeae larvae were described previously (Chapter 3). If not otherwise specified, zoeae were reared either in 1 L glass beakers or 4 L plastic buckets at approximately 11 ˚C. Glaucothoe or first crab instars that were used in experiments on the ontogeny of reversed asymmetry and impact of cheliped removal were transferred to 100 mL plastic containers with 400 μm Nitex mesh bottoms. These

cultures were partially immersed in a flow through sea tray at approximately 9.5 ˚C. Containers were cleaned every 48 to 72 hours by gently flushing out the contents in the sea tray. During the flushing process, juvenile crabs remained in the container by clinging to the Nitex mesh. At each cleaning, dead crabs and exuviae were removed and food consisting of chopped frozen krill (*Euphausia* sp.) was added. This diet was supplemented at least once per week with fragments of the bryozoan *Membranipora membranacea.* When juveniles molted to a subsequent instar they were transferred to new containers. Juvenile crabs were maintained at maximum densities of 10, 4, 4, 2, and 1 individual per container in the 1st, 2nd, 3rd, 4th, and 5th instars, respectively.

Influence of maternal asymmetry

By the time the significance of reversed asymmetry was recognized in the spring of 2006, all individuals reared that season were already past the glaucothoe stage. In July of 2006, 157 1st, 2nd, and 3rd crab instars surviving from larvae released by female 1 were visually inspected and scored for direction of asymmetry of the chelae. This was generally possible without the aid of magnification.

In subsequent years, the direction of asymmetry of the chelae was scored as soon as individuals molted to the glaucothoe stage. Glaucothoe in a dish of seawater were examined using a dissecting microscope; the direction of asymmetry was generally evident even at low magnification (Figure 4.1A). Subsequent measurement of the chelae of many glaucothoe identified as either right or left handed (see next section) confirmed near 100% accuracy of this scoring technique. Where the direction of asymmetry was too close to call by examining the whole animal, chelae were removed and measured as described in the following section.

The proportion of individuals exhibiting reversed asymmetry was determined for all available glaucothoe reared from females 8 and 13 in various culture vessels at approximately 11 ˚C. A Pearson's chi-squared test for independence was used to compare these proportions to the proportions of reversed asymmetry in juveniles reared from female 2, and glaucothoe reared from female 9 at 12 ˚C.

Characterization of the reversed asymmetry phenotype in glaucothoe

Chelae were removed from mortalities and exuviae of 252 glaucothoe (94 from female 8 and 158 from female 13) at either the joint between the carpus and merus or between the carpus and propodus. Each chela was placed in a drop of seawater on a glass slide and positioned with the inner (concave) surface facing upwards. A coverslip supported by plasticine feet was placed over the chela and gentle pressure was applied to hold specimens in position without causing damage. Chelae were photographed under bright field illumination at 50 X magnification using a Sony PowerHAD digital video camera mounted on an Olympus SZX9 dissecting microscope (Figure 4.1C). Propodus length was measured from the tip of the fixed finger to the ventral condyle (Figure 4.2) using Northern Eclipse software calibrated with a slide micrometer. The ratio of right propodus length / left propodus length was calculated for each individual.

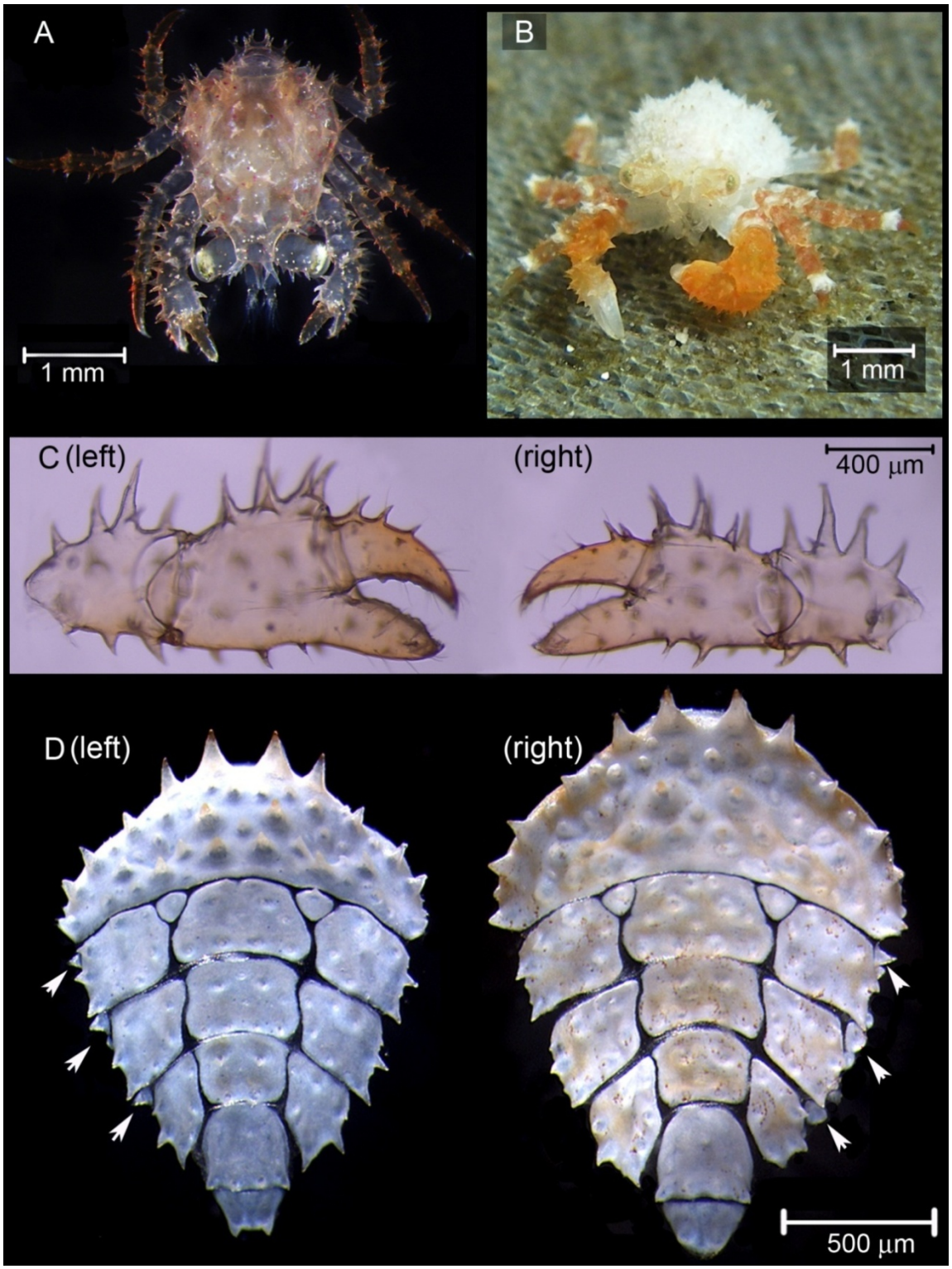


Fig. 4.1. Ontogenetic stages of *Lopholithodes foraminatus* exhibiting normal and reversed asymmetry: A. normal glaucothoe; B. 4th crab instar with reversed asymmetry of the chelae; C. left and right carpi, propodi, and dactyli from the exuvium of a left handed glaucothoe; D. abdomens of 4th crab stage females (external view) with larger (left) and (right) chelae, arrows indicate the location of marginal plates.

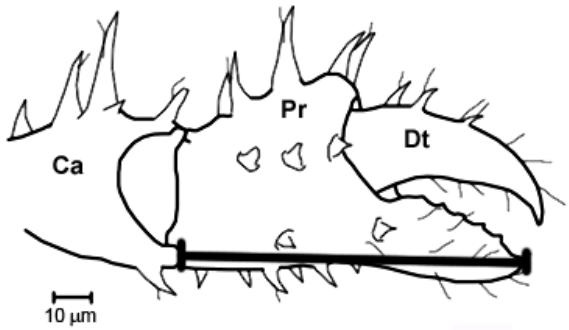


Fig. 4.2. Carpus (Ca), propodus (Pr) and dactylus (Dt) of the left chela of a *L. foraminatus* glaucthoe. The black line illustrates the measurement of propodus length.

To calculate measurement error, digital photographs of the claws of 8 left handed and 7 right handed individuals (30 photographs in total) were randomly assigned numeric file names by a third party. Propodus length was then re-measured. The measurement error was calculated as the mean of the absolute values of the differences between the first and second measurements for each claw. Error in proportion data was calculated by dividing the absolute value of the difference between the ratios of the minor to major propodi for the first and second measurements by the mean of the ratios of the minor to major propodi for the first and second measurements.

Ontogeny of asymmetry

Left handed glaucothoe from females 8 (n = 19) and 13 (n = 42), were reared through subsequent crab instars to determine if females would also develop reversed abdominal asymmetry. Those individuals that survived to the 4^{th} crab instar were sexed and scored for direction of asymmetry of the chelae and abdomen.

Effect of temperature on incidence of reversed asymmetry

Zoeae released by female 9 in February of 2008 were reared in 4 L buckets in incubators set to 8 ˚C, 12 ˚C, and 16 ˚C on a 12:12 light/dark cycle. Three replicate buckets in each incubator contained 110 larvae each. Surviving individuals were scored for direction of asymmetry of the chelae upon molting to the glaucothoe stage. The frequency of reversed asymmetry was compared between treatments using a one-way ANOVA of arcsine transformed proportion data (Sigma Stat 2.03).

Cheliped removal experiments

The right cheliped bud was removed from 26 4th stage zoeae (7 from female 13 and 19 from female 8) by pinching the base of the appendage using a pair of fine forceps. Similarly, the larger cheliped was removed from 21 right handed glaucothoe and 9 left handed glaucothoe on the day of molting to stage. All individuals were reared through subsequent crab stages to determine the impact of claw removal on the ontogeny of directional asymmetry.

Weight of normal and reversed glaucothoe

To determine if the direction of asymmetry is correlated with a difference in larval growth, 10 normal and 10 reversed glaucothoe were removed from one bucket at each temperature in the ‘effect of temperature’ experiment described above. Dry weight of each individual was determined as described previously (Chapter 3). The effect of handedness on dry weight was tested using a two-way analysis of variance (ANOVA) with handedness as the treatment factor and temperature as a blocking factor (Sigma Stat 2.03).

RESULTS

Adult reversed asymmetry

Female 13, which showed reversed asymmetry, had a carapace length of 8.85 cm, carapace width of 10.42 cm, left propodus length of 3.41cm and right propodus length of 2.88 cm; yielding a ratio of minor to major propodus length of 0.845 (compared to a minor to major ratio (± standard deviation) of 0.82 ± 0.06 calculated for right handed females by Goddard, 1997). The reversed female also exhibited complete reversed abdominal asymmetry with large lateral plates on the right, small lateral plates and marginal plates on the left, and the telson oriented to the left of the midline. Pleopods 3-5 were present on the right side only.

A total of 56 female, 43 male and 12 unsexed (abdomen missing or not examined) adult crabs were scored for direction of asymmetry. All but one (female 13) had a larger right chela and all females except female 13 had normal abdominal asymmetry with larger left lateral plates and marginal plates only present on the right.

Influence of maternal asymmetry

Frequencies of reversed asymmetry in juveniles reared from female 2 and glaucothoe reared from females 8, 9 and 13 are presented in Figure 4.3. The percentage of reversed individuals ranged from 22.7 % (female 8) to 28.4% (female 9) and was independent of maternity; Pearson's χ^2 test of independence, $\chi^2_{(3,\, N = 795)} = 1.56$, $P = 0.67$.

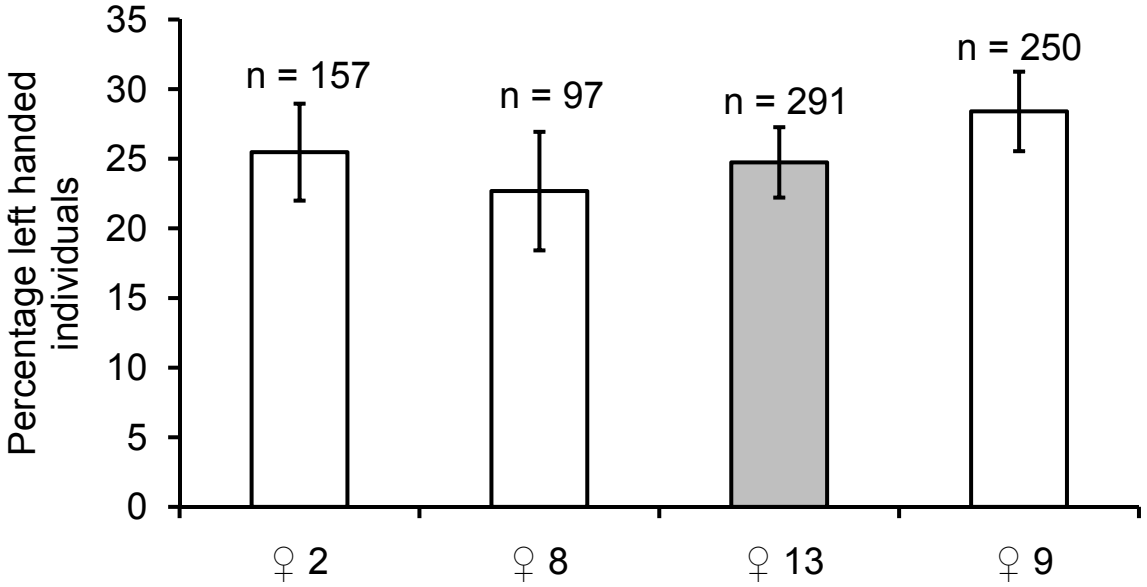


Fig. 4.3. Incidence of reversed asymmetry of the chelae in laboratory reared juvenile crabs (female 2) or glaucothoe (females 8, 13, and 9) of *Lopholithodes foraminatus* at 11 - 12 ˚C. Error bars indicate standard error and the grey bar identifies glaucothe reared from the female exhibiting reversed asymmetry.

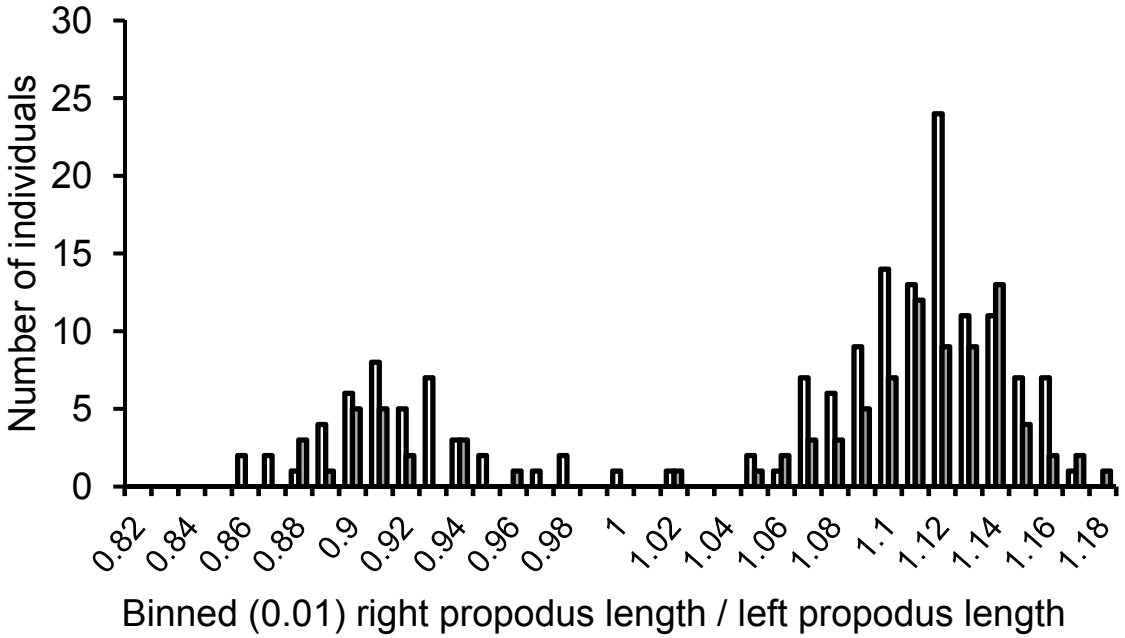


Fig. 4.4. Frequency distribution of the ratio of right propodus length / left propodus length for 252 *Lophlithodes foraminatus* glaucothoe reared from females 8 (filled bars, n = 94) and 13(open bars, n = 158).

Characterization of reversed asymmetry in glaucothoe

The ratio of right propodus length to left propodus length among laboratory reared glaucothoe exhibited a bimodal distribution (Figure 4.4). Very few individuals had chelae

that were close to symmetrical. Figure 4.1C illustrates typical left and right chelae of a left handed glaucothoe (exuvium). Mean percentage error in the measurement of propodus length (±SD) was 0.4% ± 0.3%. This resulted in a mean percentage error in the ratio of minor propodus length to major propodus length of 0.6% ± 0.6%.

Ontogeny of asymmetry

In some left handed individuals, small marginal plates appeared on the left-hand side in the 3rd crab instar, while minute pleopod buds appeared on the right. By the fourth crab instar, marginal plates were visible on both sides in males (n = 9) and on the left side in all females (n = 11) (Figure 4.1D-left). Abdominal asymmetry was the reverse of that observed in normal females with a larger right chela (Figure 4.1D-right, and see Chapter 3). Reversed asymmetry of the chelae was maintained through development in both males and females (Figure 4.1B).

Effect of temperature on incidence of reversed asymmetry

The incidence of reversed asymmetry in glaucothoe from female 9 that completed larval development at 8 ˚C, 12˚C, and 16 ˚C was 30.8 %, 28.4% and 32.4% respectively (Figure 4.5). No significant differences were detected in the incidence of reversed asymmetry of the chelae among the 3 temperatures (One way ANOVA of arcsine transformed proportion data, $F_{(2, 6)} = 0.392$, $P = 0.692$).

Cheliped removal experiments

Twenty three of the 26 4th stage zoeae that underwent right cheliped bud removal survived to molt to the glaucothoe stage. No regeneration of the right claw was observed in glaucothoe, and in the first crab instar the right cheliped was present only as a

regenerate limb bud with membranous exoskeleton. By the 2nd instar a fully formed regenerating right cheliped was present; usually smaller than or close to the size of the left cheliped. Two males and two females survived to the 4th crab instar. All 4 had a larger right chela and the females exhibited normal abdominal asymmetry.

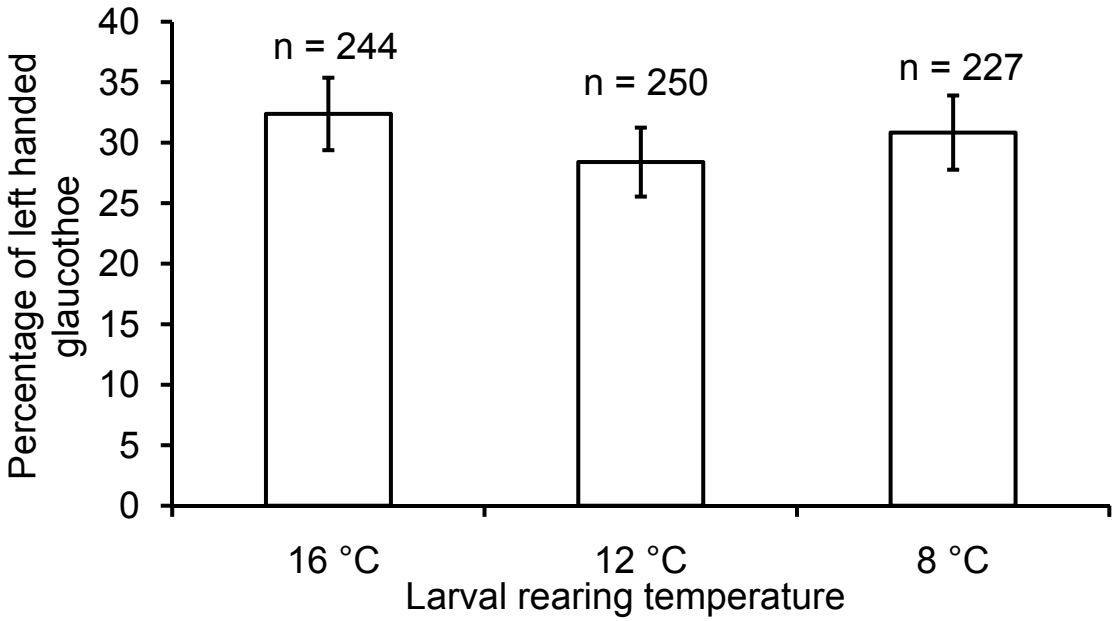


Fig. 4.5. Incidence of reversed asymmetry of the chelae in *Lopholithodes foraminatus* glaucothoe from female 9 reared in 4 L plastic buckets in incubators set to 8 ˚C, 12 ˚C, and 16 ˚C on a 12/12 light/dark cycle. Data were pooled for 3 replicate buckets. Error bars indicate standard error.

Glaucothoe from which the major claw was removed exhibited no regeneration during the glaucothoe stage. A regenerate limb bud was present in the first crab instar and a generally smaller completely formed cheliped was normally present in the second crab instar. Of the 9 left handed glaucothoe from which the left cheliped was removed, all 3 individuals surviving at the 4th crab stage had a larger left claw. The single female also had a reversed abdomen. Six of the 21 right handed glaucothoe from which the right cheliped was removed also survived to the 4th crab stage. Of these, 4 were females with a larger right claw and normal abdominal asymmetry; one was a female with nearly

symmetrical chelae and normal abdominal asymmetry, and one was a male with a larger right claw.

Weight of normal and reversed glaucothoe

The dry weight of left-handed and right-handed glaucothoe was very similar at all three rearing temperatures (Figure 4.6). No significant effect of handedness on dry weight of glaucothoe was detected (two-way ANOVA, $F_{(1, 54)} = 0.246$, $p = 0.622$).

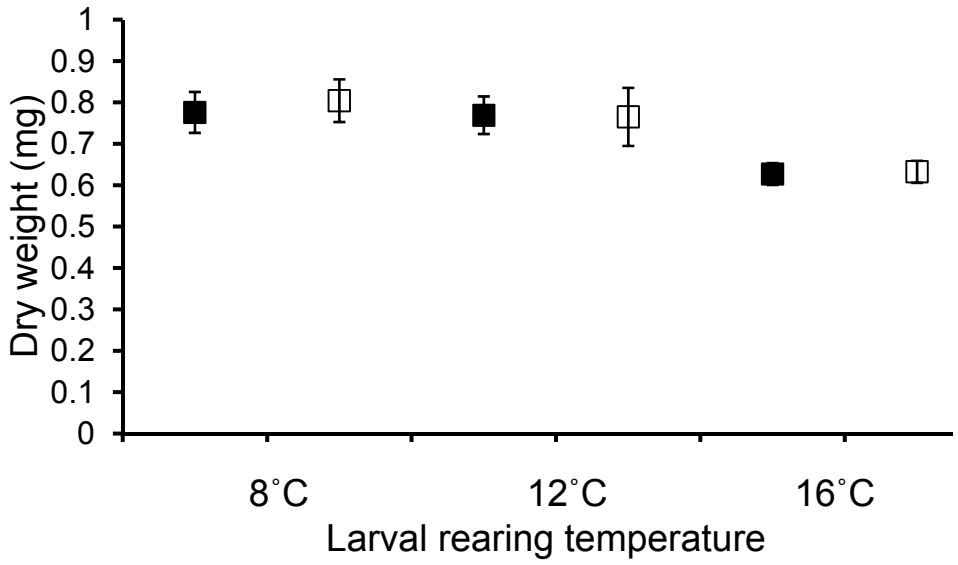


Fig. 4.6. Mean dry weight (± 95% confidence intervals) of *Lopholithodes foraminatus* glaucothoe reared in 4 L plastic buckets in incubators set to 8 ˚C, 12 ˚C, and 16 ˚C. Filled squares indicate left-handed individuals while open squares indicate right handed individuals.

DISCUSSION

The high incidence of reversed asymmetry (approximately 25%) observed in offspring of both normal and reversed female *Lopholithodes foraminatus* is difficult to interpret given that reversed asymmetry is apparently very rare among adults in the field (1 out of 111 individuals examined). Two hypotheses can be proposed to explain this elevated incidence of reversed asymmetry: some aspect of laboratory culture may be

inducing reversal; or reversed glaucothoe may occur in the field but they either switch to normal handedness or experience higher mortality as a result of ecological or intrinsic disadvantages associated with the reversed phenotype. Regardless of proximate explanation, a central enigma still remains: if the handedness of lithodid crabs is not under tight developmental control, why has it remained constant during an evolutionary history of at least 15 million years (Cunningham et al., 1992)?

Potential induction of reversed asymmetry by laboratory rearing

The most parsimonious explanation for the elevated incidence of reversed asymmetry observed in the present study is induction by laboratory rearing. Environmental influences have been known to reverse the handedness of bilaterally asymmetrical organisms. Abnormal rearing temperature can increase the frequency of reversed handedness in the directionally asymmetrical development of flounder (Aritaki et al., 2004) and sea stars (Newman, 1925). In *Caenorhabditis elegans,* maternal exposure to low temperature also results in an elevated incidence of reversed asymmetry (Wood et al., 1996). Elevated temperature was identified as a possible cause of reversed asymmetry in the present study due to the elevated frequency of reversed asymmetry of the chelae (approximately 20%) observed in glauchothoe of *Paralithodes camtschaticus* that had been reared at a higher temperature (7 – 8 ˚C) than experienced in the field (< 5˚C) (A. Epelbaum, pers. com., 2007). Rearing temperatures for *L. foraminatus* zoeae in 2006 and 2007 (11 – 12 ˚C) were also warmer than mean surface temperatures in the northern Strait of Georgia in the winter and spring (8 - 9.5 ˚C) (Department of Fisheries and Oceans, 2008). However, the lack of significant differences between the incidence of reversed asymmetry in *L. foraminatus* glaucothoe reared at or below natural temperatures

(8 ˚C), and those reared at 12 ˚C and 16 ˚C (Figure 4.5), indicates that elevated temperature during rearing was not responsible for the high incidence of reversed asymmetry observed in this study.

An alternative cause of elevated reversed asymmetry in *Lopholithodes foraminatus* juveniles could be abnormal physical characteristics of the rearing environment. For example, lack of a substrate to allow for differential exercise of the chelae results in development of symmetrical claws in the American lobster (Govind & Pearce, 1986). However, in cases where an environmental trigger determines the handedness of antisymmetrical or directionally asymmetrical decapods, the handedness can be reversed by cheliped removal. In the antisymmetrical snapping shrimp *Alphaeus heterochelis,* the presence of an undamaged major snapping claw is necessary to prevent development of the minor claw into a snapper (Read & Govind, 1997). Similarly, in some directionally asymmetrical brachyuran crabs, asymmetry reversal can be induced throughout life by the loss of a major cheliped (Vermeij, 1977; Abby-Kalio & Warner, 1989; Ladle & Todd, 2006). Major cheliped removal at the glaucothoe stage had no effect on the ontogeny of asymmetry in *Lopholithodes foraminatus.* All but one individual surviving to the 4th crab instar reverted to original handedness. The one female with approximately symmetrical chelae had likely lost her right cheliped a second time in the 2nd or 3rd crab instar. As with all other females surviving to the 4th crab instar, she exhibited abdominal asymmetry corresponding to her handedness at the glaucothoe stage.

In some decapods, the direction of left-right asymmetry is determined during a critical window, after which it becomes fixed. In American lobsters, differential exercise of one chela during the 4th and 5th juvenile stages leads to its development into a crusher

claw. Removal of one claw at or before this stage will result in the remaining claw becoming a crusher; however, after this stage claw laterality is fixed and cannot be reversed by subsequent claw removal (Govind & Pearce, 1989a, 1989b). Fiddler crabs in the genus *Uca* exhibit a similar pattern of handedness fixation. Small males (< 2.5 cm carapace width) have symmetrical chelae, but apparently discard one at random. The remaining chela develops into a major claw while a minor claw regenerates on the side of autotomy. As with lobsters, the direction of asymmetry cannot be reversed by subsequent cheliped loss (Yamaguchi, 1977). In the present study on *Lopholithodes foraminatus*, removal of the right cheliped bud from 4th stage zoeae tested the possibility that handedness was determined in the molt to the glaucothoe stage, and could not be subsequently altered. The development of normal asymmetry in juvenile crabs that had experienced right cheliped removal as zoeae suggests that the direction of asymmetry is determined earlier in development than in fiddler crabs (Yamaguchi, 1977), American lobsters (Govind & Pearce, 1986), snapping shrimp (Young et al., 1994), and some predatory brachyuran crabs (Ladle & Todd, 2006).

It is interesting to note that a larger right chela is also apparent by the glaucothoe stage in pagurid hermit crabs (e.g. McLaughlin et al., 1989; McLaughlin & Gore, 1992; McLaughlin et al., 1992; Kornienko & Korn, 2006). In arguing against a hermit crab ancestry for the Lithodidae, McLaughlin et al. (2007) suggested that cases of reversed asymmetry indicate that the handedness of lithodids may be controlled by a 'binary switch' mechanism. The authors contrasted this putative mechanism with the universal inheritance of directional asymmetry in pagurids. However, my observation that handedness is determined early in the development of *L. foraminatus,* suggests that the

development of handedness in lithodids may have more in common with that of pagurids than with that of other decapods. This would not be surprising given the developmental (Macdonald et al., 1957; Gould, 1992), morphological (Richter & Scholtz, 1994), and molecular (Cunningham et al., 1992; Morrison et al., 2002; Zaklan, 2002; Tsang et al., 2008) support for evolution from 'hermit to king.'

No mechanism for induction of reversed asymmetry was detected in the present study. However, this does not preclude the possibility that reversal was induced by some aspect of laboratory rearing. It seems unlikely that laboratory conditions could have had an impact during embryogenesis, as the incidence of asymmetry was similar in the offspring of females held in captivity for very different periods prior to hatching (see Chapter 2). Any search for an induction cue should therefore focus on the zoeal period. The influence of diet and water quality during larval development on handedness frequencies could be investigated.

Zoeal mandibular asymmetry could also provide evidence as to the stage of development at which reversed asymmetry becomes evident. The morphology of the right and left mandibles of all zoeal stages of *L. foraminatus* is distinctly different (see Chapter 3). Mandibular asymmetry is also illustrated in all other descriptions of decapod zoeae familiar to this author. While the direction of mandibular asymmetry was always the same when noted (Chapter 3: approximately 5 individuals of each zoeal stage), no attempt was made to quantify the frequency of possible asymmetry reversal. If reversed asymmetry of the zoeal mandibles occurs at a frequency similar to reversed asymmetry of the chelae in glaucothoe, it would indicate that direction of lateral asymmetry is

determined early in development and is likely not effected by laboratory rearing conditions.

Alternative explanations for an elevated incidence of reversed asymmetry

If reversed asymmetry is not a consequence of laboratory rearing, some alternative explanation must exist for the discrepancy between the incidence of asymmetry observed in field collected adults (1 / 111) and laboratory reared glaucothoe (approximately 25%). One possibility is that normal asymmetry of the chelae is not established until later in development, as is the case in other decapods (Yamaguchi, 1977; Govind & Pearce, 1986; Young et al., 1994; Ladle & Todd, 2006). If this were the case, glaucothoe would presumably exhibit a continuous asymmetry distribution, including symmetrical individuals. The bimodal asymmetry distribution observed in the present study (Figure 4.4) suggests that glaucothoe with larger left or right chelae constitute distinct left-handed and right-handed phenotypes.

Further support for the distinct nature of the reversed phenotype is provided by the maintenance of reversed asymmetry of the chelae through juvenile development. All females with reversed chelae surviving to the 4th crab stage also exhibited reversed abdominal asymmetry. This result was corroborated by the cheliped removal experiments discussed above. It seems unlikely that abdominal asymmetry, once established, would subsequently be reversed. The direction of asymmetry of the chelae in glaucothoe apparently predicts the direction of asymmetry of external morphology in subsequent development. This result is important, because if it also holds true for other lithodid species, it would alleviate the need for rearing past the glaucothoe stage in subsequent studies on the incidence of reversed asymmetry in lithodid crabs.

An alternative explanation for the low incidence of reversed asymmetry in adult *L. foraminatus* relative to laboratory reared glaucothoe is reduced fitness resulting from negative ecological consequences of reversed asymmetry in the wild. It has been suggested that the larger right claws of many brachyuran crabs provide an advantage in peeling open the shells of predominantly dextral marine snails (e.g. Ng & Tan, 1985; Shigemiya, 2003). In species that feed primarily on gastropods, left-handed crabs could be at a significant disadvantage. Little is known about the ecology of *Lopholithodes foraminatus.* The species has been described as a deposit feeder which also preys on bivalves exposed by its digging (MacGinitie & MacGinite, 1949), and it enthusiastically consumes ophiuroids in the laboratory (pers. obs.). A congener, *Lopholithodes mandtii*, was utilized as the 'non-predatory' crab in a study testing the response of a gastropod to the effluent of predators (Marko & Palmer, 1991). It seems unlikely that gastropods form an important part of the diet of adult *L. foraminatus.* However, as the habitat and ecology of early ontogenetic stages of the box crab are totally unknown, it is possible that gastropods could constitute an important component of diet during the juvenile period. It is hard to imagine other asymmetrical factors in the habitat of these crabs that could prejudice the survival of reversed individuals.

Reduced survival to adulthood in reversed individuals could also result from intrinsic deleterious traits associated with the reversed asymmetry phenotype. In humans, reversal of visceral asymmetry is a consequence of non-motile cilia, and individuals exhibiting the reversed phenotype also exhibit pathology resulting from the underlying cause of that phenotype (Afzelius, 1976). Similarly, reversal of eye-side in the Pleuronectidae (right-eyed flounders) results in looping of the optic nerve of the right eye

around the optic nerve of the left eye. It has been suggested that increased mortality associated with this abnormal arrangement may explain why the reversed phenotype is more common in juvenile pleuronectids than in adults (Parker, 1903; Fornbacke et al., 2002). The lack of a significant difference between dry weights of normal and reversed *L. foraminatus* at the glaucothoe stage (Figure 4.6) suggests that asymmetry reversal is not associated with reduced growth during larval development. Attempts to compare the subsequent survival of left and right handed juveniles in the lab were confounded by extremely high mortality in both groups. Investigation of the incidence of reversed asymmetry in juvenile lithodid crabs from the field could shed light on the consequences of reversed asymmetry for subsequent survival.

Heritability of handedness

In species exhibiting directional asymmetry or biased antisymmetry, handedness is normally heritable (Palmer, 2004; 2005). Determination of the direction of asymmetry early in development also tends to indicate that handedness is under cytogenetic rather than environmental control (Palmer, 1996). Directionally asymmetrical traits in animals which exhibit heritable reversals include visceral polarity in mice (e.g. Yokoyama et al., 1993); cuticle chirality in *Caenorhabiditis elegans* (Bergmann et al., 1998); eye side in flounder (Policansky, 1982) and coiling direction in snails (e.g. Freeman & Lundelius, 1982). The early establishment of handedness in the present study, the clear prevalence of the right handed phenotype, and failure to detect an environmental induction cue for reversed asymmetry suggests that handedness could be heritable in *L. foraminatus*.

The approximately 25% incidence of reversed asymmetry observed in glaucothoe reared from all 4 females is suggestive of a Mendelian inheritance ratio. However, if

reversed asymmetry were a recessive trait controlled at a single locus, female 13 would need to have been homozygous recessive to have exhibited the reversed phenotype. If this was the case, she would have produced 100% normal, 50% reversed, or 100% reversed offspring depending on whether she mated with a homozygous dominant, heterozygous, or homozygous recessive male respectively. Also, in order to produce 25 % reversed offspring, all 3 other females would need to have been heterozygous and have mated with heterozygous males. Assuming the population was in Hardy-Weinberg equilibrium and given a homozygous recessive frequency of 1/111, the frequency of heterozygotes would be approximately 17%. It would therefore be unlikely to randomly encounter 3 broods that were the product of matings between heterozygotes.

Even if the handedness of *L. foraminatus* is apparently not controlled by single-locus, diallelic Mendelian inheritance this does not rule out the possibility that the direction of asymmetry is inherited. The heritability of reversed asymmetry phenotypes is sometimes controlled by alternative mechanisms. For example, the pulmonate *Lymnea peregra* exhibits maternal inheritance in which the offspring of homozygous recessive females express the recessive phenotype, regardless of their genotype. Reversed asymmetry apparently results from the effect of a cytoplasmic factor in oocytes (Freeman & Lundelius, 1982). In starry flounder (*Platyichthys stellatus*), eye-sidedness is heritable, but is likely controlled by more than one locus (Policansky, 1982). The *iv* mutation in mice is inherited as an allele at a single locus. However, this mutation randomizes the direction of visceral asymmetry. This means that 50% of homozygous recessive individuals exhibit reversed polarity of the visceral organs, while the other 50% are normal (Layton, 1976; Brown & Wolpert, 1990). If reversed asymmetry in lithodid crabs

is indeed heritable, comparison of the frequency of reversed asymmetry in the offspring of a large sample of females may provide insight into the mechanism of heritability.

The enigma of reversed asymmetry in lithodid crabs

Juvenile and/or adult reversed asymmetry has now been described for 8 lithodid species in 4 genera (Campodonico, 1978; Stevens & Munk, 1991; Sandberg & McLaughlin, 1998; Crain & McLaughlin, 2000; Zaklan, 2000; McLaughlin & Paul, 2002; Motoh & Toyota, 2006; the present study). However, reversal is apparently rare among adults in the wild. For example, Campodonico (1978) identified only one female *Paralomis granulosa* with reversed abdominal asymmetry out of a total of 4,193 females examined. In hermit crabs occupying dextrally coiled gastropod shells, the importance of dextral asymmetry of the abdomen is obvious. However, given the lack of an obvious ecological constraint on the direction of asymmetry in lithodids, and given the apparent lability of this trait, it is unclear why it has been maintained in the Lithodidae through at least 15 million years of evolution (Cunningham et al., 1992).

One potential fitness disadvantage of reversed asymmetry is reproductive incompatibility. In snails, individuals exhibiting a reversed direction of coiling may be unable to reproduce with normally coiled individuals because the position of the shell forces face to face mating, but genitalia are on the wrong side of reversed individuals (Asami et al., 1998; Okumura et al., 2008). *L. foraminatus* does exhibit laterality in mating behavior, with the male guarding the female by grasping her major claw with his minor (see Chapter 2). Also, the male transfers spermatophores to the pleopods of the female with his 5th pereopods (Powell & Hurd, 1972), and it is possible that the success of this transfer could be effected if the pleopods of the female are on the wrong side. The

capture of a brooding female exhibiting reversed asymmetry does indicate that reversed females can reproduce successfully, however; if reversed individuals have relatively lower reproductive success this would still reduce their relative fitness.

Unless an induction cue resulting from laboratory conditions can be identified, the high incidence of reversed asymmetry in juvenile *Lopholithodes foraminatus* will remain difficult to explain. Reversals of normal left-right asymmetry through environmental induction and heritable mutation have provided powerful insights about evolution and development. If a high incidence of reversed asymmetry proves to be repeatable in lithodid rearing experiments, it may prove to be an important subject for future research.

REFERENCES

Abby-Kalio, N.J. and Warner G.F. (1989) Heterochely and handedness in the shore crab *Carcinus maenas* (L.) (Crustacea: Brachyura). *Zoological Journal of the Linnean Society*, 96, 19-26.

Afzelius B.A. (1976) A human syndrome caused by immotile cilia. *Science*, 193, 317-319.

Aritaki M., Ohta K., Hotta Y., Tagawa M. and Tanaka M. (2004) Temperature effects on larval development and occurrence of metamorphosis-related morphological abnormalities in hatchery-reared spotted halibut *Verasper variegatus* juveniles. *Nippon Suisan Gakkaishi,* 70, 8-15. (Japanese with English abstract and figure legends)

Asami T., Cowie R.H. and Ohbayashi K. (1998) Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. *The American Naturalist*, 152, 225-236.

Bergmann D.C., Crew J.R., Kramer J.M. and Wood W.B. (1998) Cutical chirality and body handedness in *Caenorhabditis elegans. Developmental Genetics*, 23, 164-174.

Brown N.A. and Wolpert L. (1990) The development of handedness in left/right asymmetry. *Development*, 109, 1-9.

Campodonico G.I. (1978) Un caso de inversión en la asimetría abdominal de las hembras de *Paralomis granulosa* (Jacquinot) (Decapoda, Anomura, Lithodidae). *Anales del Instituto de la Patagonia*, 9, 231-232.

Crain J.A. and McLaughlin P.A. (2000) Larval and early juvenile development in the Lithodidae (Decapoda: Anomura: Paguroidea) reared under laboratory conditions. 1. subfamily Lithodinae: *Lopholithodes mandtii* Brandt, 1848. *Invertebrate Reproduction and Development*, 37, 43-59.

Cunningham C.W., Blackstone N.W. and Buss L.W. (1992) Evolution of king crabs from hermit crab ancestors. *Nature*, 335, 539-542.

Fisheries and Oceans Canada (2008) Inside Passage: Northern Strait of Georgia: winter and spring. In *Range Profiles Data Page*. Retrieved Jan 22, 2009, from: http://www-sci.pac.dfo-mpo.gc.ca/osap/data/SearchTools/rangeprofiles_e.htm

Fornbacke M., Gombrii M. and Lundberg A. (2002) Sidedness frequencies in the flounder *Platichthys flesus* (Pleuronectiformes) along a biogeographical cline. *Sarsia*, 87, 392-395.

Freeman G. and Lundelius J.W. (1982) The developmental genetics of dextrality and sinistrality in the gastropod *Lymnaea peregra. Wilhelm Roux's Archives of Developmental Biology*, 191, 69-83.

Goddard J. (1997) Size at maturity in box crabs, *Lopholithodes foraminatus*, from Oregon. Report prepared for the Oregon Department of Fish and Wildlife. 7 pp.

Gould S.J. (1992) We are all monkeys' uncles. *Natural History*, 6, 14–21.

Govind C.K. and Pearce J. (1986) Differential reflex activity determines claw and closer muscle asymmetry in developing lobsters. *Science*, 233, 354-356.

Govind C.K. and Pearce J. (1989a) Critical period for determining claw and closer muscle asymmetry in developing lobsters. *Journal of Experimental Zoology*, 249, 31-35.

Govind C.K. and Pearce J. (1989b) Delayed determination of claw laterality in lobsters following loss of target, *Development*, 107, 547-551.

Jones D.S. and George R.W. (1982) Handedness in fiddler crabs as an aid in taxonomic groupings of the genus *Uca* (Decapoda Ocypodidae). *Crustaceana*, 43, 100-102.

Jensen G.C. (1995) *Pacific Coast Crabs and Shrimps*. Monterey, California: Sea Challengers.

Kornienko E.S. and Korn O.M. (2006) The larval development of *Pagurus proximus* (Decapoda: Anomura: Paguridae) reared in the laboratory. *Journal of the Marine Biological Association of the United Kingdom*, 86, 369-381.

Ladle R.J. and Todd P.A. (2006) A developmental model for predicting the handedness frequencies in crabs. *Acta Oecologica*, 30, 283-287.

Layton W.M. (1976) Random determination of a developmental process – reversal of normal visceral asymmetry in mouse. *Journal of Heredity,* 67, 336-338.

Levin M. and Palmer A.R. (2007) Left-right patterning from the inside out: widespread evidence for intracellular control. *BioEssays*, 29, 271-287.

Levin M. and Aw S. (2008) What's left in asymmetry? *Developmental Dynamics*, 237, 3453-3463.

MacDonald J.D., Pike R.B. and Williamson D. (1957) Larvae of the British species of *Diogenes*, *Pagurus*, *Anapagurus*, and *Lithodes* (Crustacea, Decapoda). *Proceedings of the Zoological Society of London*, 128, 209-257.

MacGinitie G.E. and MacGinitie N. (1949) *Natural History of Marine Animals*, New York: McGraw-Hill, p. 300.

Mariappan P., Balasundaram C. and Schmitz B. (2000) Decapod crustacean chelipeds: an overview. *Journal of Biosciences*, 25, 301-313.

Marko P.B. and Palmer A.R. (1991) Responses of a rocky shore gastropod to the effluents of predatory and non-predatory crabs: avoidance and attraction. *Biological Bulletin*, 181, 363-370.

McLaughlin P.A., Gore R.H. and Buce W.R. (1989) Studies on the provenzanoi and other pagurid groups: III. The larval and early juvenile stages of *Pagurus kennerlyi* (Stimpson) (Decapoda; Anomura; Paguridae) reared in the laboratory, *Journal of Crustacean Biology,* 9, 626-644.

McLaughlin P.A., Crain J.A. and Gore R.H. (1992) Studies on the provenzanoi and other pagurid groups: VI. Larval and early juvenile stages of *Pagurus ochotensis* Brandt (Decapoda; Anomura; Paguridae) from a northeastern Pacific population, reared under laboratory conditions, *Journal of Natural History*, 26, 507-531.

McLaughlin P.A. and Gore R.H. (1992) Studies on the provenzanoi and other pagurid groups: VII. The zoeal and megalopal stages of *Pagurus armatus* (Dana) (Decapoda; Anomura; Paguridae) reared in the laboratory, *Journal of Crustacean Biology*, 12, 448-463.

McLaughlin P.A. and Lemaitre R. (1997) Carcinization in the Anomura – fact or fiction? I. Evidence from adult morphology. *Contributions to Zoology*, 67, 79-123.

McLaughlin P.A. and Paul A.J. (2002) Abdominal and pleopod changes in *Lithodes aequispinus* Benedict, 1895 (Crustacea: Decapoda: Anomura: Lithodidae) from megalopa to juvenile. *Proceedings of the Biological Society of Washington*, 115, 138-147.

McLaughlin P.A., Lemaitre R. and Tudge C.C. (2004) Carcinization in the Anomura - fact or fiction? II. Evidence from larval, megalopal and early juvenile morphology. *Contributions to Zoology*, 73, 165-205.

McLaughlin P.A., Lemaitre R. and Sorhannus U. (2007) Hermit crab phylogeny: a reappraisal and its "fall-out". *Journal of Crustacean Biology*, 27, 97-115.

Motoh H. and Toyota K. (2006) A case of reversed asymmetry in a lithodid crab, *Paralomis hystrix*. *Cancer*, 15, 25-27.

Morrison C.L., Harvey A.W., Lavery S., Tieu K., Huang Y. and Cunningham C.W. (2002) Mitochondrial gene rearrangements confirm the parallel evolution of the crab-like form. *Proceedings of the Royal Society Series B*, 269, 345-350.

Newman H.H. (1925) An experimental analysis of asymmetry in the starfish, *Patria miniata*. *Biological Bulletin*, 49, 111-138.

Ng P.K.L. and Tan L.W.H. (1985) 'Right handedness' in heterochelous calappoid and xanthoid crabs- suggestions for a functional advantage. *Crustaceana*, 49, 98-100.

Okumura T., Utsuno H., Kuroda J., Gittenberger E., Asami T. and Matsuno K. (2008) The development and evolution of left-right asymmetry in invertebrates: lessons from *Drosophila* and snails. *Developmental Dynamics*, 237, 3497-3515.

Palmer A.R. (1996) From symmetry to asymmetry: Phylogenetic patterns of asymmetry variation in animals and their evolutionary significance. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 14279-14286.

Palmer A.R. (2004) Symmetry breaking and the evolution of development. *Science*, 306, 828-833.

Palmer A.R. (2005) Antisymmetry. In Hallgrimsson B. and Hall B.K. (eds) *Variation,* New York: Academic, pp. 359–397.

Parker G.H. (1903) The optic chiasma in teleosts and its bearing on the asymmetry of the Heterostomata (flatfishes). *Bulletin of the Museum of Comparative Zoology at Harvard University*, 40, 221-242.

Policansky D. (1982) Flatfishes and the inheritance of asymmetries. *The Behavioral and Brain Sciences*, 5, 262-265.

Powell G.C. and Hurd C.L. (1972) Observations of initial king crab egg attachment. *Transactions of the American Fisheries Society*, 101, 557–559.

Read A.T. and Govind C.K. (1997) Claw transformation and regeneration in adult snapping shrimp: test of the inhibition hypothesis for maintaining bilateral asymmetry. *Biological Bulletin*, 193, 401-409.

Richter S. and Scholtz G. (1994) Morphological evidence for a hermit crab ancestry of lithodids (Crustacea, Decapoda, Anomala, Paguroidea). *Zoologischer Anzeiger*, 223, 187-219.

Sandberg L. and McLaughlin P.A. (1998) Crustacea, Decapoda, Paguridae. In *Marine Invertebrates of Scandinavia*, *10.* Oslo: Universitesforlaget, 113 pp.

Shigemiya Y. (2003) Does the handedness of the pebble crab *Eriphia smithii* influence its attack success on two dextral snail species? *Journal of Zoology, London*, 260, 259-265.

Smith D.L. and Palmer A.R. (1994) Effects of manipulated diet on size and performance of brachyuran crab claws. *Science*, 264, 710-712.

Spéder P., Ádám G. and Noselli S. (2006) Type ID unconventional myosin controls left-right asymmetry in *Drosophila. Nature,* 440, 803-807.

Spéder P., Petzoldt A., Magali S. and Noselli S. (2007) Strategies to establish left/right asymmetry in vertebrates and invertebrates. *Current Opinion in Genetics and Development*, 17, 351-358.

Sumner F.B. and Huestis R.R. (1921) Bilateral asymmetry and its relation to certain problems of genetics. *Genetics*, 6, 445-485.

Stevens B.G. and Munk J.E. (1991) Lateral asymmetry in the thoracic segmentation of a king crab, *Paralithodes camtschaticus* (Tilesius, 1815) (Decapoda, Anomura), from Kodiak, Alaska. *Crustaceana*, 61, 317-320.

Sucharit C., Asami T. and Panha S. (2007) Evolution of whole-body enantiomorphy in the tree snail genus *Amphidromus*. *Journal of Evolutionary Biology*, 20, 661-672.

Trott T.J. (1987) The prevalence of left-handedness in the painted ghost crab *Ocypode gaudichaudii* H. Milne Edwards & Lucas (Decapoda, Brachyura, Ocypodidae). *Crustaceana*, 52, 213-215.

Tsang L.M., Ma K.Y., Ahyong S.T., Chan T.-Y. and Chu K.H. (2008) Phylogeny of Decapoda using two nuclear protein-coding genes: Origin and evolution of the Reptantia. *Molecular Phylogenetics and Evolution*, 48, 359-368.

Van Valen L. (1962) A study of fluctuating asymmetry. *Evolution*, 16, 125-142.

Vermeij G.T. (1977) Patterns in crab claw size: the geography of crushing. *Systematic Zoology*, 26, 138-151.

Wood W.B., Bergmann D. and Florance A. (1996) Maternal effect of low temperature on handedness determination in *C. elegans* embryos. *Developmental Genetics*, 19, 222-230.

Yamaguchi T. (1977) Studies on the handedness of the fiddler crab, *Uca lactea*. *Biological Bulletin,* 152, 424-436.

Yokoyama T., Copeland N.G., Jenkins N.A., Montgomery C.A., Elder F.B. and Overbeek P.A. (1993) Reversal of left-right asymmetry: a situs inversus mutation. *Science*, 260, 679-682.

Young R.E., Pearce J. and Govind C.K. (1994) Establishment and maintenance of claw bilateral asymmetry in snapping shrimps. *Journal of Experimental Zoology*, 269, 319-326.

Zaklan S.D. (2000) A case of reversed asymmetry in *Lithodes maja* (Linnaeus, 1758) (Decapoda, Anomura, Lithodidae). *Crustaceana*, 73, 1019-1022.

Zaklan S.D. (2002) Review of the family Lithodidae (Crustacea: Anomura: Paguroidea): Distribution, biology, and fisheries. In Paul A.J., Dawe E.G., Elner R., Jamieson G.S., Kruse G.H., Otto R.S., Sainte-Marie B., Shirley T.C. and Woodby D. (eds), *Crabs in Cold Water Regions: Biology, Management, and Economics*. Fairbanks: University of Alaska Sea Grant College Program, pp. 751-845.