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The natural history of *Bathypolypus arcticus* (Prosch), a deep-sea octopus.

by

James B. Wood

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
April 2000

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by James B. Wood

in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Dedicated to Deborah and David Wood

and

In memory of Mr. J. Gregory

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Abstract

There is a major deficit in knowledge about deep-sea and cold-water cephalopod life processes and cycles. Because the morphology of deep-sea cephalopods differs from that of shallow-water cephalopods, it is likely that there are life history, behavioral and physiological differences as well. The main purpose of this work was to study the behavior, physiology and natural history of a deep-sea octopus to provide a comparison for the life processes of the better studied short-lived shallow-water species.

This thesis describes the collection, low-temperature husbandry protocols, copulation, fecundity, brooding, development, and hatching of *Bathypolypus arcticus*, a deep-sea octopus. The natural diet of *B. arcticus* includes brittle stars, a prey not normally consumed by shallow-water cephalopods. Hatchling *B. arcticus*, like hatchlings of near-shore species, grow best when fed live crustaceans and do not thrive when fed frozen foods. Hatchlings do not thrive on a diet exclusively of brittle stars, likely because brittle stars are only ingested when preferred prey cannot be found. The growth rates of *B. arcticus* are very low (0.117%/d at 2.4°C to 0.779%/d at 9.9°C) compared to those of other octopuses. While age at maturity of all cephalopods is affected by temperature, low temperature by itself does not explain the slow growth rates found. The age at maturity of *B. arcticus* living at 4°C is conservatively estimated to be at least six years. They lay very large eggs for their size and have a correspondingly low fecundity. They are semelparous and take over 400 days to brood their eggs at 7.5°C. Low-quality diets, low activity levels, low growth rates, low fecundity, large eggs and long life spans indicate that *B. arcticus* does not follow the typical "live fast and die young" cephalopod paradigm.

List of abbreviations and symbols used

Average	Avg
Average and Standard deviation	x±y
Days	d
e	2.718
Feeding rates	FR
Grams	g
Gross growth efficiency	GGE
Growth rate (instantaneous relative growth rate)	G
Joules	J
Log base 10	Log_{10}
Mean weight of octopuses	MOW
Meters	m
Million years ago	mya
Natural log	ln
Sample size	n
Standard deviation	Std
Temperature in degrees Celsius	°C
Time, final	T_2
Time, initial	T_1
Total food ingested	TFI
Weight, final	W_2
Weight, initial	W_1

Where:

$$G = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \times 100$$

$$GGE = ([W_2 - W_1] \times 100) \div TFI$$

$$FR = (TFI \times 100) \div (MOW \times \text{no. days})$$

Acknowledgments

Keeping octopuses in the laboratory, particularly hatchlings that do not grow on frozen or prepared food, requires large amounts of live food. Food such as brittle stars had to be collected by divers, *Gammarus* spp. (amphipods) and *Crangon* sp. (sand shrimp) were collected locally at the Rainbow Haven salt marsh. The burrowing amphipod *Corophium volutator* was sifted from Windsor "sucking-mud" flats by "the goo crew". Some of the many volunteers that donated their time to help collect cephalopod food are: Joyce Chew, Nicole Chiasson, Mike Greenwood, Dr. Chris Harvey-Clark, Jeremy Keddy, Alison King, Michelle Lam, Terri Lownds, Fred Watts and Deborah Wood.

A warm thank you to honors student Shelly Brown for helping with the feeding experiment, to volunteer Terri Lownds for assisting with the weight stress experiment (see the appendix) and helping out wherever needed, and to Nicole Chiasson and Catriona Day for caring for post-experimental octopuses and/or cuttlefish.

Thanks to Joan Manual, for among other things, informing me that "shit can, and often does, happen" when keeping sensitive deep-sea animals alive. A good dose of paranoia/reality led to the installation of many redundant systems and fail safes in my deep-sea octopus room and much of my long term success is due to this. I would also like to thank the Dalhousie Aquatron staff who are on call 24 hours a day, 7 days a week, 365 days a year, 5.3 years a PhD.

I am grateful to Alison King, my wife Deborah Wood and mother-in-law Shirly Goldstein for critically proof reading this here thing. Thanks to S. v. Boletzky and J. Voight for their helpful comments on Chapter 4. A thank you is also due to John Cigliano who commented on the mating video, Dave Gaudet for filming the mating, and to Rebekah McClean for illustrating the mating sequence. I am grateful to Bruce Carlson, director of the Waikiki Aquarium, for weighing a hatchling *Nautilus* and Greta Pecl for weighing five *Idiosepieus pygmaeus* for this project. I also wish to thank Roland Anderson and Jennifer Mather for their comments on Chapter 5.

This project would not have been possible without the help of the Canadian Department of Fisheries and Oceans. I am especially grateful to Ellen Kenchington, Dale Roddick, Mark Lundy, Brenda Bradford and the crew of the FRV J. L. Hart. Without their help there would have been no octopuses to study. This DFO group collected octopuses and kept them here in Halifax for me even before I arrived. As octopuses are only collected in the summer, this saved a year of my time and it helped lure me away from Florida to the frozen northern tundra of Nova Scotia.

I thank my supervisor, Ron O'Dor, for his humor, helpful support, ideas and especially the freedom to follow my own drum; Jeff Hutchings for ensuring that I'm the best I can be; and to Shelly Adamo for ideas, enthusiasm, encouragement and the occasional shot of motivation.

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Preface

The thesis chapters that have been published, or have been submitted for publication, are listed below:

Chapter 2. Wood, J., Brown, S., and O'Dor, R.K. (submitted). First growth rates of deep-sea octopus Bathypolypus arcticus on five diets. Canadian Journal of Zoology.

Chapter 3. Wood, J., and O'Dor, R.K. (submitted). Rearing deep-sea octopus (Bathypolypus arcticus) at temperatures from 2-10°C. Marine Ecology Progress Series.

Chapter 4: Wood , J.B., Kenchington, E., and O'Dor, R.K. 1998. Reproduction and embryonic development time of *Bathypolypus arcticus*, a deep-sea octopod (Cephalopoda: Octopoda). Malacologia 39(1-2): 11-19.

Chapter 5. Wood, J.B., and O'Dor, R.K. (2000). Do larger cephalopods live longer? Marine Biology 136: 91-99.

Chapters 2, 3, 4, and 5 are in the formats required by the journal they were submitted to. Chapter 1 gives a general introduction to cephalopods, describes the current lack of knowledge present in the field and presents the questions that the thesis will address. The summary (Chapter 6) outlines the new contributions of this thesis and compares them with other cephalopods, molluscs and fish. The thesis ends with suggestions for future research.

Chapter 1

Introduction

Introduction

Cephalopoda: Past and Present

Cephalopoda is the class in the phylum Mollusca that contains cuttlefish, octopuses, squid and the chambered nautilus. "The characteristics of modern cephalopods which most readily distinguish them from the other molluscs are their size and the impression they convey of activity and vigor" (Boyle 1987). Cephalopods tend to be larger than the molluscs in other classes with most members 10 to 100 cm in length (Boyle 1987). Other common molluscan classes include Bivalvia (scallops, oysters and clams), Gastropoda (snails and slugs), Scaphopoda (tusk shells) and Polyplacophora (chitons). Most molluscs are protected by a heavy shell and are benthic and not capable of rapid movement. There is strong evidence that the formation of carbonate shells limits the growth rate of molluscs and other marine invertebrates and that this limitation is an important evolutionary cost to having a shell. In addition to physically limiting growth, energy must be expended in order to form and transport the shell (Palmer 1981).

Although they are molluscs, cephalopods are similar in many ways to teleost fish in their morphology, physiology, ecology and behavior (Hanlon and Messenger 1996). Boyle (1987) states "the common cephalopods are unusually large and mobile invertebrates showing a versatility of behavior more associated with vertebrates".

The first written records of cephalopods were from Aristotle in 330 BC. He described several Mediterranean species and observed that octopuses had well-developed brains despite being cold-blooded (Aristotle 1948, Aristotle 1968). Cephalopods have well-developed sense organs and the most developed brains of all invertebrates. The size of their brains, in relation to their body weight, is higher than most fish and reptiles (Packard 1972). The well-developed brains of cephalopods are capable of coping with much more sensory input than a snail or limpet (Hanlon and Messenger 1996).

Cephalopods first appeared in the Lower Cambrian period approximately 570 million years ago (Hanlon and Messenger 1996). A diversity of ectocochleate (externally shelled) cephalopods once dominated the world's oceans, primarily preying on arthropods and molluscs. Many theories exist about the evolution of modern endocochleate cephalopods, competition with vertebrates being a central theme in all of them (Packard 1972, Arnold 1990).

This competition with vertebrates continues today. Aronson (1991) has demonstrated that octopus population density is negatively correlated with the abundance of large fish. In another study, Mather and O'Dor (1991) observed that octopuses spend less time foraging and more time secluded in their den than would be expected if they were maximizing growth. They propose that this is due to predation risk. Mather (1982) found that octopus density was limited by the availability of shells in which they could hide.

Although coleoid cephalopods do not have a heavy defensive shell to protect them from predators, they have evolved a well-developed nervous system, a circulatory system capable of supporting rapid movement and a number of behavioral tricks (including ink decoys, ink clouds, rapid jetting and rapid color, texture and shape changes) that are specifically designed to fool their predators, which are primarily vertebrates (Appendix 1). CephBase (Wood et al. 1999, http://www.cephbase.dal.ca/) lists over 325 published cephalopod predation records. All predators listed are either birds, fish, marine mammals or other cephalopods. These predators are active, mobile and have well-developed senses; many have well-developed vision (Hanlon and Messenger 1996).

Modern cephalopods can be divided into two groups: the externally shelled nautiloids which despite their abundance in the Paleozoic and early Mesozoic era, are now extinct except for the six species in the genus *Nautilus* (Sweeney and Roper 1998) and the coleoids such as cuttlefish, squid and octopuses. Coleoid cephalopods account for over 99% of all extant species and they are the cephalopods capable of rapid movement, ink decoys and changing their color, texture and shape. In coleoids, there has been a trend toward shell reduction. For example, octopuses have almost no evidence of a shell.

Coleoid cephalopods have a rich diversity of size and habitat. All species are marine, and, with a few exceptions, they do not tolerate brackish water (Boyle 1991). Cephalopods are found in every ocean, from the warm water of the tropics to the near-freezing water at the poles. They are found from the wave-swept intertidal region to the dark, cold abyss. Cephalopods range in size from the 8 mm long, 0.175 g *Idiosepius pygmaeus* (Lewis and Choat 1993) to the giant squid *Architeuthis*. At 18 m in total length, *Architeuthis* holds the record as the world's largest invertebrate and has the largest eye of any animal on the planet (Roper and Boss 1982).

Despite this diversity, cephalopods occupy similar trophic niches in the food chain. While there is considerable diversity in the diets of other classes of molluscs, all coleoid cephalopods studied to date are predators (Appendix 2). However, cephalopods are never the top predators. They often fall prey to vertebrates such as birds, marine mammals and fish.

Despite numerous similarities with fish, there are many differences as well, particularly in the areas of life-history strategy. The life cycle of most marine molluscs and many fish is characterized by a relatively long adult life span, a short egg and juvenile period and iteroparity (Saville 1987, Rodhouse 1998). Nautilus approximates this pattern. Coleoid cephalopods, however, spend a much larger percentage of their life span as eggs and juveniles and exist as adults for a relatively short amount of time (Rodhouse 1998). Perhaps this is due to the fact that locomotion by jetting by adult cephalopods is less energetically efficient than the undulatory swimming of the cephalopods primary competition, fish. The exception to this is for very small animals such as hatchlings which have low Reynolds numbers. At low Reynolds numbers jetting is a more efficient method of locomotion as it punches through the syrupy water (O'Dor and Webber 1986, O'Dor and Webber 1991). O'Dor and Dawe (1998) argue that the inefficient locomotion of adult cephalopods and competition from teleost fish constrain them to a life history featuring rapid growth and short life spans.

While the short life spans of cephalopods are widely cited, there are few papers that compare many species of cephalopods to numerous species in other groups in order to demonstrate this. Figure 1a shows the approximate life span of coleoid cephalopods (data from Chapter 5). Age at maturity was rounded up to the nearest year as a rough approximate of maximum life span. This is valid as none of the species used have a long reproductive period after maturation. For example, of the cephalopod species used, the one that spends the largest percentage of its life span sexually mature is probably

Figure 1. Age at maturity in years for three classes of molluscs.

Figure 1a. Age at maturity in years for species of coleoid cephalopods that were used in Chapter 5, with the exception of *B. arcticus* for which I used the more recently calculated life span from Chapter 2. Age at maturity is used to roughly approximate life spans of coleoid cephalopods. Nautilus is not a coleoid cephalopod and therefore is not on the graph. Its life span is unknown but may span several decades.

Figure 1b. Life span in years of a number of gastropod species. Values from Powell and Cummins (1985).

Figure 1c. Life span in years of a number of bivalve species. Values from Powell and Cummins (1985).

Fig. 1a

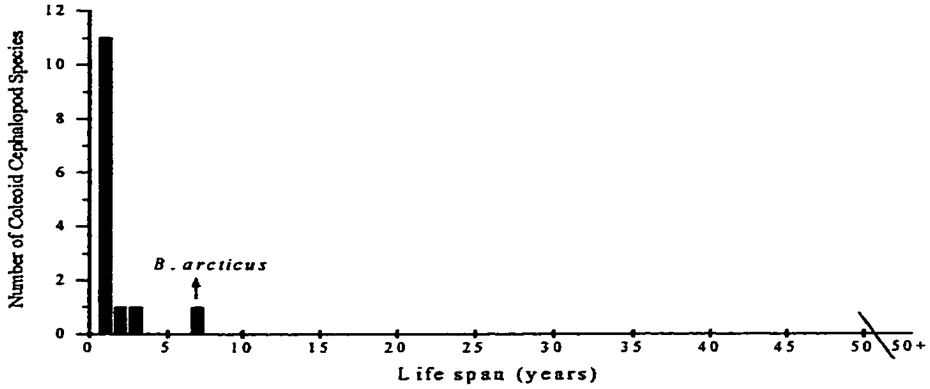


Fig. 1b

Fig. 1c

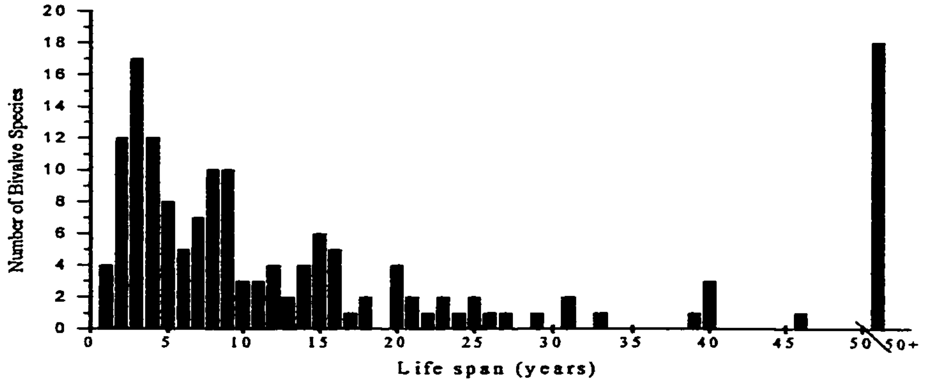


Figure 1.

Idiosepius pygmaeus. This small sepiolid matures when it is between 40 and 60 days old and has a maximum life span of 80 days (Lewis and Choat 1993). Some species of *Sepia* and *Loligo* are reproductively active for several months after they attain maturity (Boletzky 1987a, Sauer and Lipinski 1990, Hung Baeg et al. 1993, Collins et al.1995). This would place the life span of *Sepia officinalis* and *Loligo forbesi* into the two-year bin in Figure 1a. All of the octopuses listed are semelparous and after they lay their eggs they waste away by metabolizing their bodies to provide energy for brooding. The maximum estimate for the life span of the giant octopus is five years (Hartwick 1983). While this estimate is more than the value of 3 years that was used here, it is still short for a mollusc that frequently reaches sizes over 50 kg! Using age at maturity as a crude approximation of life span is reasonable for these cephalopods. Even in the cases above when life span and age at maturity are not equal, the difference does not change the conclusion that coleoid cephalopods are short-lived; there are no known species of coleoid cephalopods that live longer than ten years and most only live a year or two.

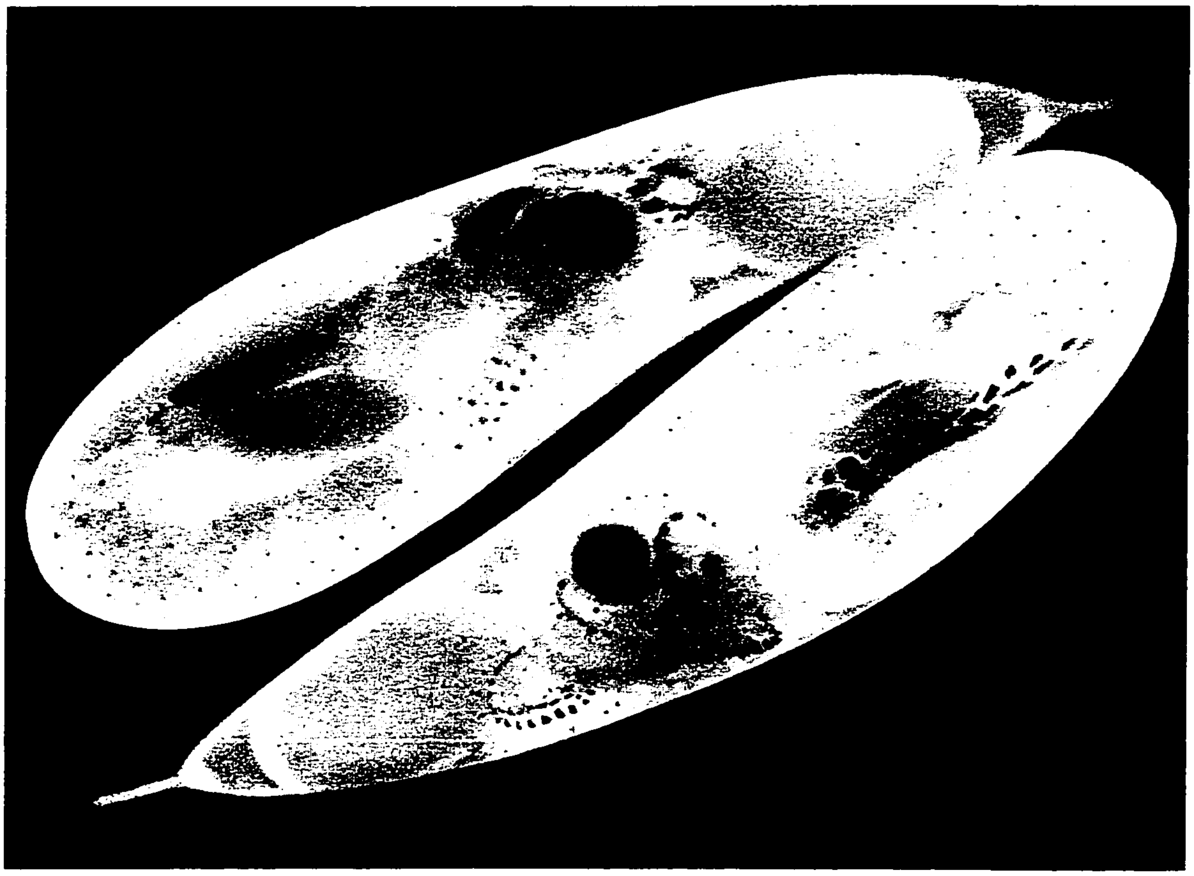
Powell and Cummins (1985) examined the maximum life span of marine and estuarine gastropods and bivalves. Values used were taken from the literature and no species was used more than once. They did not include all the species they found that had a life span of a year or less. The longevity of gastropods (Figure 1b) is much greater than the life spans of coleoid cephalopods. There are several gastropods that live over ten years and some that live over 50 years. The longevity of bivalves (Figure 1c) is also greater than the life spans of coleoid cephalopods. Bivalve species that live more than 50 years are not uncommon. Compared with those of gastropods (Figure 1b) and bivalves

(Figure 1c), the trend for short life spans of cephalopods studied to date is unmistakable. Another possible difference between cephalopods, other molluscs and many fish is the cephalopod tendency toward semelparity. While some works continue to make statements like coleoid "cephalopods are generally short lived (usually 1 year) and have monocyclic reproduction and a semelparous life history" (Rodhouse 1998), others have pointed out that there are species that exhibit true multiple spawning (Mangold et al. 1993). According to Mangold et al. (1993), cephalopods have a full range of reproductive modes from semelparity to non-seasonal iteroparity. Many of the cephalopods that exhibit reproductive strategies that vary from the semelparous model are animals that are found in the open ocean or deep sea and these are the least studied. Most of the existing cephalopod data are for warm-water or near-shore species.

Much of the work done on the life cycles of shallow-water octopuses was done by John Forsythe and Roger Hanlon in the 1980's. However, even shallow-water octopuses are poorly studied. Octopuses such as the Carribean Reef Octopus (*O. briareus* Figure 2 and 3) are very common in some areas but are rarely observed in the wild due to their nocturnal and cryptic behavior. It is known that female octopuses collected in the wild can store sperm for several months and will often lay viable eggs in captivity. These large, well-developed yolky eggs (Figure 3) do not pass through a trochophore or veliger stage like many other molluscs but are benthic upon hatching. Other species of cephalopods are planktonic for a period during which they disperse, sometimes over considerable distances. Given that few species of octopuses that have a planktonic stage have been reared to settling in the laboratory (see Villanueva et al 1996), we know almost nothing about this

Figure 2. This adult wild octopus (*Octopus briareus*) was out hunting at night in the Dry Tortugas National Park in the Florida Keys.

Figure 3 The large, well developed eggs of *Octopus briareus*.



important stage for many octopuses.

Almost all life-history data for deep, cold-water cephalopods are still unknown. A major reason for this is that cephalopods are difficult to study.

Rearing and studying natural history of octopuses: confounding factors

Field

As cephalopods live in water, they are not as easy to observe as most terrestrial animals. The deeper they live, the more expensive and complicated the life-support systems are that allow humans to observe them. Almost all cephalopod species studied so far are nocturnal which makes field observations of even shallow species difficult without disrupting their natural behavior. Cephalopods are better than any other animal at camouflage (color, skin texture, body posture, ink). With their large brains and well developed sense organs they likely have better sensory input than any other invertebrate (Hanlon and Messenger 1996). Nocturnal habits, well-developed senses and camouflage make them hard to locate in the field.

Life span and growth rate information is important for both biologists and fisheries managers. The age of individual cephalopods cannot be accurately estimated based on size, as size does not correlate well with age. For example, sibling cephalopods raised under the same conditions can be extremely variable in size at a given age (Forsythe and Van Heukelem 1987). Growth rates are even more variable between individuals that are unrelated and/or living in different environments; both of these conditions are common in the field. While there are techniques to age squid captured in the field, using their statoliths and pens (Arkhipkin and Bizikov 1997; Jackson et al. 1997; Arkhipkin and Perez 1998), there are no techniques to measure the age of field collected octopuses. Therefore, age at maturity and growth rates must be determined by life cycle studies in the laboratory or by tag and recapture studies. Soft-bodied cephalopods are not well suited to tagging. Recapture is only feasible in a few commercial species. Thus, the scientific community must heavily rely on laboratory cultures for life cycle information on cephalopods because it is extremely difficult to make accurate observations of cephalopods in the field (Boletzky and Hanlon 1983).

Laboratory

As laboratory animals, octopuses are far from the low-maintenance end of the scale. They have special requirements for housing, diet, collection, transportation, space and care. It is common knowledge that octopuses have a high propensity to escape confinement. Aquariums therefore need to be "Houdini proof" while still allowing easy access for cleaning, feeding, handling and observing the animals. Individuals may need to be kept separately as they can be cannibalistic (Boyle 1991). As all octopuses are marine, all the standard protocols for setting up and maintaining marine systems must also be implemented (see Moe 1992).

Octopuses eat a lot and hatchlings grow exponentially (Forsythe and Van Heukelem 1987). As hatchlings can only be raised on live food (DeRusha et al. 1989), collecting enough live food from the field to fuel their exponential growth can be a challenge, especially in winter. Furthermore, they are very susceptible to fatal skin ulcers when reared in batch treatments (Hanlon et al. 1984). Therefore, separate rearing chambers are also needed for the young and the space needed to do this can constrain sample size in all studies. Boletzky, Boyle, Forsythe, Hanlon, Villanueva and other workers have overcome these difficulties and have raised *Octopus bimaculoides*, *Octopus joubini*, *Octopus briareus*, *Octopus maya* and *Octopus vulgaris* in the laboratory. All of these species live near the shore in warm water. There are additional problems that must be addressed when trying to rear cold-water cephalopods in the lab.

***Bathypolypus arcticus*: A model for deep-sea octopuses**

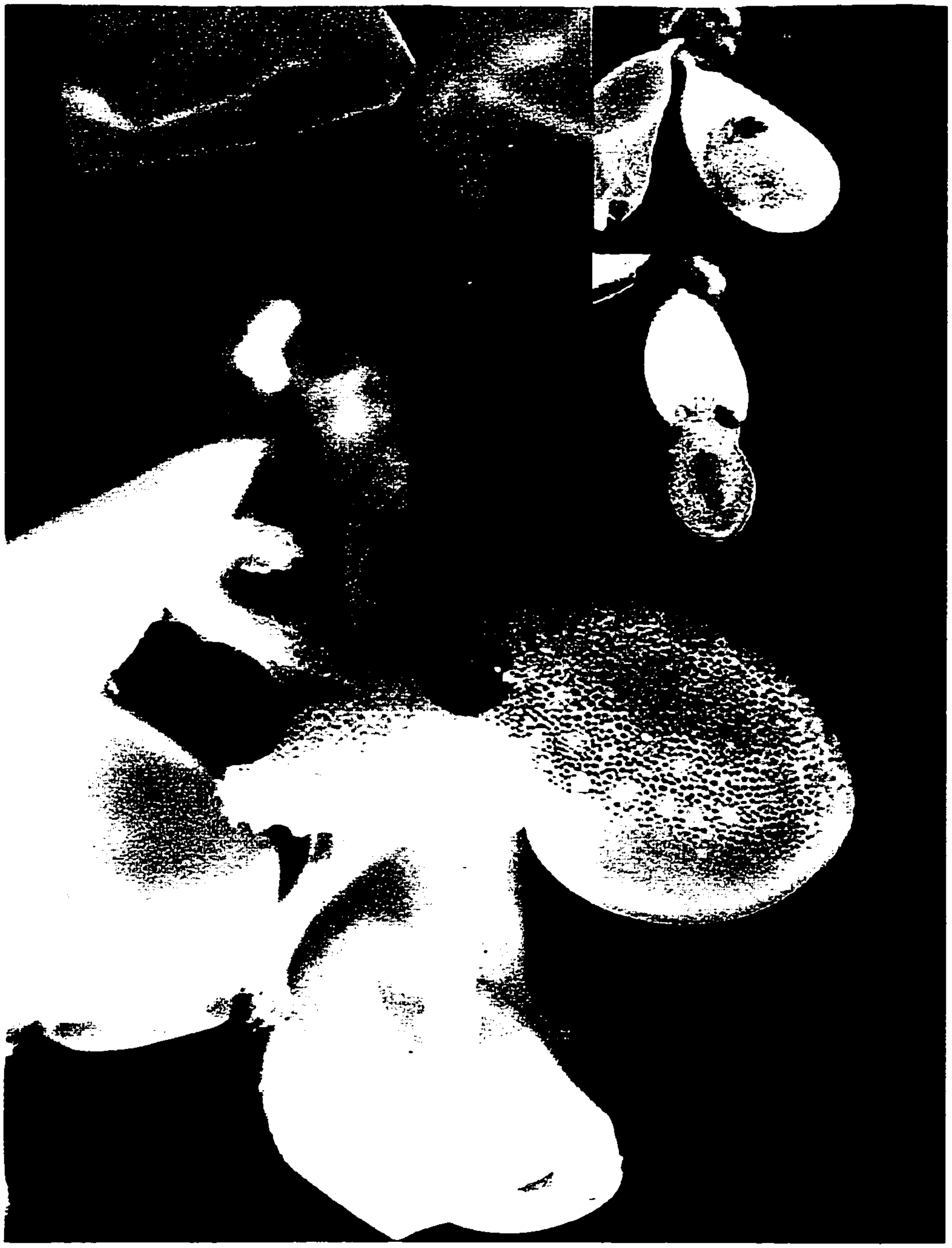
Description

B. arcticus is a small (mature females weigh between 9 and 300 g), benthic, short-armed octopus. It has been collected from Florida to Greenland in the western Atlantic Ocean and along the northern reaches of Russia to Spain in the northeast Atlantic Ocean (O'Dor and Macalaster 1983). These octopuses are remarkable because of the large ligula, the reproductive organ of males, the large eggs and low fecundity of females and the incredibly long brooding period which lasts more than 400 days (Wood et al. 1998). Hatchlings (Figure 4) weigh approximately 250 mg and are essentially miniature adults with neither a planktonic stage nor an external yolk sac (O'Dor and Macalaster 1983, Wood et al. 1998).

Habitat

O'Dor and Macalaster (1983) report that *B. arcticus* lives at 4±2 °C and is most

Figure 4. Hatchling deep-sea octopus, *Bathypolypus arcticus*.



commonly found at depths of 200-600 m on the continental slope. According to catch data, octopuses were not randomly distributed with depth but were much more likely to be collected from deeper tows, especially those over 100 m (Figure 5). Collection, transportation and rearing is discussed in Chapters 2, 3 and 4. O'Dor and Macalaster (1983) suggest that *B. arcticus* lives on sandy or muddy bottoms although they admit that their data may be biased since the sampling gear used is only implemented on such bottom types and the animals need a hard surface on which to lay their eggs. Using data provided by Peter Auster, Science Director of the National Undersea Research Center in Connecticut (pers. com.), I initially found support (Figure 6) for the conclusion that *B. arcticus* lives on mud and sand bottoms. However, as Figure 7 shows, depth was a confounding factor. A Kruskal-Wallis test revealed that the depths of the different bottom types are significantly different (p=0.002). Evidence of the bottom type from the contents of scallop dragger tows was not analyzed as it was thought to be too crude. Therefore, the preferred bottom habitat of *B. arcticus* is still not clear.

A brief history of the study of *B. arcticus*

The following is a summary of the work done on *B. arcticus* since it was described by Prosch in 1849. A few of the taxonomic papers that cursorily mention them and the handful of deep-sea benthic survey papers that list them as one of the many species collected are excluded.

Verrill described *Octopus bairdii* in 1873 from specimens taken in the Lower Bay of Fundy. He also described *Octopus lentus* and *Octopus obesus* in 1880. Kumpf's 1958 M.Sc. thesis examined 200 preserved specimens and concluded that there was only

Figure 5. Average yield per tow of *B. arcticus* at various depths based on 486 tows done during 5 scallop assessment cruises in 1995 and 1996.

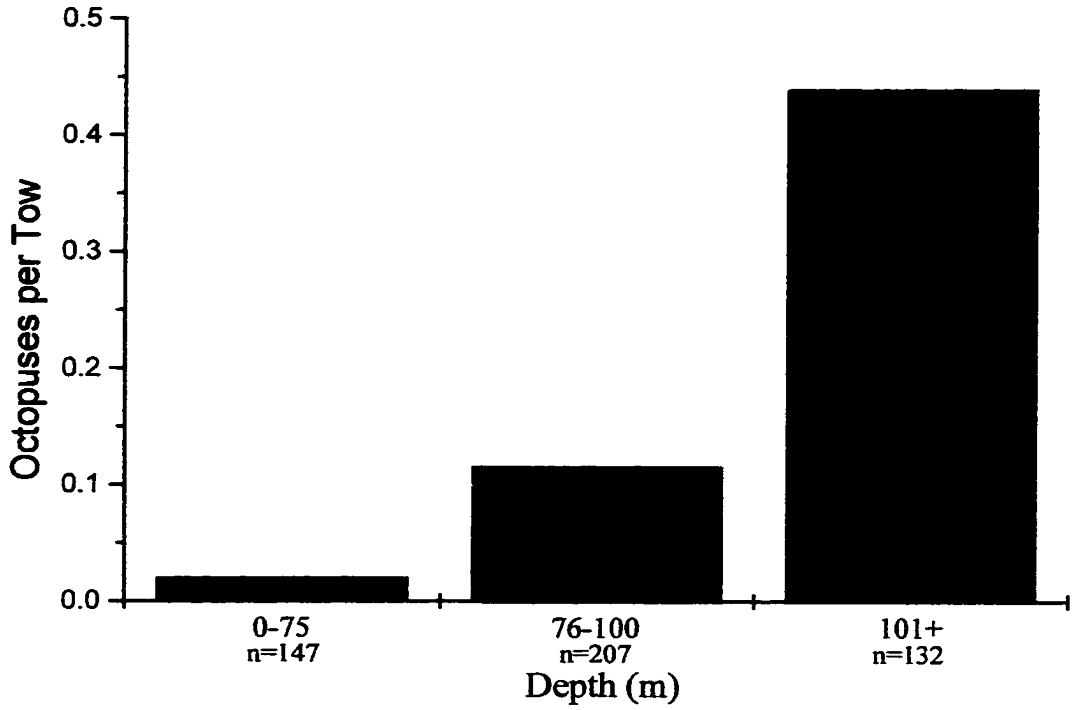


Figure 6. Number of octopuses found in tows over different bottom types. Raw data from Peter Auster. This initially supplied evidence that *B. arcticus* is found on mud and sand bottoms.

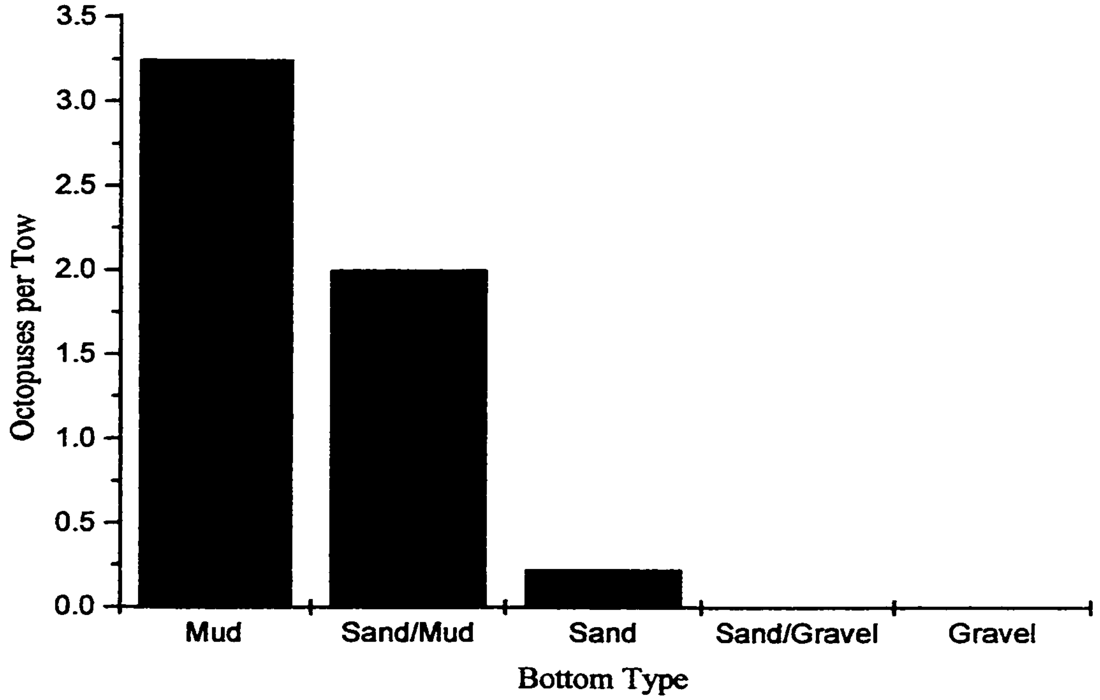
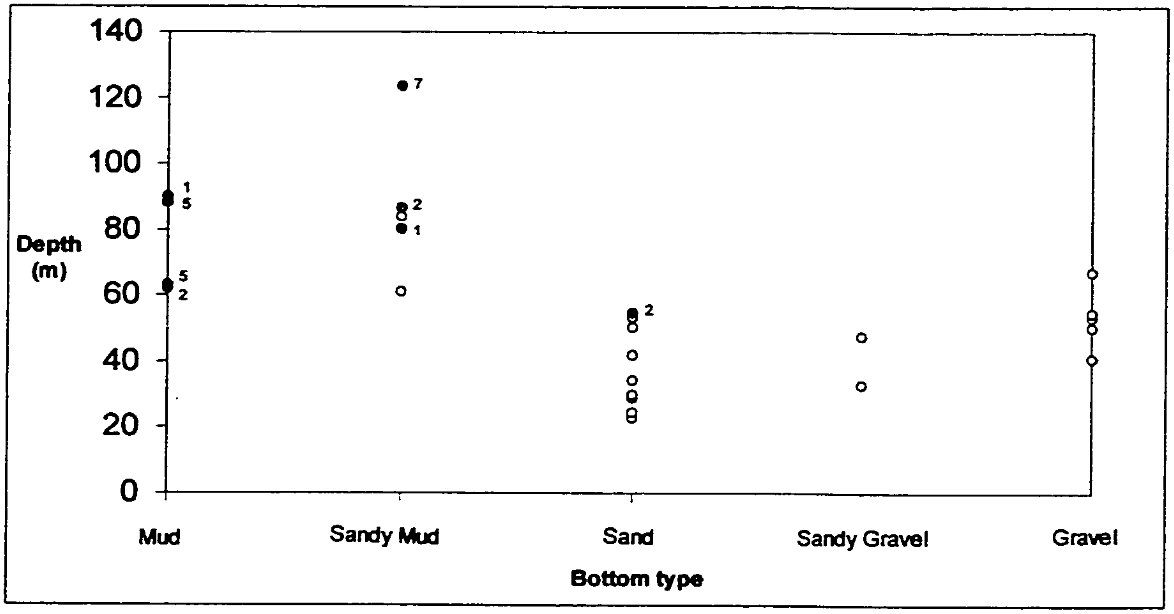


Figure 7. All octopuses in Peter Auster's data set were collected over sand or mud bottoms, although these were the also the deepest tows. Thus the preferred bottom type of *B. arcticus* is still uncertain. The numbers to the right of the dark circles indicate the number of octopuses captured in the tow. Open circles represent tows where no octopuses were captured.



one species of small deep-sea octopus off the east coast of North America. It was suggested that *O. bairdii, O. lentus* and *O. obesus* were all synonymous with *B. arcticus*. This finding was supported by Macalaster (1976). Although octopus taxonomy is in need of revision, I accept Kumpf's and Macalaster's results but I am careful to qualify my animals as the lower Bay of Fundy population, in case there are future revisions. For future study, I have donated specimens, video footage and tissue samples to numerous individuals and museums (Appendix 3).

Elizabeth Macalaster completed her M.Sc. on *Bathypolypus arcticus* at Dalhousie under the supervision of Dr. Ron O'Dor. Much of her thesis concentrates on taxonomy and analysis of preserved specimens, but her thesis was notable because it also contained the most detailed study of the natural history of *Bathypolypus arcticus*, or of any deep-sea octopus until now. Contributions from her work include: a hypothetical life cycle of 3 years based on catch weights, the discovery that brittle star arms are a primary component of *B. arcticus'* natural diet [a departure from the crab, mollusc and teleost diet of species of octopus in the genus *Octopus* (Nixon 1987)] and the first observations of living specimens including observations of feeding behavior. After Macalaster (1976) completed her thesis, preliminary observations of brooding time and offspring size were made from a single female octopus (O'Dor and Macalaster 1983). This work was a foundation for further investigation but was incomplete. Hatchling biology was not investigated because the brooding octopus ate all but 4 of her 40 eggs and only one of the 4 hatchlings lived for 2 weeks. Moreover, the temperature at which the eggs were brooded was unrecorded as were other important phenomena such as mating.

Because of the small sample size for observations of brooding (n=1) and hatchling size (n=1) it was not clear if the observed results were representative. Macalaster's (1976) thesis and observations of the 4 eggs that hatched is the sum of the behavioral, physiological and natural history work on *Bathypolypus arcticus*, the best studied deep-sea octopus.

***Bathypolypus arcticus*: An additional level of complexity**

B. arcticus are not extremely common in the field. The animals used in this thesis were obtained by collaboration with Dr. Ellen Kenchington of the Department of Fisheries and Oceans (DFO). Macalaster (unpublished data) spent many hours looking at deep-sea stills and never saw a single specimen. Few predators even find them: they make up less than 0.5 % of any species stomach contents (Kohler and Fitzgerald 1968).

Analysis of 486 DFO scallop stock assessment tows done during 5 cruises in 1995 and 1996 demonstrated that an average tow yielded 0.17 octopuses per tow. On a good day, 12 tows were done yielding approximately 2 octopuses. Collecting them in a scallop dragger damages them. The highest mortality rate (>50%) occurred within the first month. Unlike most octopuses, *B. arcticus* does not seek out lairs; thus they cannot be caught undamaged in traditional octopus pots.

In addition to the difficulties associated with studying octopuses in the laboratory already discussed, studying deep-sea octopuses poses further challenges. The water at the depths at which *B. arcticus* lives is 4±2 °C. To maintain these octopuses alive long enough to measure growth, one must keep the temperature consistently below 10 °C for months to years at a time. For most experiments, one needs to use hatchling octopuses as they are of a known age and have the most capacity to grow. Acquiring hatchling octopuses is not a trivial task due to the biological challenges involved. Hatchling *B. arcticus* have never been caught in the wild and can only be obtained by collecting a female that has mated in the wild or by collecting a female and providing an acceptable mate for her in the laboratory. If she is reproductively ready, she will lay eggs. If the eggs are fertile, and the temperature is raised by 1.5 standard deviations over the average of 4 °C to get them to develop faster, they will still take over 400 days to develop (Chapter 4). Then, if temperature and water quality remain constant, about 50 octopuses will hatch over a six-week period and only then can experiments on hatchlings begin.

However, there are more than biological challenges to consider. Power outages and chiller breakdowns are concerns. Many fail-safe mechanisms must be present to keep the water constantly cold, including insulated pipes and tanks, a secondary chiller and stashed bottles of frozen water. Feeding the hatchlings necessitated plankton tows at two in the morning in February, organization of teams of volunteers for trips to the Windsor, Nova Scotia, mud flats to sift mud and an artificial mud flat complete with tidal cycle designed to keep one of the several foods used alive through winter and spring. These and other challenges, such as supersaturation of the water with air, are discussed in Chapter 2.

After biological and technical logistics, interpretation of results can be tricky. Sample size is a limitation. Voss, in his 1988a paper on the evolution and phylogeny of deep-sea octopuses, comments on the difficulties of taxonomic work on deep-sea octopuses due to the lack of specimens: "even today, more than half of the species are represented by only two or three specimens and few are represented by as many as a dozen." Considering this and the logistical problems of collecting and maintaining octopuses in good condition, my smallest sample size of 6 is relatively large for the field.

Although keeping deep-sea octopuses poses additional challenges, they are also easier to keep than shallow-water octopuses in some ways. *Bathypolypus arcticus* do not frequently escape like shallow-water octopuses. They also eat less than warm-water species; thus less food has to be collected and maintained. If they live through the first month in the lab, they are likely to live at least a year or longer in captivity.

Despite the challenges, this work is the first to demonstrate long term success with deep-sea octopuses. I observed numerous mating sequences and have quantified brooding both in terms of time and temperature (Chapter 4) . I have had numerous specimens in the laboratory and 11 octopuses have successfully laid and brooded eggs through to hatching (Chapter 4). I kept hatchlings alive and was able to raise one to maturity (Chapter 3). There are no data anywhere in existence for any species of deep-sea cephalopod similar to what I have been able to collect.

Clearly, I believe that, despite these challenges, deep-sea and open-ocean species should be studied. I believe it is better to have some information, even if collected in a controlled laboratory environment, than to ignore a large number of species because studying them is difficult. Research should not be limited to a narrow selection of relatively easy to rear and ecologically similar species from a restricted range of taxa.

Why spend six years studying an obscure little octopus?

Given the numerous challenges outlined above you may wonder, why would anyone bother? What makes these octopuses such a special opportunity? What is it about them that makes it worth all the effort? Almost all cephalopod work has been concentrated on relatively easy to access, near-shore warm-water species (Forsythe and Van Heukelem 1987; Boyle 1991). This is especially true for studies of life processes like behavior, physiology and life history. For example, there are no growth data for any species of cephalopod found or raised below 10°C. Table 1 shows the genera in the family Octopodidae. *Octopus* is the most speciose genus and the only genus that is relatively well-studied. We have a good understanding of the life cycle of a number of its members. It is no coincidence that the best studied genus contains many common shallow-water species.

Coleoid cephalopods are thought to live fast and die young. But is this pattern applicable to all cephalopods in all environments? Little is known about deep-sea cephalopods. We can examine their stomach contents or review video tape of their behavior when confronted with a submarine. But what of life processes that occur over time? Do deep-sea cephalopods also live fast and die young? With no growth data for any species of cephalopod below 10°C, who can say if they grow slower or live longer, let alone whether this is due to a temperature effect or to adaptation to a different environment? How long do deep-sea cephalopods live? While the age of squid can be estimated using their statolith or gladus, we cannot estimate the age of octopuses. Are most deep-sea cephalopods, which presumably inhabit a more stable environment than their shallow-water cousins, iteroparous? Evidence of long life spans could challenge the "live fast and die young" paradigm or strengthen it if longer life spans of deep-sea cephalopods can be explained solely by variation in temperature. Evidence of octopuses

Table 1. Taxonomy in the family Octopodidae following Sweeney and Roper 1998.

There are a number of deep-sea genera that are poorly studied or not studied.

Which octopus species have been studied?

Family: Octopodidae (one of twelve families in **Order** Octopodida)
- **Subfamily:** Bathypolypodinae Robson, 1928
  - **Genera:** *☞*Bathypolypus* Grimpe, 1921 (only one life history paper!)
  - * *Benthoctopus* Grimpe, 1921
  - * *Grimpella* Robson, 1928 (1 specimen, likely deep-sea, not in Voss 1988)
  - * *Teretoctopus* Robson, 1929
- **Subfamily:** Eledoninae Grimpe, 1921
  - **Genera:** *☞ *Eledone* Leach, 1817
  - **Pareledone* Robson, 1932
  - **Tetracheledone* Voss, 1955
  - **Velodona* Chun, 1915
  - **Vosseledone* Palacio, 1978
- **Subfamily:** Graneledoninae Voss, 1988
  - **Genera:** **Bentheledone* Robson, 1932
  - **Graneledone* Joubin, 1918
  - **Thaumeledone* Robson, 1930
- **Subfamily:** Megaleledoninae Taki, 1961
  - **Genera:** * *Megaleledone* Taki, 1961 (found from120 m to 803 m, deep-sea, though not listed by Voss 1988)
- **Subfamily:** Octopodinae Grimpe, 1921
  - **Genera:** *Ameloctopus* Norman, 1992
  - *Aphrodoctopus* Roper/Mangold, 1992
  - *Cistopus* Gray, 1849
  - *Enteroctopus* Rochebrune/Mabille, 1889
  - *Euaxoctopus* Voss, 1971
  - ☞ *Hapalochlaena* Robson, 1929
  - ☛ *Octopus* Cuvier, 1797
  - *Pteroctopus* Fischer, 1882
  - *Robsonella* Adam, 1938
  - *Scaeurgus* Troschel, 1857

* = There is one or more deep-sea or intermediate depth species in this genus according to Voss (1988).
☛ = The natural history of several members of this genus is relatively well studied.
☞ = The natural history of at least one member of this genus has been studied.

laying eggs over a number of years would put further strain on the idea that all coleoid cephalopods exhibit monocyclic reproduction and semelparous life history.

The natural history of some members of deep-sea groups (*Eledone* and one member of *Bathypolypus)* has also been partially investigated. However, for most of the genera in the family Octopodidae, especially the deep-sea genera, we have no idea of basic life-history traits like life span and age at maturity, nor have we observed their behavior, physiology or other life processes. The little information that exists for deep-sea cephalopods is generally limited to fields such as taxonomy, distribution, diet and evidence of iteroparity. In other words, information that can be extrapolated from preserved specimens. Voss (1988a) lists 16 morphological traits that are found in deep-sea octopuses such as loss of ink sac, large eggs, and larger reproductive organs. Deep-sea octopuses are morphologically distinct, are thought to live longer than most cephalopods and eat foods not commonly consumed by their shallow-water relatives. Perhaps the life history and behavior of deep-sea octopuses is also different. Boyle (1991) states "we should recognize that the majority of cephalopods are offshore and deepwater animals. . . Probably their range of behavior patterns and levels of sensory discrimination and activity are quite different from their coastal and surface-dwelling relatives". Mangold (1987) has already proposed that *Bathypolypus* represents the extremity of K selection in the octopus family.

What other extremes could these deep-sea animals reveal? *B. arcticus* presents a unique opportunity to increase the scope of our knowledge of cephalopods because, unlike other deep-sea octopuses, they can now be collected and kept alive in the laboratory for extended periods of time. This allows direct study of traits such as hatchling growth rate, prey choice studies and a myriad of behaviors that are not quantifiable from preserved specimens, nor predicted from near-shore species. After all, where you live influences the way you live.

This thesis attempts to answer many of these proposed questions using *Bathypolypus arcticus* as a model for other deep-sea cephalopods. In Chapter 2, the effect of diet on growth rates of hatchling deep-sea octopuses is investigated and the highest growth rate is compared to that found in other species. While temperature can explain much of the variation, the growth rates of *B. arcticus* are still comparatively low when compared to shallow-water octopuses. Chapter 3 directly investigates the effect of temperature on growth rates of hatchling *B. arcticus*. Temperature has important effects on the growth rate of *B. arcticus*; higher temperatures give higher growth rates. Extremely low growth rates were found even at high temperatures. Using the most conservative assumptions, I used these data to predict that *B. arcticus* has a life span that is at least twice as long as previously predicted. This is significant as this makes *B. arcticus* the longest lived coleoid cephalopod yet studied. In Chapter 4, I investigate the end of *B. arcticus*' life cycle. Mating had never before been observed and the over 400-day brooding period is also described. Life-history traits such as fecundity and hatchling size are also mentioned to finish the life-cycle story. In Chapter 5 my work on *B. arcticus* is compared with studies of other cephalopods by investigating the question "Do larger cephalopods live longer?" Temperature and phylogeny have confounded previous investigations of this question. I overcame these confounds by identifying them, removing

Nautilus from the sample and using physiological time (degree–days). I then found strong evidence that larger cephalopods do take longer to reach maturity. Chapter 6 summarizes the results of this thesis and speculates on future direction and possible discoveries.

Chapter 2

First growth rates of deep-sea octopus *Bathypolypus arcticus* on five diets.

Abstract

The majority of data on cephalopods are based on a small, relatively easily collected subset of species that live in shallow water and/or near the shore. Little is known about the physiology, behavior and life history of deep-sea cephalopods. This study examines the effect of five different diets on the growth and survival of hatchling *Bathypolypus arcticus*, a deep-sea octopus. 153 hatchlings were randomly assigned to one of five feeding treatments: live *Corophium volutator*, live *Gammarus* spp., frozen *Gammarus* spp., frozen haddock (*Melanogrammus aeglefinus*) and brittle star arms (*Ophiopholis aculeata*) over a six-month period. Hatchlings were individually housed, maintained at 8.3 ± 1.0°C and weighed monthly. The octopuses in the two treatments fed live crustaceans experienced positive growth rates and many survived to the end of the experiment while the octopuses in the remaining treatments had zero or negative growth rates and low survival. Interestingly, octopuses did not do well on brittle stars, the most common prey found in the guts of wild specimens. Assuming growth rates of *B. arcticus* fed crustaceans are comparable to those in the wild, they are low compared to other species of cephalopods.

Introduction

Compared with fish, cephalopods are extremely productive in terms of turnover rates. The have short life spans, high growth rates and a tendency toward semelparous life cycles (O'Dor and Webber 1986; Forsythe and Van Heukelem 1987; Rodhouse 1998). Cephalopods are unusual for invertebrates in having large brains, well-developed senses, an active life style and a large behavioral repertoire. They can jet rapidly through their environment and frequently prey on, compete with and are predated by vertebrates such as fish. They are efficient predators with high food conversion rates (Mather 1993; Hanlon and Messenger 1996).

Cephalopods are found in all the world's oceans from the tropics to the abyss yet the majority of research on them is based on a narrow selection of near-shore and tropical species. Consequently, there is very little known about open-ocean and deep-sea cephalopods (Forsythe and Van Heukelem 1987; Boyle 1991). For example, the majority of the life-history, physiology and behavioral work on members of the order Octopodida has been done on members of the genus *Octopus*. While *Octopus* is a speciose genus, it may not be representative of the other 39 genera in the order (Sweeney and Roper 1998).

The limited research on deep-sea species has been largely confined to examining preserved samples or to behavioral analysis from encounters with submersibles (Wood et al. 1998). Information on life span, fecundity, development time, growth rates, predator-prey relationships, behavior and reproduction would all provide valuable insight into these creatures of the deep, allowing scientists to compare them with their more widely studied relatives. These data could be used to support or refute hypotheses that have been proposed for all species of cephalopods and widen the data base for comparisons to other taxa across a variety of environments. For example, Hanlon and Messenger (1996) put forward a behavioral hypothesis for cephalopods; a positive correlation between number of body pattern repertoires and habitat complexity. Unfortunately, there are no data for any of the three low-complexity habitats (midwater, open-ocean or deep-mud benthos). Basic natural history data are needed to substantiate or refute this and other hypotheses. While the age of squid can be determined from statolith and pen structure (Arkhipkin and Bizikov 1997; Jackson et al. 1997; Arkhipkin and Perez 1998), there is currently no reliable way to age octopuses. Laboratory rearing and tag-recapture studies are the only methods to acquire reliable growth rate data.

It is difficult to study wild cephalopods due to their excellent vision, mobility and generally nocturnal habits. This makes them hard to sample; they are likely aware of and influenced by observers and able to hide or jet away. Even the largest invertebrate on the planet, the giant squid (*Architeuthis* sp.), has managed to escape observation in its natural habitat. Therefore, scientists must depend largely on laboratory culture to provide life-history and behavioral information (Boletzky and Hanlon 1983).

Although there are limited data comparing field and laboratory growth rates of octopuses, existing data indicate that laboratory growth rates are similar to those in the field. Van Heukelem (1976), for example, found that the growth rates of branded *Octopus cyanea* in the wild were comparable to animals fed *ad libitum* in the laboratory.

No species of deep-sea cephalopod has ever been raised from hatching to maturity in the laboratory and there are no published growth data for any species of cephalopod at temperatures below 10 °C. When we talk about life history, behavior and other traits of cephalopods, we must realize that most of our knowledge is based on a very limited selection of shallow-water or near-shore species. Should we expect deep-sea species to be different?

Voss (1988a) lists loss of ink sac, loss or reduction of crop, reduction of radula, larger eggs, larger hectocotylus and eleven other specific adaptations for deep-sea life in octopuses. If deep-sea octopuses that live in cold, dark habitats are morphologically different and possibly behaviorally different, they may not fit the typical octopus model derived from studies of shallow-water *Octopus* species. While much data can be gleaned from preserved specimens, processes that occur over time, such as growth rates, behavior and reproductive costs, are best studied with live animals.

The species *Bathypolypus arcticus* has been found to a maximum depth of 1,543 m and is classified as a deep-sea octopus by Voss (1988a, 1988b). *B. arcticus* is a small (mature females weigh between nine and 300 g, averaging 70 g), benthic, short-armed octopus that typically resides at depths between 200-600 meters along the continental slope. Specimens have been collected from Greenland to Florida in the western Atlantic Ocean and along the northern reaches of the former Soviet Union to Spain in the northeast Atlantic Ocean. In Atlantic Canada, they are relatively easy to collect for a deep-sea octopus. *B. arcticus* is a part of the fauna associated with the commercially important scallop *Placopecten magellanicus* in the Bay of Fundy (Fuller et al. 1998). They live at 4 ± 2 °C and are thought to live for three years, an exceptionally long life span for an octopus (O'Dor and Macalaster 1983). This species is characterized by a large ligula (the reproductive arm tip of males), low fecundity, and large eggs laid during a 400+ day brooding period (Wood et al. 1998). Hatchlings weigh approximately 200 mg and are essentially miniature adults, having neither a planktonic stage nor an external yolk sac (O'Dor and Macalaster 1983; Wood et al. 1998). *B. arcticus* do not hide in lairs which makes them much easier to observe than most octopuses. Given these unique characteristics, *B. arcticus* is not only a model species for deep-sea research but has a high aquarium exhibit value as well.

Finding an appropriate food source is one of the most important steps to successful laboratory maintenance of any experimental animal and is one of the most significant factors affecting growth and survival of cephalopods in the laboratory (Forsythe and Van Heukelem 1987). Crustaceans, fish and other molluscs are the dominant components of most wild cephalopods' diets. Based on middens and observations of predation, *Octopus vulgaris* eats molluscs (bivalves, gastropods, chitons) and crabs in the wild (Mather 1991). *Octopus bimaculatus* eats crustaceans, molluscs and fish in both the laboratory and the field and prefers crabs to molluscs (Ambrose 1984). Villanueva (1993) presents field data showing that the bathybenthic octopus *Octopus magnificus* primarily eats decapod crabs with hermit crabs and fish also significantly contributing to their diet.

Ophiuroids, which are not a major component of the diet of near-shore octopuses, are eaten by a significant portion of adult *B. arcticus*; 58% of adult specimens were found to have brittle stars in their stomachs (O'Dor and Macalaster 1983). No other prey item was as common (Table 1). However, Wood et al. (1998) hypothesized that ophiuroids are only consumed when a more suitable food source is unavailable. Prior observations (Wood unpublished data) indicated that *B. arcticus* eat the arms of brittle stars but almost never consume the central disk, and that *B. arcticus* in captivity shift their diets away from brittle stars if crustaceans are present. Brittle stars are of low caloric value per gram and their gonads, which presumably have greater caloric value, are in their central disk (Packer et al. 1994).

Fish species have not been isolated as a major prey item for *B. arcticus*, although fish scales were found in one of the 450 octopus stomachs examined by Macalaster (1976). However, fish have been used to culture other octopus species and are a common component of many cephalopods' natural diets (Boletzky and Hanlon 1983). Crustaceans were the second most commonly found prey in *B. arcticus* stomachs and have been successfully used to rear a variety of hatchling shallow-water octopus species (DeRusha et al. 1989; Villanueva et al. 1996).

Successful cultures of hatchling cephalopods have relied on diets of live food, typically live crustaceans, and this has limited cephalopod aquaculture. No hatchling octopus has ever been successfully reared on a frozen diet (DeRusha et al. 1989), although no one has ever tried to rear hatchlings of any deep-sea cephalopod species. *B. arcticus* prey on a variety of invertebrates (Table 1) that are not consumed by shallow-water cephalopods, and it seemed possible that if *B. arcticus* are scavengers it might be able to utilize dead food better than shallow-water species. Additionally, frozen-food sources such as frozen amphipods and frozen fish are inexpensive and convenient foods, and using

Table 1. Prey species. This was determined from the % of *Bathypolypus arcticus* which contained that type of prey in their stomachs (Modified from O'Dor and Macalaster 1983). The total does not equal 100% because more than one type of prey may be found in an individual octopuses stomach.

Prey Type	Prey (%)
Cumaceans	4.9
Sipunculids	6.7
Foraminifera	7.1
Gastropods	12.1
Bivalves	21
Polychaetes	23.1
Crustaceans	25
Ophiuroids	58

both live and frozen foods of the same type allows the tradeoffs between them to be investigated. Success with frozen food would greatly simplify the rearing of *B. arcticus* hatchlings; otherwise tens of thousands of live amphipods collected in the summer and fall must be maintained live throughout the winter.

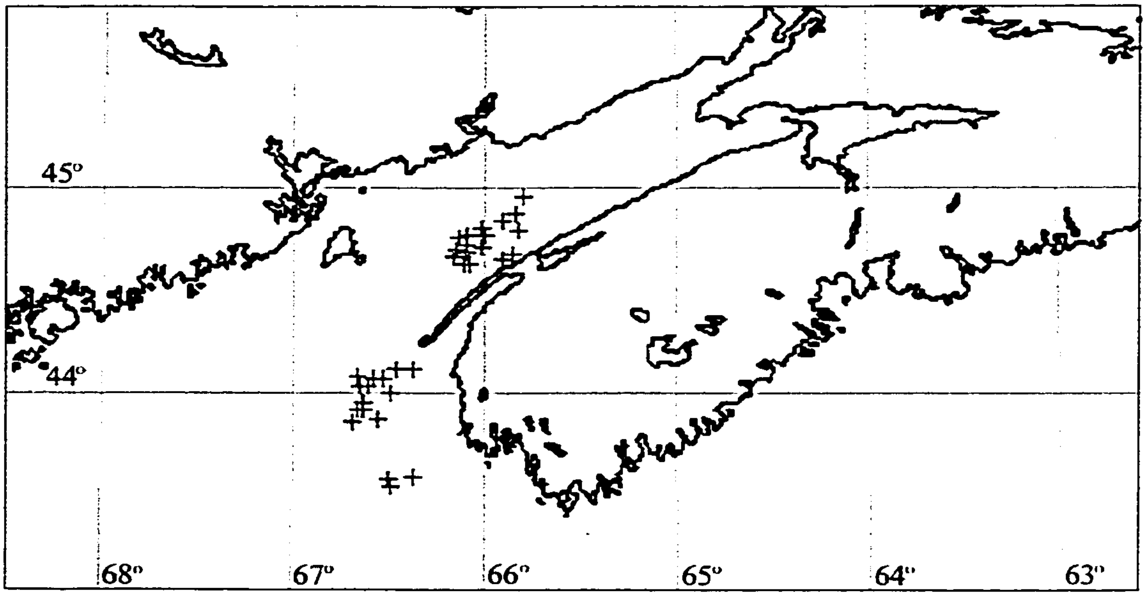
This paper examines the effects of five alternative diets on hatchling *Bathypolypus arcticus*.

Materials and methods

Adult octopuses were collected in the Bay of Fundy off of Digby, Nova Scotia, (circa 44.70 N and 65.90 W; 44.00 N and 66.50 W) in the summer of 1995 (Figure 1). They were collected in scallop trawls as by-catch during DFO scallop stock surveys; most were caught at depths of more than 100 m. Octopuses were housed in the Dalhousie University Aquatron Laboratory, and nine of the collected females laid and brooded eggs (Wood et al. 1998). The hatchlings used in this experiment emerged from late August to early September 1996 and came from seven of the nine broods. All octopuses were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

A total of 153 *B. arcticus* hatchlings were weighed and randomly assigned to one of the five treatments on the day they hatched. The five diets used in this experiment were (1) live *Corophium volutator*, (2) live *Gammarus* spp., (3) frozen *Gammarus* spp., (4) frozen haddock (*Melanogrammus aeglefinus*) and (5) bits of brittle star arms

Figure 1. Location of adult octopuses collected from the Bay of Fundy in the summer of 1995.



(*Ophiopholis aculeata*). I chose five diet treatments based on the natural diet of *B. arcticus,* prior success rearing near-shore octopuses in the laboratory and local availability of prey items. While the dietary requirements of *B. arcticus* are not known, we do know their natural diet is somewhat different from that of better-studied shallow-water octopuses.

Both amphipods and brittle stars were collected for use prior to and during the course of the experiment. The *C. volutator* were kept in a mud flat tank (2.13 m long, 0.61 m wide, 0.30 m deep) complete with tidal cycles. These amphipods were fed *Chaetoceros muelleri* and *Isochrysis* sp. (T-iso) several times a week, and reproduced under these conditions. *Gammarus* spp. were kept in a large fiberglass tank filled with damp eel grass (*Zostera* sp.) while brittle stars were kept in a flow-through aquarium. Frozen fish and frozen amphipods were kept in a freezer at -14°C. The size of the prey items was roughly equal to or less than the mantle length of a *B. arcticus* hatchling. i.e., amphipods used were approximately 8 mm or less at the start of the experiment. The species of brittle star used, *Ophiopholis aculeata,* is abundant in the area from which the octopuses were collected (Fuller et al. 1998).

The nutritional content of the four food types was analyzed by Doug Singer of the Canadian Institute of Fisheries Technology following the methods outlined in Woyewoda et al. (1986). Two replicates of each food type were analyzed and samples were carefully prepared to ensure that there were no contaminants. For example, hundreds of thousands of *C. volutator* were collected, washed and individually picked out of the sieve with tweezers and placed on a paper towel to absorb external water.

Carbohydrates were determined by the subtraction method. A value of zero was used for the carbohydrates for the haddock (fish) sample since the calculated value was −0.13%. Small negative values are likely caused by small amounts of variance in the measured values and a very low amount of carbohydrates in the sample and are commonly rounded to a zero value (Singer, pers. com.). The USDA Nutrient Database for Standard Reference (http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl) also used the subtraction method to determine the amount of carbohydrate in haddock and also reported a value of zero.

Total energy in kJ per gram of food was calculated using the standard values of 20.5 kJg^{-1}, 38kJg^{-1} and 17 kJg^{-1} for protein, lipids and carbohydrates, respectively (Withers 1992).

Additional octopuses hatched after the main experiment started. A polychaete treatment was not done as part of the main experiment as collecting enough worms was not feasible. However, three of the octopuses that hatched later were fed polychaetes *ad libitum* but the sample size wasn't large enough to draw any meaningful conclusion. In addition, another fifteen hatchling octopuses were used in a starvation treatment. These treatments will not be statistically compared with those in the main experiment due to time and size confounds (day old octopuses in the starvation treatment were slightly smaller 0.175 ± 0.029g). However, the starvation treatment gives a crude baseline comparison and examines the species ability to deal with food limited conditions.

Hatchling octopuses were individually housed in rearing chambers made from PVC pipe. The chambers were 10 cm in diameter and 20 cm high. The bottom of the chamber was covered with Nitex micron screening to allow old water to exit while the top remained open to permit access for feeding. Chambers were individually supplied with clean, chilled seawater at a rate of 3 $ml \cdot s^{-1}$ by a drip-feed line. See Wood and Wood (1998) for details on why this design of rearing chamber was used and how they were constructed.

The chambers were placed in three large flow-through tanks. Tanks A and C measured 70 x 132.5 cm, and tank B measured 59 x 210 cm (all measurements are of internal dimensions). Tank B housed approximately 50% of the test animals. The flow-through system was supplied with water from two header tanks, one containing warm water (average temperature of 10°C) and the other containing cold water - nominally at 2°C, but temperature fluctuated with user demand and the temperature of the incoming ambient water. A degassing tower was positioned on top of each header tank to release supersaturated gases and prevent gas bubbles from forming in or on the animals. Water temperatures were measured three times a week. Additionally, a minimum/maximum thermometer was used and the week's minimum and maximum temperatures recorded. Temperatures were adjusted manually to a nominal 8°C by mixing heated and chilled water. The hatchlings were maintained at an average water depth of 12 cm. The laboratory was continuously lit by an R-40 250 watt GE infrared light (peak wavelength 1200 nm). The room should appear dark to the octopuses as most cephalopods have a single visual pigment that is maximally sensitive to light in the 470-500 nm range (Hanlon and Messenger 1996). Indirect light from a single 25 watt light was used when necessary while feeding the octopuses or for routine maintenance.

The hatchlings were fed their respective diets three times per week thereby ensuring that the food supply was *ad libitum*. The frozen diets and brittle star arms were fed to the octopuses by hand. Before feeding, any remaining non-live food or debris from the previous feeding was removed. To feed octopuses brittle stars or frozen foods, I tilted the rearing chamber on its side and slid the food down to the hatchling. Often, bumping the food fragments into the animal stimulated a feeding response. Animals that did not eat the food on the first attempt were given at least 2 other tries before moving on to the next animal. Uneaten food from the current feeding was always left in the chamber for the animal to consume at a later time. Live food was added as need to ensure *ad libitum* feeding, no attempt to induce these octopuses to eat was made.

The wet weights of the hatchlings were measured to the nearest milligram approximately every 25 days for a six-month period, using a Mettler P163 scale. Attempts to remove excess water directly from the animal's skin can cause damage and mortality, so individuals were weighed in a modified weighing tray which had its bottom removed and replaced with a micron screen (Wood et al. 1998). This allowed excess water to be removed by blotting through the micron screen and without direct skin contact.

Instantaneous relative growth rates (G), as percent increase in body mass per day, were calculated for those animals that survived to the end of the experiment by using the following equation [1]:

[1] $G = ([\ln W_2 - \ln W_1] \times 100) \div (t_2 - t_1)$,

where W_1 and W_2 are the initial and final wet weights of the hatchlings in grams and t_1 and t_2 are the initial and final ages in days (DeRusha et al. 1989). The instantaneous relative growth rate was then adjusted for temperature by dividing by the average temperature. This effectively transforms time to degree-days. Since ectotherms require a certain amount of time *and* temperature for growth (Begon et al. 1990), using degree-days to measure time allows comparison with other octopus species reared at higher temperatures (Wood and O'Dor 2000).

The survivorship (number of hatchlings alive) in each treatment was recorded at least three times a week. For six of the hand feedings in October 1996, I also recorded which animals accepted food and which did not. I counted the number of times food was accepted for each octopus and then calculated the percentage of food acceptance for each of the octopuses in the three hand-fed treatments.

During the last weighing period, from January 8 to May 2, 1997, food intake was monitored in addition to growth. To determine food intake, the wet weights of the food portions were recorded. Debris was siphoned out at the midpoint of this interval and again at the end of the experiment. Uneaten food portions were separated and weighed and then subtracted from the weight of the food offered to estimate the amount of food ingested. These feeding intake data were used to calculate gross growth efficiency (GGE) and feeding rate (FE). Gross growth efficiency was determined using equation [2]:

$$[2]\quad GGE = ([W_2 - W_1] \times 100) \div TFI,$$

where TFI is the total food ingested. Following DeRusha et al. (1987), only the octopuses that grew in the last period were used in the GGE calculation [2].

Feeding rates (FR), as a percentage of the body wet weights per day, were also calculated for this period using the equation [3]:

[3] $FR = (TFI \times 100) \div (MOW \times \text{no. days})$,

where TFI is the total amount of food ingested during the 28-day period (DeRusha et al. 1989) and MOW is the mean weight of octopuses in the treatment. All animals that were alive at the end of this period were used in the feeding rate calculation. The chambers containing octopuses fed live *Gammarus* amphipods (n=3) were covered with a clear acrylic lid to prevent the crustaceans from escaping for this part of the experiment.

Results

Hatchlings were maintained at an average temperature of 8.3 ± 1.0°C. All three of the tanks experienced some short-term temperature fluctuations. Over the 6-month study, the lowest minimum temperature was 2.5°C and the highest maximum was 10.5°C.

Day old hatchling octopuses (n=153) weighed 0.199 ± 0.025 g. Hatchlings were frequently observed clinging to the side of their chamber. They appeared to prefer sites just below the water surface but were observed at all depths. The hatchlings were relatively inactive but occasionally swam if disturbed.

Feeding behavior observed for hatchlings was very distinctive. After grasping the food item with their arms, the food was pulled under the web. Unsuitable or unwanted food was dropped or rejected by a blast of water from the siphon. Accepted food was ingested.

The hatchlings were observed ingesting food in all five of the treatments. Rejection of food items by hatchlings was also observed in the non-live treatments. Of the non-live treatments, brittle star arms and frozen haddock pieces were accepted less often, 20.1% and 16.7%, respectively, than frozen amphipods which were accepted 59.4% the time.

Capture of live food items was occasionally observed but rejection of live prey was not. However, half-eaten portions of *C. volutator* were occasionally removed. The animals in the live treatments did not often consume food when I was watching them. Consumption was confirmed by the decrease in prey items and from the pieces of partially eaten prey remaining in the chambers.

The octopuses in the *C. volutator* treatment (n=38) had the highest growth rate with the average final weight at day 200 of 0.76 g (Figure 2) compared to the final weight of 0.38 g for the octopuses in the live *Gammarus* spp. treatment (n=23). The octopuses in the three non-live food treatments, frozen *Gammarus* spp. amphipods (n=34), frozen haddock (n=29) and brittle star arms (n=29), had negative growth rates. All of the hatchlings in the non-live food treatments died before the end of the experiment. Data for individual octopuses is documented in Appendix 4.

The instantaneous relative growth rates of survivors were determined. Those in

Figure 2. The changes in wet weight of hatchling octopuses over time. The fitted exponential growth equations (where y is octopus weight in grams and t is time in days) are: live *C. volutator*: $y=0.199e^{0.0067t}$ ($r^2=0.75$, $p<0.0001$), live *Gammarus* spp.: $y=0.207e^{0.0030t}$ ($r^2=0.34$, $p<0.0001$), frozen *Gammarus* spp. $y=0.194e^{-0.0019t}$ ($r^2=0.00$, $p=0.69$), *Ophiopholis* sp.: $y=0.197e^{-0.0024t}$ ($r^2=0.11$, $p=0.004$), frozen haddock: $y=0.202e^{-0.0025t}$ ($r^2=0.15$, $p=0.001$). Octopuses only gained weight when fed live food.

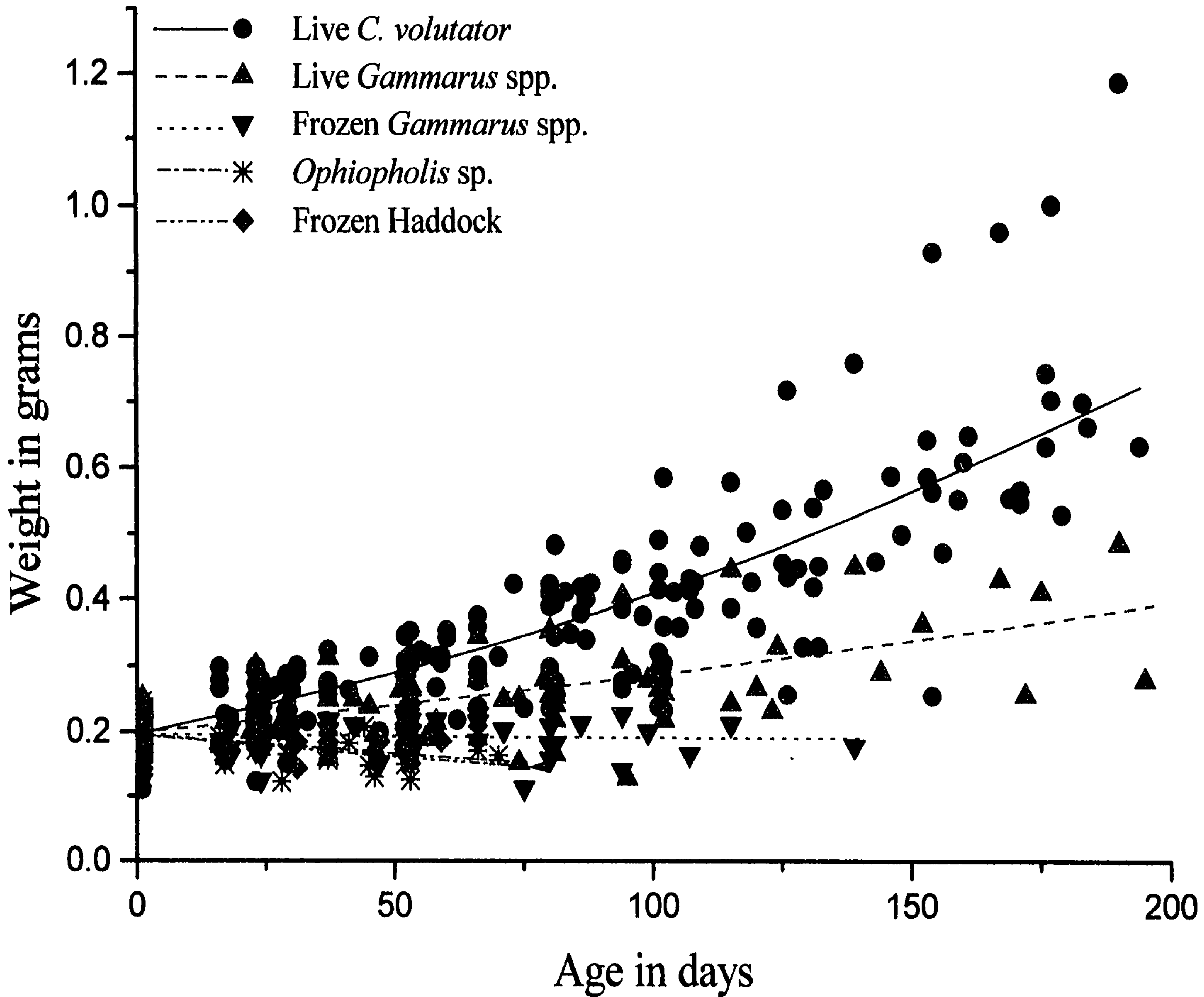

Live *C. volutator*
Live *Gammarus* spp.
Frozen *Gammarus* spp.
Ophiopholis sp.
Frozen Haddock
Weight in grams
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Age in days
0
50
100
150
200

Figure 2

the *C. volutator* treatment (n=13) had an overall daily growth rate of 0.70 %/d, while those in the live *Gammarus* spp. treatment (n=3) had a rate of 0.33 %/d. The temperature adjusted instantaneous relative growth rate (mean growth rate divided by mean temperature to provide a degree-day comparison) was 0.081 %/d for the octopuses in the *C. volutator* treatment and 0.039 % for the octopuses in the live gammarid amphipod treatment. Compared to other octopuses (Table 2), even these temperature-adjusted growth rates are low.

The percent survival in the five treatments over the duration of the experiment is presented in Figure 3. The first treatment with 0% survival was the *O. aculeata* treatment at 74 days with the frozen haddock treatment closely following at 75 days. The longest lived octopus in the starvation treatment died on day 68 and the median life span of these octopuses was 57 days. Octopuses in the frozen *Gammarus* spp. treatment reached 0% survival at 144 days. At the end of the 6-month experiment, the octopuses in the live *Gammarus* spp. treatment had a 12.5 % survival rate while those in the *C. volutator* treatment had a 33.3% survival rate.

Food intake data were recorded between the last two weighing periods so that GGE and FE could be calculated. The daily feeding rate of hatchlings in the *C. volutator* treatment was 3.24 ± 0.93 %, and those in the live *Gammarus* spp. treatment had a daily feeding rate of 2.02 ± 0.34 %. The gross growth efficiency for the hatchlings with positive growth rates in this period in the *C. volutator* treatment (n=12) was 20.1 ± 9.9 %/d. The GGE for the single octopus with positive growth in the live *Gammarus* spp. amphipod treatment was 14%.

Table 2. Comparison of the instantaneous relative growth rates (G) and physiological instantaneous growth rate for some cephalopods.

Species	**G**	**Temperature**	**Physiological G**	**Source**
Bathypolypus arcticus	0.67%/d	8.3°C	0.08	This Study
Octopus bimaculoides	2.2%/d	18°C	0.12	Hanlon and Forsythe 1985
O. "joubini"	2.5%/d	25°C	0.10	Hanlon and Forsythe 1985
O. briareus	2.7%/d	25°C	0.11	Hanlon and Forsythe 1985
O. maya	4.1%/d	25°C	0.16	Hanlon and Forsythe 1985
O. digueti	4.7%/d	25°C	0.19	DeRusha et al. 1987

Figure 3. Percent survival for octopuses in the five treatments. Survival of octopuses was highest in the two live food treatments.

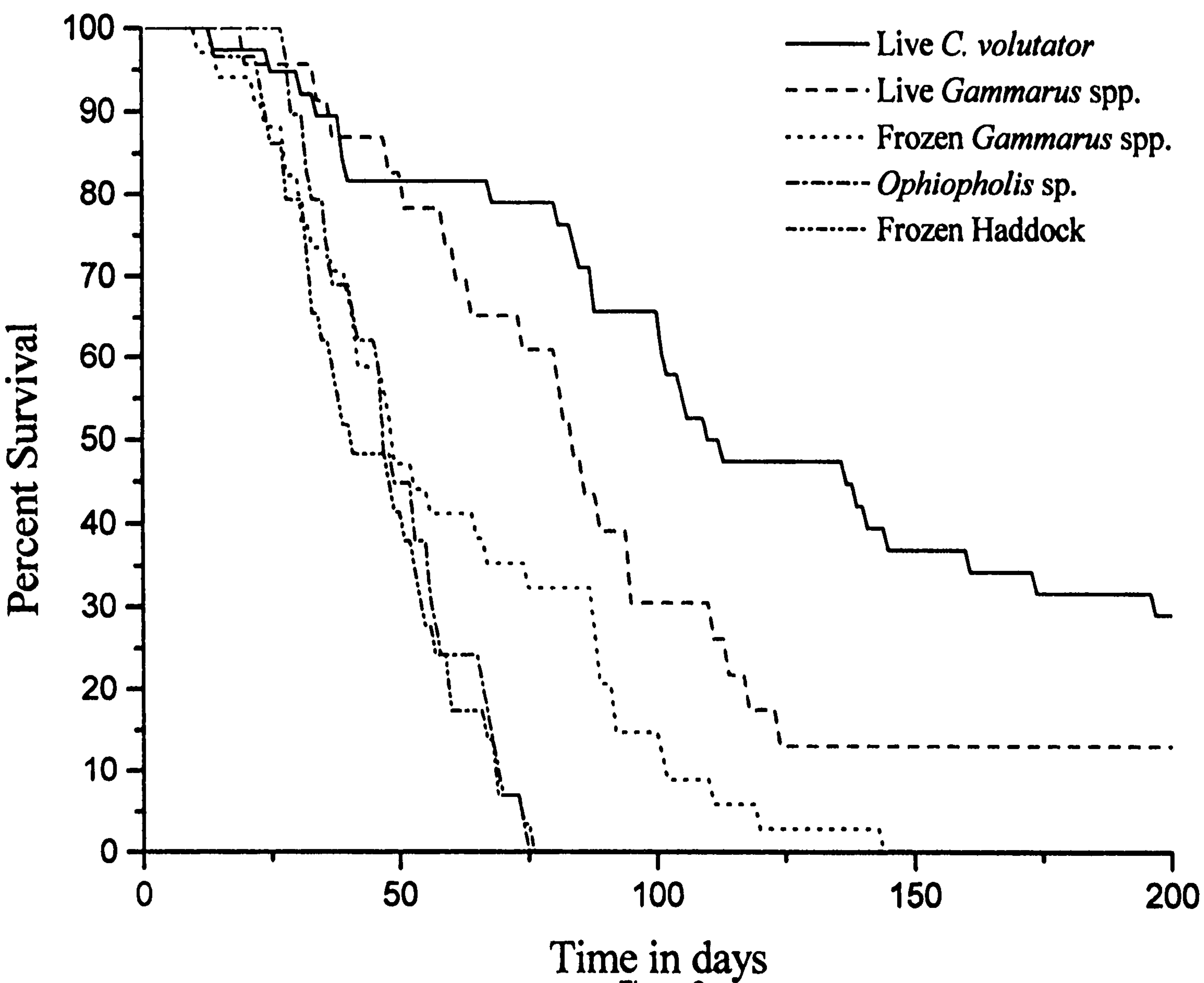

100
90
80
70
60
50
40
30
20
10
0
Percent Survival
0
50
100
150
200
Time in days
Live *C. volutator*
Live *Gammarus* spp.
Frozen *Gammarus* spp.
Ophiopholis sp.
Frozen Haddock

Figure 3

The proximate analysis results and energy per gram of food ingested is presented in Table 3. *Gammarus* amphipods had almost twice the energy per gram of any of the other foods while brittle star arms contained the least amount of energy. Both the *Gammarus* sp. and frozen-fish treatment had high amounts of protein compared with the other treatments.

Discussion

The octopuses in the two live food treatments had positive growth rates as well as hatchlings that survived to the end of the study. I conclude that live crustacean diets promote survival and growth of the deep-sea octopus *Bathypolypus arcticus*. This is consistent with what is known from feeding experiments on shallow-water cephalopods. Cephalopods studied to date display a clear preference for living prey over frozen, pelleted and dead food sources (Boletzky and Hanlon 1983; Lee et al. 1991). This requirement for live prey has limited cephalopod rearing and no diet of non-live food has been found to promote adequate growth and survival in hatchling octopuses (DeRusha et al. 1989). Although the natural diet of *B. arcticus* (Table 1) is different from the near-shore species studied to date, the constraint that hatchlings require live food is still true.

It is not understood why hatchling octopuses do well on live foods and not on dead or frozen ones. Both the frozen *Gammarus* and frozen-fish treatments contained a high percentage of protein which cephalopods are especially efficient at metabolizing (O'Dor and Wells 1987) and which have a relatively high amount of energy per gram.

Table 3. Nutritional content and energy per gram for the four food items used.

Sample	**%Protein**	**%Lipids**	**%Moisture**	**%Ash**	**%Carbohydrate**	**Energy (kJ/g)**
Corophium sp.	9.62	1.09	80.78	6.77	1.74	2.68
Gammarus spp.	19.45	5.44	65.65	7.67	1.79	6.36
Ophiopholis sp.	6.09	0.59	43.97	46.14	3.21	2.02
Fish	17.00	0.64	81.18	1.31	0	3.73

Hatchlings exhibited a specific behavior while ingesting food which made it easy to tell when they were feeding. They would elevate themselves on the tips of their arms with their bodies extended away from the surface of attachment (Macalaster 1976). If *B. arcticus* are behaving like other octopuses, during this posture they are releasing salivary enzymes and toxins into an external pouch created by the web and partial external digestion aids the suckers in dissection of large prey. In *Eledone* and *Octopus*, these enzymes paralyze the prey and destroy musculo-skeletal attachments (Hanlon and Messenger 1996).

Frozen fish was not often accepted but the frozen *Gammarus* was readily accepted and ingested. DeRusha et al. (1989) found that frozen foods were accepted readily by all the shallow-water octopus species they tested, although only octopuses older than 10 weeks survived. At least with frozen crustacean foods, the problem is not getting the animals to feed.

It has been suggested that non-live foods are nutritionally deficient or imbalanced (DeRusha et al. 1989; Lee et al. 1991). The use of such foods may be suitable if supplemented by live prey items. In this study, the variation between the success of the octopuses in the live and frozen amphipod treatments provides further evidence that the freezing process affects the food items to make them less palatable or nutritous to the hatchlings. We currently do not know why hatchling octopuses do not thrive on frozen foods. DeRusha et al. (1989) found that bacterial enzymatic activity caused a continual protein breakdown and ammonia build-up in frozen shrimp which decreased its nutritional value. Freezing can cause lipids to oxidize and proteolytic enzymes may cause texture changes when the foods freeze and thaw. Perhaps ice crystals cause rips in cell membranes which allow essential water soluble vitamins to diffuse out before ingestion.

One reason for the discrepancy between the growth and survival rates of hatchlings in the live and frozen food treatments are differences in the octopuses' ability to utilize the nutritional content of the two food types. An alternative hypothesis is that the different methods used to deliver live and non-live food were responsible for the different growth rates. A separate experiment to compare live and freshly dead crustaceans was attempted but ended prematurely during a systems failure.

However, I believe that nutritional differences, and not feeding method, were responsible for the success of octopuses in the live amphipod treatment over octopuses in the frozen one. This hypothesis is supported by the observation that octopuses readily accepted and ingested frozen amphipods but failed to grow on them. Clearly, more work needs to be done in this area.

Further work on artificial diets for cephalopods should consider using a crustacean base. This study found that crustaceans were accepted more frequently than fish and *O. aculeata* arms. DeRusha et al. (1989) found that frozen shrimp were readily accepted by *Octopus maya*, *Octopus bimaculoides*, *Sepia officinalis* and *Lolliguncula brevis*. They suggested that shrimp would be a good food to analyze for feeding attractants. In their investigation of pelleted diets, Lee et al. (1991) stated that chemical attractants may cause food to be accepted but that feeding suppressants in prepared food may also be present to cause the rejection of food.

Cephalopods, in general, have poor assimilation efficiency of lipids but are efficient at metabolizing protein and carbohydrate (O'Dor and Wells 1987). *C. volutator* is a major food source of migrating shorebirds. *C. volutator* has a relatively low total lipid content of 1.74% ± 0.30% lipids in summer/fall and 1.3% to 1.7% lipids in winter (Napolitano and Ackman 1989), although studies of other gammarid amphipods (Torres et al. 1994) have shown a higher lipid content relative to protein content. I found a lower lipid content in my *C. volutator* samples (average = 1.09%). These differences may be due to population nutritional differences, reproductive timing differences (eggs are likely high in lipids) or to the fact that the samples, like the food actually used to feed the octopuses, were maintained in the laboratory prior to analysis. The *Gammarus* spp. amphipods had more than twice the protein and overall energy content than the most successful food, *C. volutator*, but they also had high lipids which could effect digestion (Table 3).

Although differences in nutrient content could contribute to higher growth and survival of hatchlings in the *C. volutator* treatment, I believe that the behavior of the amphipods is the primary cause. *C. volutator* is a burrowing amphipod that moves relatively slowly. This species is thought to be relatively passive as it is a selective deposit feeder (Murdoch et al. 1986) and it is likely easier for *B. arcticus* to catch and consume than *Gammarus* spp. which swim relatively quickly and may be difficult to catch. In addition, hatchlings may experience added stress if physically harmed by *Gammarus* amphipods. Hanlon and Forsythe (1985) found that hatchling octopuses could attack and capture prey from 1/3 to two times their mantle length. However, the deep-sea octopus *B. arcticus* does not seem able to capture and hold onto prey much larger than their mantle length.

The octopuses in the frozen *Gammarus* spp. treatment survived twice as long as those in the other two non-live treatments, suggesting that this diet is of some benefit to the hatchlings. Amphipods are a crustacean food source and, as a result, are likely to be more suitable than either the frozen haddock or the brittle star arms.

The fact that the brittle star arms were accepted but the octopuses in the treatment did not do well supports the hypothesis that *B. arcticus* only eats brittle stars as a last resort. Brittle stars are a common benthic group with densities of 240-970 m^2 at depths of 150-275 m in the Gulf of Maine (Packer et al. 1994). Hatchlings may not be able to assimilate brittle star arms as efficiently as adults. For example, adult *Octopus maya* are able to assimilate frozen shrimp and they grow well on them while hatchlings do not (DeRusha et al. 1989). Hatchling cephalopods will often accept a variety of foods but they only grow when fed live food (DeRusha et al. 1989).

Using a combination of field and laboratory work, Ambrose (1984) found that the shallow-water octopus, *Octopus bimaculatus,* consumes more than 55 prey species from three phyla but strongly prefers crustaceans to all other prey. However, crustaceans only made up 5% of the diet of these wild octopuses. Ambrose concluded that *O. bimaculatus* diets reflect a compromise between prey preference and prey availability. Similarly, *B. arcticus* may not prefer low-energy brittle stars but may eat them because they are frequently encountered and easy to catch.

The animals were fed species-appropriate foods based on diets in the wild and what has worked for other species of octopuses. They were kept at appropriate temperatures in low light conditions. There is no evidence that octopuses in the wild are more successful than those fed live crustaceans *ad libitum* live. Although low compared to other octopuses, laboratory growth rates of octopuses fed live crustacean diets may be at least as fast as the growth rates of octopuses in the field given the frequency of low energy brittle stars in the diet of wild *B. arcticus*. If the fastest growth rates found in this experiment are similar to the growth rates of wild octopuses, *B. arcticus*, a species already considered to have an extremely long life-span for a coleoid cephalopod, take much longer to reach maturity than the three-year life span proposed previously by O'Dor and Macalaster (1983). Mortality levels are also reasonable given the long time span of the experiment.

Temperature plays a large role in growth, temperate cephalopods growing more slowly than the subtropical and tropical species (Jackson and Choat 1992; Wood and O'Dor 2000). Forsythe and Hanlon (1988) demonstrated that a temperature increase of 5 °C accelerated reproduction and decreased life span by 20% in *Octopus bimaculoides*. Forsythe (1993) developed a model based on laboratory growth rates of *Loligo forbesi* at various temperatures. This model demonstrated that even a 1 °C increase in temperature during the 90-day exponential growth phase causes a doubling of weight such that a 2 °C increase in temperature is predicted to result in animals that would grow five times larger! DeRusha et al. (1987) demonstrated that decreasing temperature by 4 °C extended the life span of *O. digueti* and *O. joubini* by 3-4 months. Wood and O'Dor (2000) found that temperature is a very important factor for interspecific comparison of size at maturity and life span in cephalopods.

Table 2 compares the octopuses in the *C. volutator* treatment, the fastest growing treatment in this experiment, with the growth curves from other species of octopuses. The growth rates of octopuses in the *C. volutator* treatment are low compared to species of warm-water near-shore octopuses. The hatchlings in the *C. volutator* treatment had a final wet weight of 0.76 g at 200 days while the lowest wet weight at 200 days for the other species cited is approximately 15 grams.

Many near-shore cephalopod species have been successfully reared from hatchlings to adults in the laboratory and their growth rates have been determined. To facilitate comparisons between octopus species of varying sizes, the instantaneous relative growth rate is often used (Forsythe 1984). Reported growth rates of cephalopods range from 2.2 to 4.7 % d^{-1} at varying temperatures (Table 2). These rates are much higher than the growth rate of the *B. arcticus* in the best treatment. To facilitate comparisons with *B. arcticus*, I use physiological growth rate which controls for temperature by using degree-days as the time variable in the instantaneous relative growth equation (Wood and O'Dor 2000). Although hatchling octopuses typically grow faster than older octopuses (Forsythe and Van Heukelem 1987), the growth rate of hatchling *B. arcticus* in the most successful treatment is lower than lifetime growth rates of the other octopuses.

The gross growth efficiency of *B. arcticus* was only 20.9 ± 9.9% (Table 4) which is lower than those reported in studies of other cephalopods. Cephalopods typically have GGE values between 40% and 60% (O'Dor and Wells 1987). *O. digueti* was reported to have a GGE of 48% (DeRusha et al. 1987) while both *O. joubini* and *O. maya* had values of 40% (Table 4) (Van Heukelem 1976; Forsythe 1984). The low GGE of *B. arcticus*

Table 4. Comparison of the Gross Growth Efficiency (GGE) and food intake for several species of octopus.

Species	**GGE (%)**	**Feeding Rate (%)**	**Source**
Bathypolypus arcticus	20.9 ± 9.9	3.24 ± 0.93	This Study
Octopus digueti	48	4.7	DeRusha et al. 1987
O. "joubini"	39.7	N/A	Forsythe 1984
O. maya	40	9.2	Van Heukelem 1976

suggests that metabolic efficiency is lower, one reason they grow slower.

Feeding rate and GGE calculations depend on the amount of food actually ingested. I observed only a few exoskeleton remains when only part of the prey was eaten. I believe that the octopuses typically eat all of the prey but perhaps the exoskeleton plates are simply too small to be easily detected. If remains of prey were not noticed due to their small size the actual feeding rates would be even lower and GGE higher. Feeding rates, as a percent of body wet weight per day, for the hatchlings in the *C. volutator* treatment was 3.24 %. The hatchlings in the live gammarid amphipod treatment had a feeding rate of 2.02 %. These intake values are also lower than those reported for other cephalopods. DeRusha et al. (1987) reports a value of 4.7 % body weight per day for *O. digueti* while the feeding rate of *O. maya* hatchlings was 9.2 % (DeRusha et al. 1989). However, these feeding rate values for *O. digueti* and *O. maya* were obtained at temperatures of 22- 24 °C.

Warm water octopuses do not survive long without food (Wood pers. ob.) but hatching *B. arcticus* lived up to 68 days (median 57 d) without food. *B. arcticus* appear to be adapted to an environment where food is often limiting and predation pressure is relaxed.

B. arcticus, being a deep-sea octopus, has lower feeding rates than near shore species. These octopuses are much less active than their tropical counterparts (Wood pers. ob.). Low feeding rate, low GGE and a natural diet consisting of low energetic yield prey are consistent with the low growth rates found for *B. arcticus* and a shift away from the live fast and die young strategy of traditionally studied near shore cephalopods.

There are challenges for studying deep-sea cephalopods in addition to finding appropriate diets. Some of them are maintaining cold water over an extended period of time and collecting and transporting specimens. The effect of pressure on the growth and survival of deep-sea cephalopods has yet to be tested. Hopefully, as the husbandry requirements of deep-sea cephalopods become understood, we will be able to perform more controlled experiments and start filling the many gaps in our knowledge.

The deep-sea octopus *Bathypolypus arcticus* can be successfully studied in the laboratory. Females often lay eggs and, after the 400+ day brooding period, the hatchlings can be raised in the laboratory. Octopuses will grow and survive if fed live crustacean prey. Of the two live diets, *C. volutator* promoted the best growth and survival rates. Results indicate that non-live diets are not suitable for long term rearing of hatchling *B. arcticus*. However, frozen crustacean diets, such as frozen *Gammarus* spp., may be suitable for short-term emergency rations.

There is much that is unknown about the life history and behavior of open-ocean and deep-sea cephalopods. Based on what we now know about *B. arcticus*, we can expect that, in addition to morphological differences (Voss 1988a) and behavioral differences (Hanlon and Messenger 1996), deep-sea cephalopods are likely to have life-history and physiological differences such as lower growth and assimilation rates and a prolonged life span even when temperature is controlled. More research is need to understand the physiological, life-history and behavioral differences between shallow-water and deep-sea animals.

Chapter 3

Rearing deep-sea octopus (*Bathypolypus arcticus*) at temperatures from 2 - 10°C.

Abstract

Thirty-six, 18 to 70 day old *Bathypolypus arcticus* were randomly assigned to 2°C, 6°C and 10°C temperature treatments and were reared for 170 days. Octopuses in the warmer treatments grew larger than those in cooler treatments. Those in the 10°C treatment weighed an average of 1.481 g at the end of the experiment while those in the 2°C treatment only weighed 0.415 g. *B. arcticus* grow more slowly than warm-water octopuses and only part of their low growth rate appears to be due to the low temperatures at which they live. The prior estimate of a three-year life span for *B. arcticus* is reviewed and is considered an underestimate. I suggest that these octopuses take at least 6 years to reach maturity in the wild which is considerably longer than the typical one year life span of near-shore octopuses. Being the first long-term experiment on hatchling deep-sea cephalopods, I also discuss some problems and solutions particular to the rearing of deep-sea cephalopods.

Introduction

Cephalopods have been characterized as having higher growth rates and shorter life spans than other molluscs (Rodhouse 1998). Various laboratory experiments and field observations have shown that most species of coleoid (squid, octopuses, cuttlefish) cephalopods typically live from one-half to two years (Jackson et al. 1997; Wood and O'Dor 2000). Knowledge of cephalopod growth comes exclusively from shallow- water and frequently near-shore species. We know almost nothing about the growth of deep-sea cephalopods although they constitute a large number of cephalopod species (Forsythe and Van Heukelem 1987) and live in the largest habitat on earth (Merret and Haedrich 1997). It has not been possible to assess whether deep-sea cephalopods conform to the cephalopod paradigm of rapid growth rates and short life-spans because of this lack of data (Daly et al. 1998). This report investigates this problem with the first detailed study of Canada's most common Atlantic octopus, *Bathypolypus arcticus*, a co-faunal species in scallop beds (Fuller et al. 1998), distributed off the continental shelf to depths over 1,500 m (Voss 1988a).

Laboratory studies have shown that temperature has a strong effect on the growth rates, life span, and the size at maturity of many cephalopods (Forsythe and Van Heukelem 1987; Forsythe and Hanlon 1988). For example, Forsythe and Hanlon (1988) found that a temperature increase of 5 °C accelerated growth rates, reduced time to maturity and decreased life span by approximately 20% in *Octopus bimaculoides*, a common octopus in shallow water off California. Wood and O'Dor (2000) concluded that temperature is also an important factor for interspecific comparisons.

Furthermore, Forsythe and Hanlon (1988) suggested that a change in temperature is likely to have a greater effect on demographics of short-lived semelparous cephalopods than longer-lived iteroparous fish. To clarify this point, suppose there are unfavorable environmental conditions one year for offspring. The offspring of iteroparous fish will experience extremely high mortality that year, but most of the adults will survive to reproduce in the following years. In short lived semelparous cephalopods however, a single bad year will severely depress the population. Populations of cephalopods that live for several years would be expected to have a fraction of its members coming into maturity each year. Such a population would be expected to be effected less by environmental variability in temperature and other parameters.

Bathypolypus arcticus is a small deep-sea octopus with large eggs (circa 0.25g) and benthic hatchlings. O'Dor and Macalaster (1983) estimated a life span of at least three years for this octopus based on catch weights. Based on this three-year estimate, *B. arcticus* is often used as an example of a long-lived coleoid cephalopod (Mangold 1987). *B. arcticus* is most common at depths of 200-600 m and at temperatures of 4 ± 2°C in the Atlantic Ocean. O'Dor and Macalaster (1983) reported that brief exposure to a temperature of 10-12°C was fatal to these octopuses although Wood et al. (1998) were able to successfully acclimate them to 11°C.

While there are techniques to age squid using their statoliths and pens (Arkhipkin and Bizikov 1997; Jackson et al. 1997; Arkhipkin and Perez 1998), a reliable method to age octopuses has not yet been developed. Sibling cephalopods raised under the same conditions can be extremely variable in size (Forsythe and Van Heukelem 1987). Growth rates are even more variable between unrelated individuals and those living in different environments. For these reasons, aging individual octopuses based on size is not considered valid and laboratory studies or tag-and-release methods must be used.

The primary objective of this study was to investigate the effect of temperature on the growth rate of a juvenile cold-water, deep-sea octopus such as *B. arcticus*. An essential first step was to establish successful methods for rearing such octopuses in the laboratory. Developing techniques to rear this and other deep-sea cephalopods in the laboratory can provide basic information such as life span, growth rates, and reproductive behavior and pattern needed to understand both the physiology of cephalopods and their role in the ecosystem.

From an ecological perspective, one critical question is, "How long does *B. arcticus* live in the wild?" I first make predictions about *B. arcticus* life span by assuming it continues to grow exponentially to maturity at the rates I found in this experiment. Next, I looked at octopuses of known life spans and growth rates and determined how much an assumption of uniform exponential growth underestimated their life span. This was used as a guide to interpret the *B. arcticus* data.

Materials and methods

Rearing deep-sea cephalopods

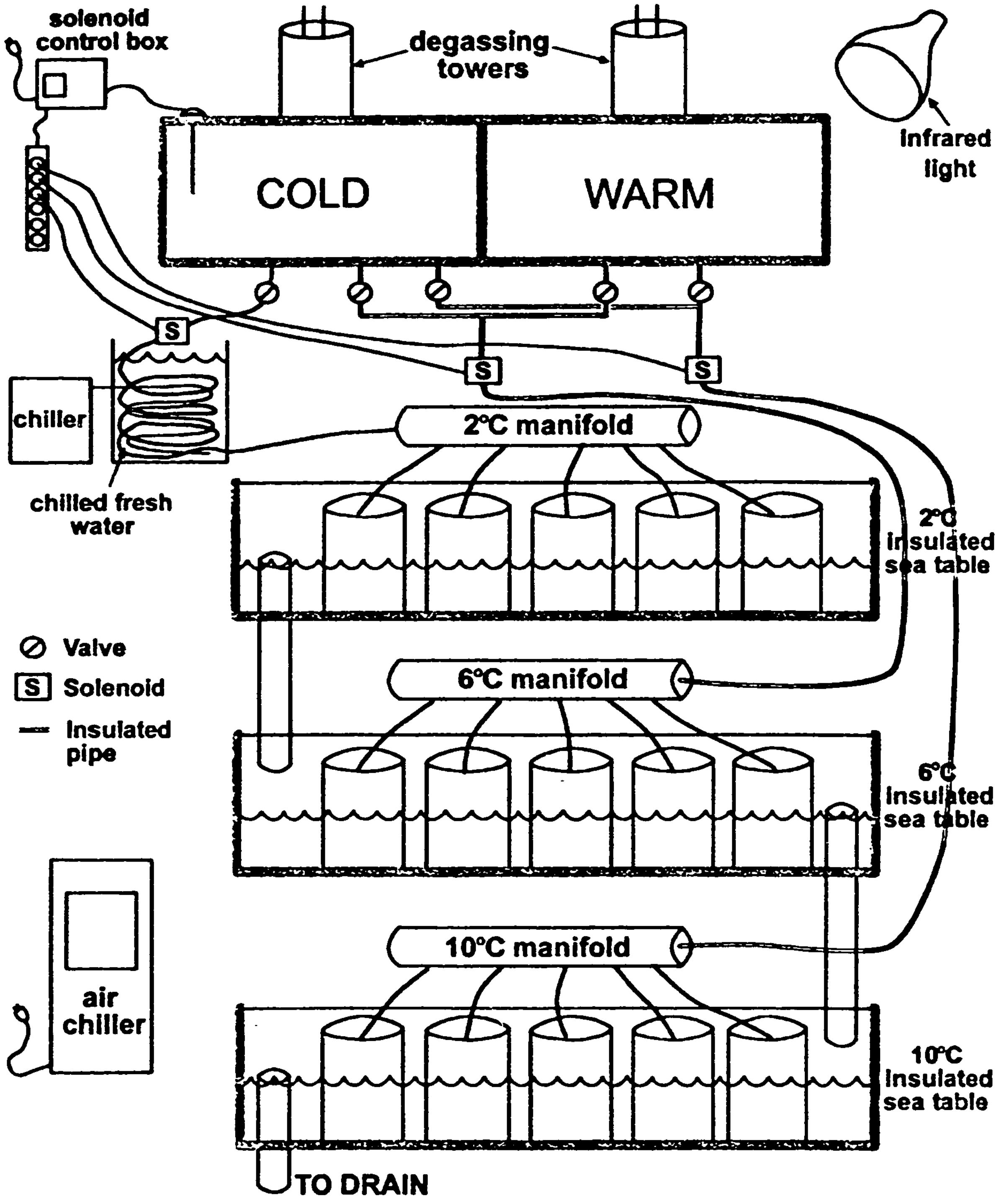
Fresh chilled or ambient (depending on season, 2-7°C) and warm (13-17°C) seawater was provided by the Aquatron system at Dalhousie University. Chilled, ambient, and warmed incoming water passed through a degassing tower before flowing into their respective header tanks (Figure 1). 6°C and 10°C temperatures were achieved by mixing water from the cold and warm header tanks. After temperatures had been measured, fine adjustments were done manually by altering the amount of water coming from each header tank. In theory, modern technology can maintain water temperature within ±0.1°C accuracy. In practice, theory breaks down several times per year.

An additional chiller was needed to keep water at 2°C for the coldest treatment. The chiller's copper cooling coils were painted with plastic enamel and placed in freshwater in an insulated tank. Incoming seawater passed through a large coil of flexible tubing that was immersed in the insulated tank. Although energetically inefficient, this system eliminated any chance that copper, which is deadly to cephalopods, would leach into the seawater. The room was illuminated with a 250 watt infrared light.

A temperature probe connected to solenoid valves controlled the flow of water leaving the header tanks (Figure 1). If the incoming water warmed, such as during or immediately after a power outage, chiller breakdown, or other joyful surprises, this safety feature would cause the solenoids to stop the flow of water to the well-insulated rearing chambers. When the temperature returned to normal, the valves reopened returning water flow to the rearing chambers.

The octopus rearing chambers and natural tanks were placed in three vertically stacked insulated sea tables. The coldest treatment was on top. The waste water from the rearing chambers in the cold treatment went into the sea table and then flowed down to

Figure 1. Simplified diagram of the flow-through system and room set-up used in this experiment. Ambient water was used instead of chilled water in the winter when the ambient temperature was low.

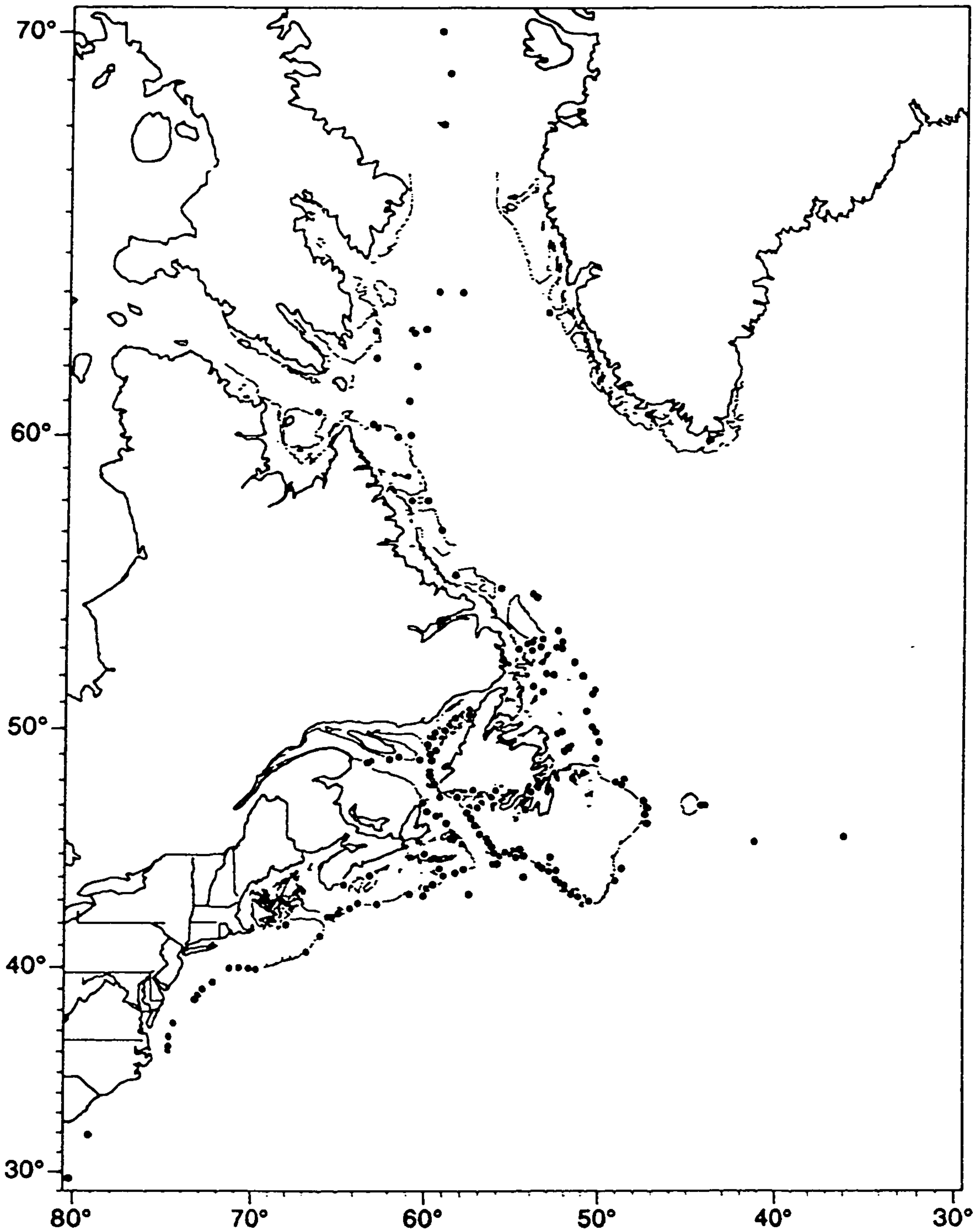


the next sea table and so on. Thus, the octopus rearing chambers were bathed by colder water which helped keep them cool longer during emergencies. However, each rearing chamber had its own separate supply of incoming water. No octopus in the 2°C, 6°C and 10°C treatments received water that had been exposed to another octopus. An air chiller was also used in the wet laboratory. It lowered the room temperature to 15°C which helped maintain cold conditions during emergencies. A stock pile of two liter plastic bottles of frozen water was distributed in various freezers and used to keep the water cold during longer emergencies.

Octopuses in the 2°C, 6°C and 10°C treatments were fed gammaridean amphipods (*Gammarus* spp. and/or *Corophium volutator*) *ad libitum*. Amphipods offered were in the range of 0.5 - 1.2 times the octopuses's mantle length. The amphipods used were smaller than those from the preliminary experiment as octopuses appeared to have problems handling the larger ones. Diets were occasionally supplemented with mysid shrimp (*Mysis*) when an adequate supply was available to provide each octopus with one. The end of the tail of each shrimp was removed to handicap it and make it easier for the hatchlings to catch. A wider variety of food was offered to the octopuses in the natural treatment including marine worms (*Nereis*), mussels (*Mytilus*), brittle stars (primarily *Ophiopholis aculeata*) and mysids (*Mysis*) in addition to the amphipods listed above. These foods were chosen based on stomach contents reported by O'Dor and Macalaster (1983).

The hatchling octopuses used in this experiment came from two females that were collected in scallop drags in the summer of 1994 from the lower Bay of Fundy (Figure 2).

Figure 2. Distribution of *Bathypolypus arcticus* along the east coast of North America. Data from Macalaster (1976).



The collecting, mating, and brooding time of the females that laid the eggs used in this experiment have been described previously (Wood et al. 1998).

Estimate of life span

A separate preliminary experiment was done with 45 hatchling octopuses. These octopuses were randomly assigned to one of four treatments on November 13, 1995. In a preliminary experiment, octopuses were weighed by placing them directly on a Kimwipe to absorb excess water before transferring them to the scale. This appeared to damage the octopuses so a modified weighing tray was designed for the primary experiment. As this was the first time *B. arcticus* hatchlings had ever been reared in the laboratory, a variety of food items were offered including amphipods, plankton, mysid shrimp, and mussels. Amphipods, the primary food, were 1/3 to 2 times the mantle length of the octopuses; the size range recommended by Hanlon and Forsythe (1985) for feeding hatchling octopuses.

The data presented here were generated from the primary experiment. Thirty-six octopuses from a single brood were used. These octopuses were randomly assigned to one of four treatments (2°C, 6°C, 6°C natural environment and 10°C). The 2°C and 6°C temperatures used were chosen based on the temperatures of 4 ± 2°C at which *B. arcticus* naturally occur. The 10°C temperature was used to enable me to compare my data to those of temperate shallow-water cephalopods reared at the low end of their temperature range.

Octopuses in the 6°C natural environment treatment were batch-reared in a single plastic tank that measured 50 cm by 33 cm, with water 18 cm deep. This tank contained sand and small rocks to provide a more natural environment. A wider variety of prey was offered. This was done to see if the relatively sterile individual rearing chambers used in the 2°C, 6°C, 10°C treatments depressed growth rates. Wood and Wood (1999) present evidence that sterile environments can cause abnormal and potentially lethal behavior in hatchlings of some species of octopuses.

Octopuses assigned to the 2°C, 6°C, 10°C treatments were kept in individual rearing chambers constructed of 20.5 cm lengths of 10.5 cm diameter PVC pipe with Nitex screening glued to the bottom with aquarium safe silicon. These independent chambers did not contain sand or rocks and octopuses were fed only amphipods *ad libitum* and occasionally a mysid shrimp when there was enough to feed all the octopuses in all three treatments. Each chamber was immersed halfway to its top in seawater. Fresh seawater was continuously supplied via 0.32 cm (1/8 inch) airline tubing.

Temperatures were recorded for the 2°C, 6°C and 10°C treatments three times a week with a mercury thermometer. Weekly minimum and maximum temperatures were also recorded from these three treatments with a digital min/max thermometer. The octopuses in the natural treatment were only disturbed for weighing and the treatment was supplied with water from the same manifold as the other 6°C treatment.

Octopuses were weighed to the nearest milligram at the start of the experiment, and again at days 70, 120, and 170. Octopuses were weighed in a modified plastic weighing tray. The bottom of the weighing tray was cut out and Nitex micron screening was taped in its place. Prior to weighing an octopus, water was removed by placing an absorbent paper towel under the screening. This method wicked away excess water without directly touching and possibly damaging the octopuses.

Growth rates were calculated by two or three different methods to ensure that the growth rates that yielded the most conservative life span estimates (i.e. the fastest growth rates) were used to estimate *B. arcticus* longevity in the wild. In the first method, the instantaneous relative growth rate (Forsythe and Van Heukelem 1987) was calculated for each animal that lived to the end of the experiment and gained weight. Using only survivors that gained weight should produce growth rates slightly higher than average. Workers traditionally do not report the variability of growth rates of cephalopods which makes meaningful comparisons between the growth rates of various species difficult. Standard deviations of G and G/T were calculated.

The second method was to fit a linear regression line to the natural log of the average weights of all octopuses in each treatment at days 1, 70, 120 and 170. The 6°C and 10°C treatment had octopuses that lived almost a year after the experiment ended. For the third method, a linear regression of the natural log of the weight vs age of the longest lived octopus in these two treatments was also done.

Results

Average final weights and standard deviations in grams at 170 days were 0.415 ± 0.146g, 0.534 ± 0.070g, 0.528 ± 0.174g and 1.481 ± 0.330g for the octopuses in the 2°C, 6°C, 6°C natural and 10°C treatments, respectively (Table 1). All but one of the surviving octopuses gained weight in the experiment. The average growth rates in percent per day for surviving octopuses were 0.117 ± 0.149, 0.221 ± 0.041, 0.159 ± 0.163

Table 1. Average weights (in grams), standard deviation and sample size for octopuses in each of the temperature treatments.

Treatment	day 1	day 70	day 120	day 170
2°C	0.368±0.030 (n=10)	0.341±0.047 (n=9)	0.352±0.056 (n=9)	0.415±0.146 (n=5)
6°C	0.367±0.030 (n=8)	0.416±0.050 (n=8)	0.450±0.091 (n=8)	0.534±0.070 (n=3)
6°C Natural	0.389±0.058 (n=8)	0.441±0.081 (n=7)	0.520±0.125 (n=7)	0.528±0.174 (n=6)
10°C	0.390±0.060 (n=10)	0.626±0.154 (n=10)	1.026±0.324 (n=6)	1.481±0.330 (n=3)

and 0.779 ± 0.207, respectively (Table 2). Data from individual octopuses is recorded in Appendix 5.

The growth rates of *B. arcticus* are much lower than those of other species, even when degree days are used. Regressions for the average weights of all octopuses alive on day 1, 70, 120 and 170 are presented in Table 3. The slope of these lines is very similar to the growth rates for the octopuses in the 6°C and 10°C treatments. The octopuses in the 2°C treatment had a lower slope since growth rates were calculated from survivors that gained weight while the regression was done on the average weight of all octopuses alive at the time including one that lost weight but lived to the end of the experiment.

Octopuses in the warmer treatments grew faster (Figure 3). Of the 36 octopuses, 46% survived to the end of the experiment and the highest survival rates were in the natural treatment. Weights of five individual octopuses that lived over 200 days beyond the beginning of the experiment are presented in Table 4. Some of the octopuses lived for quite a while after the experiment ended (Table 4). Two of these octopuses, one in the 6°C and one in the 10°C treatment, lived for a year and a half. The octopus in the 6°C treatment weighed 3.8 g at an age of 583 days (562 days after the experiment began). The octopus in the 10°C treatment weighed 19.3 g and was 587 days old (also 562 days since the experiment began). Unfortunately an electrical failure stopped the flow of water and killed these octopuses. At this time the female kept at 10°C was near maturity and contained full-sized eggs.

A regression was done on age versus natural log of weight for both of these octopuses. The growth of the single long-lived 6°C octopus fit the equation

Table 2. Growth rates (G) and physiological growth rates (G/T) of various species of hatchling octopuses. Growth rates from all octopuses that survived to the end of the experiment and gained weight are presented with standard deviations. See the slopes on Table 3 for the growth rates from the regression equations. Even when temperature is corrected for by using degree-days, *B. arcticus* grow very slowly compared with octopuses that occur in shallow-water. Note: The growth rate (G) is 100% times the slope of the regression equations in Table 3.

Species	G %/d	°C	G/T	Reference
B. arcticus (n=5)	0.117±0.149	2.4	0.049±0.062	This experiment
B. arcticus (n=3)	0.221±0.041	6.0	0.037±0.007	This experiment
[1]*B. arcticus* (n=6)	0.159±0.163	6.0	0.026±0.027	This experiment
B. arcticus (n=3)	0.779±0.207	9.9	0.079±0.021	This experiment
B. arcticus (n=3) (fed *Gammarus* spp.)	0.33	8.3	0.04	Wood (unpublished data)
B. arcticus (n=13) (fed *Corophium* sp.)	0.67	8.3	0.08	Wood (unpublished data)
Octopus bimaculoides	2.76	18	0.15	Forsythe and Hanlon 1988
O. bimaculoides	4.59	23	0.2	Forsythe and Hanlon 1988
O. briareus	4.62	25	0.18	Forsythe and Van Heukelem 1987
O. joubini	7.05	25	0.28	Forsythe and Van Heukelem 1987
O. maya	6.06	25	0.24	Forsythe and Van Heukelem 1987
O. tetricus	5.14	20	0.26	Forsythe and Van Heukelem 1987

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[1] This is the 6°C natural treatment

Table 3. Regression equations of the natural log of the average weights of octopus (Table 1) versus their age at day 1, 70, 120 and 170. As these regressions were done using the average weight of all octopuses, instead of just those that survived till the end of the experiment, the slopes of the lines are somewhat different than the growth rates of just the survivors. Regression equations for octopuses A and D (Table 4) are also presented separately. Slopes of the lines are equal to instantaneous relative growth rates if multiplied by 100%.

Octopus	equation	std error X	std error b	r^2
2°C treatment	$y=0.000628x - 1.0564$	0.000717	0.0788	0.28
6°C treatment	$y=0.00214x - 1.0195$	0.00028	0.0302	0.97
10°C treatment	$y=0.00869x - 0.9736$	0.00040	0.04427	0.99
Octopus A (6°C)	$y=0.00398x - 1.2018$	0.00021	0.0662	0.97
Octopus D (10°C)	$y=0.00768x - 1.1126$	0.00037	0.1182	0.98

Figure 3. Average sizes of octopuses in the four experimental treatments. Octopuses kept at 10°C grew much faster than those at lower natural temperatures.

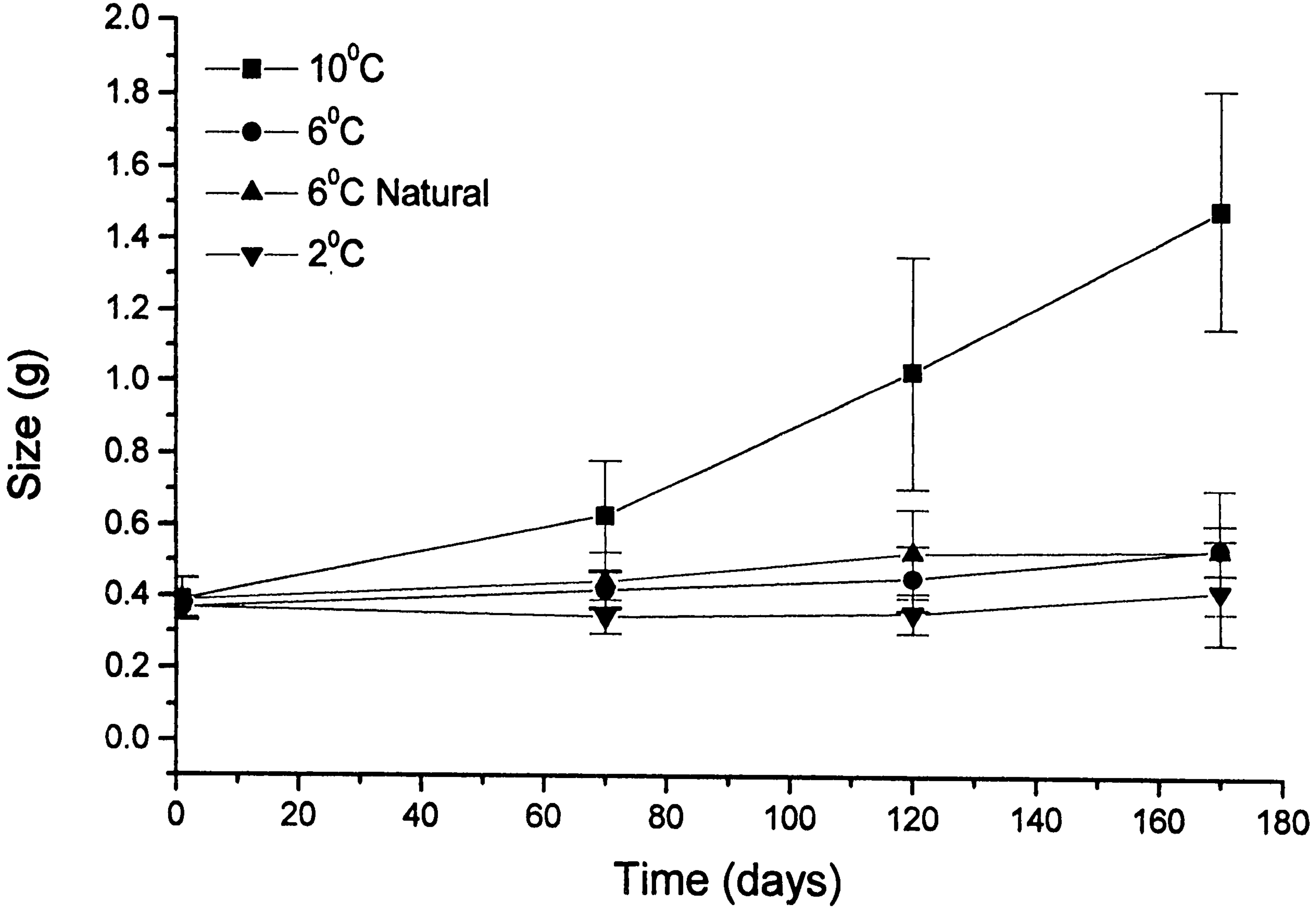

2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Size (g)
0
20
40
60
80
100
120
140
160
180
Time (days)
10^{0}C
6^{0}C
6^{0}C Natural
2^{0}C

Figure 3

Table 4. Weight in grams of five of the octopuses that lived over 200 days from the beginning of the experiment.

Treatment	day 1	day 70	day 120	day 170	day 213	day 270	day 314	day 409	day 462	day 562
6°C Octo A	0.39	0.48	0.53	0.61	0.69	0.84	1.00	1.53	2.15	3.80
6°C Octo B	0.35	0.39	0.54	0.48	0.56	0.51				
6°C Octo C	0.35	0.43	0.58	0.50	0.53	0.49				
10°C Octo D	0.33	0.66	1.01	1.79	2.74	3.54	4.23	10.92	16.64	19.30
10°C Octo E	0.41	0.70	1.06	1.51	2.25					

$y=0.00398x - 1.2018$ ($r^2 = 0.97$) while the octopus in the 10°C treatment fit the equation $y=0.00768x - 1.1125$ ($r^2 = 0.98$). The slope of the equation for the single 10°C octopus (0.00768 or 0.768%) fits very closely with the average growth rate of 0.779%/d ± 0.207%/d and is similar to the regression equation derived from the weights of all octopuses over the 170 day experiment ($y=0.00869x - 0.9736$). However, the single long-lived octopus in the 6°C treatment grew much faster over its life than expected based on the growth rates of all the octopuses that survived the 170 day experiment or the slope of the regression line done on their average weights (i.e. $0.00398 = 0.398\% > 0.221\% \pm 0.041\% \approx 0.00214 = 0.214\%$).

The 2°C treatment was kept at 2.44 ± 0.89°C during the experiment. The minimum and maximum temperatures for this treatment were 1.6°C and 6.7°C. On day 169, the incoming water stopped flowing to the 2°C tank after failure of a secondary fail-safe. This caused the tank to warm up to 14.2°C. This did not immediately kill the octopuses in the treatment. They were barely alive for the final weighing at day 170 and found dead on the following day. Including this anomalous point, the average and standard deviation for the 2°C treatment was 2.61 ± 1.68°C. The 6°C treatment was kept at 5.99 ± 0.98°C with a minimum recorded temperature of 2.7°C and a maximum of 9.9°C. The 10°C treatment averaged 9.90 ± 0.84°C with a minimum temperature of 3.1°C and a maximum of 13.3°C during the 170 day experiment. The temperatures were within ± 2°C of the target temperatures 93%, 93% and 94% of the time for 2°C, 6°C and 10°C, respectively.

Discussion

Rearing deep-sea octopuses.

Octopuses with large benthic hatchlings are among the easiest cephalopods to rear. Hanlon and Forsythe (1985) report that these hatchlings are able to capture prey that is anywhere from 1/3 to 2 times their mantle length. I have observed a single week old *Octopus "joubini"*, a semitropical shallow-water octopus, capture three amphipods all more than 1.5 times the octopuses' mantle length within five minutes. Such crustaceans including mysid shrimp are commonly used to feed hatchling cephalopods (Boyle 1991; Lee et al. 1991).

In contrast, the deep-sea octopus *Bathypolypus arcticus* is not as aggressive or as quick as the near-shore species others have studied. At Dalhousie, I also observed hatchling *O. joubini* and *O. bimaculoides* capturing *Gammarus* amphipods in the size ranges that Hanlon and Forsythe (1985) report, but *B. arcticus* was unable to. Large amphipods seemed to be harder to catch and hold on to and may have bit back, damaging the octopuses. Preliminary experiments also showed that hatchlings were usually not able to handle amphipods other than *Corophium volutator* that were more than 1.5 times their mantle length unless the amphipods were handicapped. There were noticeable differences between prey suitable for other octopuses and those for the deep sea octopus, *B. arcticus*.

C. volutator is a burrowing amphipod that ingests diatoms in the size range of 4 to 63 µm and are, therefore, less likely to attack octopuses (Gerdol and Hughes 1994). Preliminary experiments showed that these amphipods were the best food offered since they are relatively slow moving and not likely to be able to damage octopuses. Although they were very abundant in the Bay of Fundy mud flats during the summer and early fall, my supply of *C. volutator* ran out near the end of the experiment and small *Gammarus* amphipods were substituted instead.

I did not observe a *B. arcticus*, hatchling or adult, capture a mysid shrimp of any size unless the shrimp was wounded. Adults are able to capture *Crangon*, but these shrimp bury themselves in the sand, while mysid shrimp generally swim in the water column. Experiments investigating prey capture, nutritional requirements, and the effect of diets on growth rates of *B. arcticus* and other deep-sea octopuses are needed.

One of the main challenges limiting the study of the life history of deep-sea creatures in the laboratory is that they must be kept in chilled water for long time periods. For instance, *B. arcticus* broods eggs for over 400 days at temperatures around 7.5°C (Wood et al. 1998). After collection, during brooding and during rearing experiments the animals must be kept cold continuously despite power outages, chiller breakdowns and other such problems. The slow growth rates of deep-sea octopuses require much longer experiments. For example, in the time it took for one octopus at 6°C to grow from hatching to 3.8 g I could have raised three generations of the Florida Pygmy Octopus (*Octopus* sp. "*joubini*"). It will take long-term dedication and a bit of luck to raise any species of deep-sea octopus from hatching to a second generation in the laboratory. Any researcher doing this type of work should have at least one fail-safe mechanism on their system and a plan for inevitable equipment breakdowns.

Octopuses in the 6°C natural treatment did not have a higher growth rate than the octopuses in the 6°C treatment despite the greater variety of food and environmental enrichment. Although the batch culture had the highest survival rate during the experiment, most of the octopuses in it died shortly after the end of the experiment. Octopuses in other batch cultures of *B. arcticus* have been kept at Dalhousie since this experiment and they seem to do well initially but then die. Hanlon et al. (1984) reports that hatchling *Octopus joubini* and *O. briareus* that are reared in high-density batches develop skin ulcers which are fatal. In the same study, octopuses kept in the same closed seawater system but reared in individual containers did not develop ulcers. Perhaps for similar reasons, hatchling *B. arcticus* do not do well in group cultures. However, adult *B. arcticus* seem to do fine when kept in a group for extended periods of time. Most species of adult octopuses are not kept in group cultures as they can be prone to cannibalism if conditions are not ideal (Boyle 1991) but I have seen no evidence of this in *B. arcticus*.

Life spans of deep-sea octopuses

The purpose of this section is not to fix an exact length on the life-span of *B. arcticus,* but to suggest that previous estimates may be unreasonably short. Using *B. arcticus* as a model, longer life spans may also be generalized among other deep-sea octopuses.

Previous studies report a life span of at least three years for *B. arcticus* (O'Dor and Macalaster 1983). If animals in the 10°C treatment continued to grow at the same rate as hatchlings they would reach an average adult size of 70 g in approximately two years. 70 g was the average size of maturity reported by O'Dor and Macalaster (1983) for wild octopuses living at 4±2°C. Allowing for a year to lay and brood eggs, octopuses at

10°C would fit the proposed 3 year life span. However, *B. arcticus* are rarely found at 10°C in nature; their typical temperature range is 4 ± 2°C. Water in their preferred depth range of 200 to 600m (O'Dor and Macalaster 1983) would only reach 10°C near vents or seeps (Figure 2). The results of this experiment indicate that warmer water significantly increases the growth rate of *Bathypolypus arcticus*.

B. arcticus at natural temperatures of 4 ± 2°C would be expected to live longer; but how much longer? Here, I estimate the life span of *B. arcticus* based on assumptions that err conservatively towards shorter life span estimates. These assumptions pertain to the animal's size at maturity, growth pattern and growth rates. A hatchling size of 0.245 g will be assumed in all estimates as this was the average size of hatchlings used in this experiment. This size at hatching is higher than the size of 0.15 g reported by O'Dor and Macalaster (1983). A larger initial hatching size will shorten estimated life span in the model.

Stearns (1992) notes that in general, cold-blooded animals not only grow faster at warmer temperatures, they also mature earlier at smaller sizes. Upon re-examining Macalaster's (1976) data, Wood (unpublished data) did not find a significant correlation between the size of mature female *B. arcticus* and the temperature at which they were caught or the average yearly temperature at that location. Wood (unpublished data) has observed strong sex ratio biases on some collecting trips and no octopuses smaller than 1 g have been collected. It is conceivable that octopuses migrate up the continental shelf to warmer water before laying eggs. Such migrations would confound attempts to correlate size at maturity with bottom temperature. However, Macalaster (1976) found no evidence of migration.

There are, however, data to support Stearns' (1992) hypothesis. The pattern of increased growth rates and decreased size at maturity with increasing temperature has been found in other octopuses (Forsythe and Hanlon 1988). Furthermore, the average water temperature at the depths from which the octopuses were collected in the Bay of Fundy is around 6.9°C (Figure 2) and average female weight is approximately 45 g when these octopuses lay eggs (Wood et al. 1998). Octopus D (Table 4) which was raised at 10°C did not lay eggs before it died but did have full-sized eggs when it was 20 g. These sizes are lower than the 70 g average weight reported by O'Dor and Macalaster (1983) for octopuses living at 4±2°C and are consistent with Stearns' (1992) predictions and data from other octopuses (Forsythe and Hanlon 1988). Using these smaller weights at maturity deceases *B. arcticus* life span in my model. The parents of these octopuses were collected from the Bay of Fundy where temperatures average about 6.9°C. So the value of 45 g for mature females gives times to maturity of 12.2 years at 2°C and 7.5 years at 6°C. Other models below assume average female weights at maturity of 25 g at 10°C, 45g at 6°C and 70 g at 4°C, unless otherwise stated.

The models used likely underestimate the life span of *B. arcticus* as they assume that the octopuses continue to grow at an exponential rate throughout their life span. Forsythe and Van Heukelem (1987) report that laboratory studies have consistently shown that the growth of benthic octopuses occurs in two phases over their life cycle. The first phase is exponential, then growth slows down to the logarithmic phase. The exponential equation is $W_t=se^{Gt}$ where W_t equals weight at time t, s equals starting weight, G equals the growth rate and t equals time in days (Forsythe and Van Heukelem 1987).

If I assume that *Octopus bimaculoides*, *Octopus* sp. "*joubini*" and *Octopus maya* continue to grow at their early exponential rate my calculated values would only be 57%, 52% and 67% of their true age at maturity. Conservatively inferring that *B. arcticus*' age at maturity reported above is 70% of the actual age at maturity and assuming that the average size of adults is only 45 g, rather than 70 g, octopuses living in the temperature range of 4 ± 2°C would take 10.7 to 17.4 years to reach maturity!

The last assumption I make to estimate the life span of *B. arcticus* as conservatively as possible is that of growth rate; the fastest growth rates observed for each temperature is used. For the 2°C treatment I used the relative instantaneous growth rate of surviving octopuses that gained weight (0.117%/d or 0.00117) as this rate is higher than the slope of the regression of the average weights of all octopuses in the treatment including the one that lived to the end of the experiment but lost weight. For the 6°C treatment I used the growth rate of the single octopus that lived for a year and a half (0.389%/d). This single animal grew over four standard deviations faster than the average octopus in the 6°C treatment. The highest growth rate (0.869%/d) for the octopuses in the 10°C treatment was also used, although all three growth rates were similar; in any case, these octopuses would almost never experience such high temperatures in nature. To give a conservative idea of natural life span at 4°C, I averaged the slopes for the 2°C and 6°C lines.

Figure 4 uses these consistently conservative models to speculate on the minimum average age of maturity for wild *B. arcticus*. According to the model, wild animals live at

Figure 4. Conservative estimates of age at maturity for *B. arcticus* at 4 different temperatures. The longest lived octopuses in the 6°C and 10°C treatment are shown for comparison along with the entire life span of the Florida Pygmy Octopus (*Octopus* sp. "*joubini*"). This model predicts that on average, *B. arcticus* living at their natural temperatures of 4°C take at least 6 years to reach maturity.

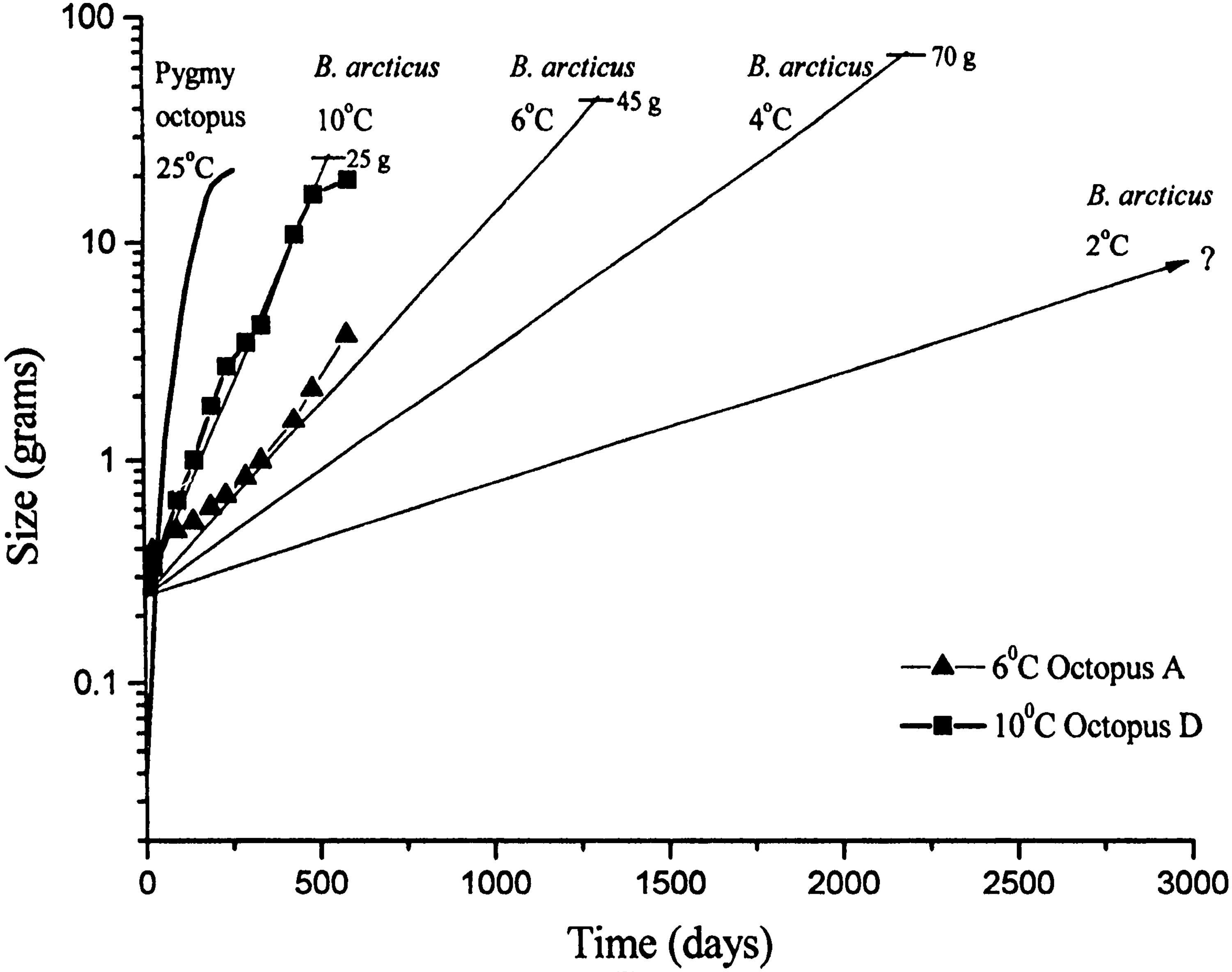

100
10
1
0.1
Size (grams)
0
500
1000
1500
2000
2500
3000
Time (days)
Pygmy octopus 25°C
B. arcticus 10°C
25 g
B. arcticus 6°C
45 g
B. arcticus 4°C
70 g
B. arcticus 2°C
?
6⁰C Octopus A
10⁰C Octopus D

Figure 4

4°C, mature at 70 g, hatch at an average size of 0.245 g and grow exponentially at a rate of 0.253 %/d throughout their lives. This model predicts that these octopuses take just over six years to reach maturity. This is twice the previous estimate of their life span and, if true, makes *B. arcticus* the longest lived coleoid cephalopod yet studied. Furthermore, I predict that other deep-sea octopus species that live in even colder polar waters and that mature at larger sizes will take even longer to reach maturity (Wood and O'Dor 2000). This suggests that there may be a large group of cephalopods that does not fit the 0.5 to 2 year life span paradigm.

The above estimates indicate time from hatching to maturity. To estimate life span, brooding time must also be included for females. *B. arcticus*, besides being long-lived, also has the longest brooding time recorded for any cephalopod. One octopus took 419 days at 7.3°C to brood her eggs while another took 407 days at 7.8°C (Wood et al. 1998). These times would likely be even longer at 4°C. Again, using a conservative estimate of only a year for brooding, *B. arcticus* at natural temperatures of 4°C lives for seven years. To put this in perspective, in the same amount of time at least 10 generations of the similarly sized Florida Pygmy Octopus (*Octopus* sp. "joubini") could be raised at 25°C (Figure 4).

Due to the long life span of *Bathypolypus arcticus*, these estimates have important impacts on expected mortality rates in this species. *B. arcticus* is also known for its low fecundity and large eggs; the large size of eggs is especially interesting given the small size of adults. O'Dor and Macalaster (1983) report that a typical 70 g female only lays 90-100 eggs and Wood et al. (1998) found that 9 females whose average weight was around 45g laid 44 ± 34 eggs (n=9). The latter is an incredibly small number when compared with the hundreds to hundreds of thousands of eggs laid by other species of octopuses (Sweeney et al. 1992). Mangold (1987) suggested that *B. arcticus* represents the extremity of K-selection within the family Octopodidae. However, large egg size and the corresponding reduction in fecundity is one of 16 modifications for deep-sea life listed by Voss (1988a).

In terms of egg production per year, *B. arcticus* is the low extreme for coleoid cephalopods studied to date. Assuming six years to reach maturity and additional year to brood, an octopus laying a hundred eggs is only producing an average of 14.3 eggs per year. This low fecundity resembles that of *Nautilus* which produces a maximum of only 11 large eggs per year once mature (Ward 1987). Unfortunately, we do not have age-specific mortality rates for any species of cephalopod. With such low egg production and long life spans, *B. arcticus* mortality would have to be extremely low compared to other coleoid cephalopods and most other similarly sized marine invertebrates and vertebrates.

Are these results believable? While there are few data on deep-sea or cold-water octopuses to compare, Wood (unpublished data) found growth rates of 0.33%/d and 0.67%/d for *B. arcticus* fed two types of amphipods *ad libitum* at 8.3 °C. By raising *B. arcticus* at 10 °C, which is 3 standard deviations above their natural temperatures, I can compare the growth rate of *B. arcticus* to the only other published information on the growth rates of cephalopods in cold water. Mangold and Boletzky (1973) reported a growth rate of 0.9%/d for *Octopus vulgaris* at 10 °C which is very similar to the growth rates I found for *B. arcticus* at 10 °C.

Coleoid cephalopods are commonly thought to live for only six months to two years and even the giant octopus, *Octopus dofleini*, which reaches sizes over 50 kg, is thought to live only about 3 years (Cosgrove 1976; Hartwick 1983). Compared to near-shore species, *B. arcticus* has very slow growth rates. Even when time is measured in degree-days (Wood and O'Dor 2000), the growth rate of *B. arcticus* is an order of magnitude less than near-shore species of a similar age (Table 2).

These low growth rates of *B. arcticus* could be a laboratory artifact. There are few studies comparing field and laboratory growth rates of common octopuses and certainly no such data exist for any deep-sea cephalopod. However, the limited information for shallow-water octopuses indicates that laboratory growth rates are comparable to growth rates in the field (Van Heukelem 1976). A comparison of the growth rates of laboratory reared and wild deep-sea octopuses is unfortunately not likely to be made any time soon as the logistical problems of doing long-term field experiments on cephalopods, especially deep-sea cephalopods, are considerable.

Pressure may or may not be a confounding factor in my experiment. The only work on the effect of pressure on cephalopod physiology was done by Belman (1978) on the vertically migrating squid *Histioteuthis heteropsis*. He examined the effect of pressure on oxygen consumption in the range of 1 to 136 atm and did not find an effect. For logistic reasons, I have not investigated the effect of pressure on the benthic *B. arcticus*, although I encourage future researchers to investigate the influence this parameter may have on growth rate.

While the similarity in growth rates between my deep-sea octopus and *Octopus vulgaris* at 10°C are encouraging, my octopuses were hatchlings and the *O. vulgaris* used were older. On the other hand, my *ad libitum* diets of live crustaceans are more nutritious (Wood unpublished data) than the brittle star-dominated and possibly food-limited diets that *B. arcticus* eat in the wild (O'Dor and Macalaster 1983; Wood unpublished data). Therefore, it is also possible that wild octopuses eating their natural, low-quality diet grow even slower, further increasing actual life span and reinforcing my model as conservative.

Conclusions

Although many questions remain unanswered, I conclude that the deep-sea octopus *B. arcticus* lives much longer than previously thought and that the life spans of other cold water cephalopods may also be very long. I found that temperature has a strong effect on growth; only octopuses at abnormally warm temperatures grow at rates that fit a 3 year life span model. Almost nothing is known about the behavior and life history of deep-sea cephalopods. Hopefully, the methods delineated here will be used to rear and study other species of deep-sea cephalopods in the laboratory, providing information to support or refute the life-history theories discussed here. The behavioral differences, slow growth rates, large offspring size, low fecundity, long life span and low mortality rates discussed here all point to a strategy that dramatically differs from the near shore species on which we base our knowledge of cephalopods.

Chapter 4

Reproduction and embryonic development time of *Bathypolypus arcticus*, a deep-sea octopus (Cephalopoda: Octopoda).

...Worse, in terms of outright scariness,
Are the suckers multifarious... Bill Watterson, Calvin and Hobbes

Abstract

Mating, brooding, and embryonic development rate of *Bathypolypus arcticus*, a deep-sea octopus, are described. Live specimens of *B. arcticus* were collected in the Bay of Fundy, Canada, and kept in a flow-through system in the laboratory. Two of the octopuses laid and brooded viable eggs. Brooding and embryological development took over a year at average temperatures of 7.3 °C and 7.8 °C. Brooding females ate occasionally only during the first months of brooding and did not leave their eggs until shortly before they died. Hatchlings weighed 208 ± 17 mg (SD) from the first batch and 283 ± 20 mg (SD) from the second batch. There was no evidence of multiple spawning.

Mating of *B. arcticus* was also observed. The usually smaller male sits upon the female, enveloping much of the female's mantle in his web, and he inserts his large ligula into her mantle. One or two large spermatophore are transferred by a combination of mantle pumping and arm groove peristalsis. A filmed mating sequence lasted 140 seconds.

Introduction

Bathypolypus arcticus (Prosch 1849) is a small incirrate octopus that rarely exceeds 200 g (O'Dor and Macalaster 1983). It has been found to depths of 1543 m (Voss 1988a,b) and is classified as a deep-sea octopus by Voss (1988a). *B. arcticus* is most common at depths of 200-600 m and is widely distributed in the Atlantic Ocean (O'Dor and Macalaster 1983). Assuming the three-year life span estimated by O'Dor and Macalaster (1983), females brood eggs for a larger percentage of their life than any octopus studied thus far. Like all deep-sea octopods, female *B. arcticus* lay large eggs from which well-developed young hatch. It has been suggested that *B. arcticus* represents the extremity of K-selection within family the Octopodidae (Mangold 1987).

Mature male *B. arcticus* have the largest ligula relative to body size of any octopus. The ligula is part of the hectocotylus, the modified third right arm which males use to transfer their large spermatophores while mating. Mating has not been previously described for *B. arcticus* - what males do with their hectocotylus was unknown. Two mating positions have been observed in the family Octopodidae: a distant position where the male and female are separated except for the hectocotylus, and one where the male mounts the female (Mangold 1987). Mangold notes that mating may last from a few minutes to several hours.

As much as there is to be learned from easily-collected cephalopods, perhaps even more is yet to be learned from those species that dwell in the deep-sea (Forsythe and Van Heukelem 1987). Little laboratory work has been done with deep-sea cephalopods due to difficulties in collecting undamaged live specimens and continually providing cold water. The only previous laboratory information on brooding and embryonic development of deep-sea octopuses is from O'Dor and Macalaster (1983). They reported that a single female *B. arcticus* laid eggs in August 1978 and brooded them until they hatched in July 1979. O'Dor and Macalaster state that the temperature varied between 3 °C and 10 °C but temperatures were not recorded. The female was essentially ignored until the spring of 1979 when the eggs were discovered to be developing. She only had 4 of 40 eggs left in July 1979 and was not offered food while brooding. This female *B. arcticus* died shortly after her eggs hatched.

Very little is known about the life history of deep-sea octopuses. This report describes the first detailed observations of mating, brooding, and embryonic development time of *Bathypolypus arcticus*.

Methods

Eighteen *B. arcticus* were collected from the Bay of Fundy off Digby (circa 44.70 N and 65.90 W) on June 8-21 and below Brier Island (circa 43.80 N and 66.30 W) on August 22 - September 1, 1994. The FRV J. L. Hart, a 20 m LOA trawler belonging to the Department of Fisheries and Oceans (DFO), was used. Animals were collected in scallop trawls as incidental catch during Department of Fisheries and Oceans (DFO) scallop stock surveys. The majority of animals came from the deeper tows done during the cruise which were at depths of 75 to100+ m. While on the boat, specimens were housed in a portable cooler for as long as a week and kept at temperatures below 10°C. Additional specimens were collected in the same manner in the summer of 1995.

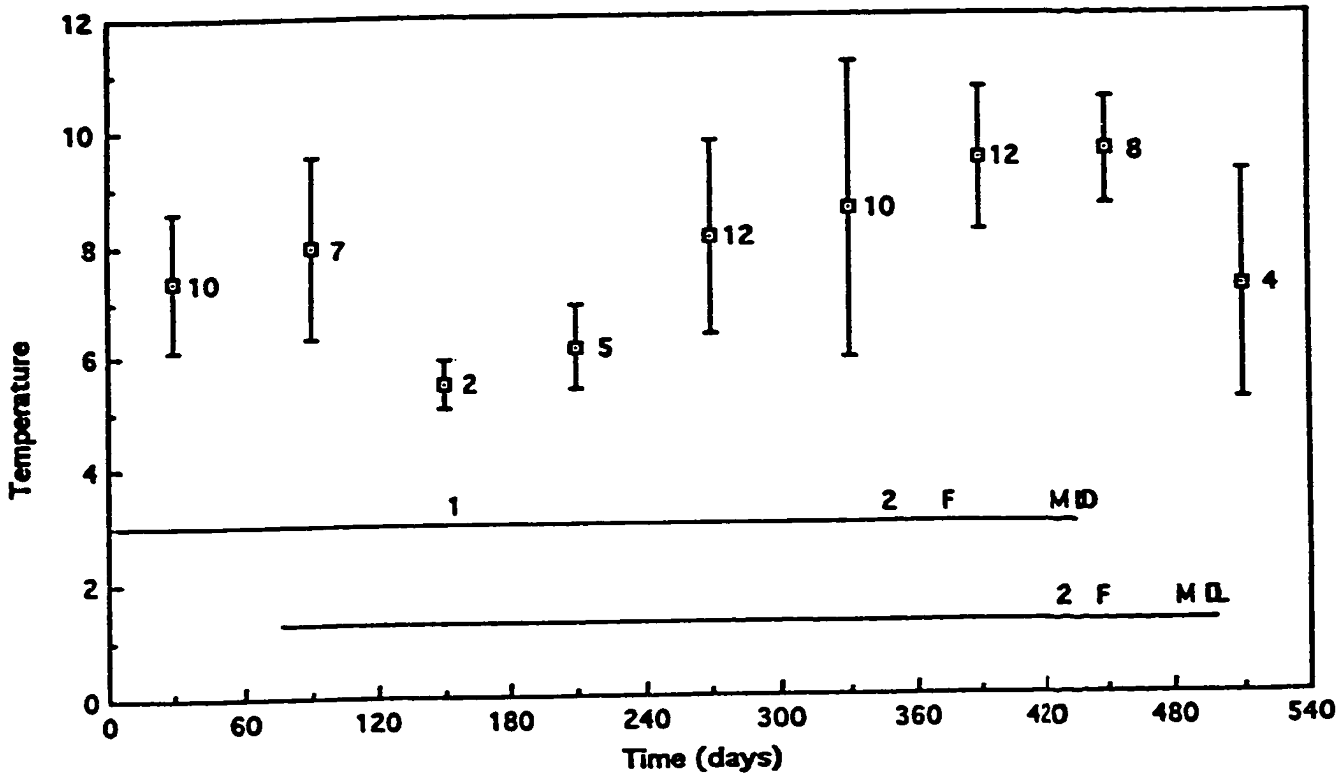
The *B. arcticus* collected in 1994 were kept together in a flow-through system at DFO's Halifax laboratory. The animals were housed in a 91 by 91 cm fibreglass tank (internal dimensions). The walls of the tank were 4 cm thick and contained chilling coils. Water depth was 31 cm. Water temperature was controlled by adjusting the amount of ambient and heated water that entered the system. Temperature varied with the incoming water and user demand.

Initially, temperature was recorded with a mercury thermometer (Figure 1). In late December, 1994, a min/max thermometer was added to the system to record temperature fluctuations.

The octopuses collected in 1995 were kept in two flow-through fiberglass tanks at Dalhousie University. Most of the females laid eggs and only one brood was infertile. Temperature was recorded but will not be presented here. The number of eggs laid in these fertile broods was assessed in May, 1996, by removing the females and photographing their eggs with an underwater camera. Eggs were much easier to count in the static photographs. However, I did knock eight eggs off when removing the females from their broods which are included in the totals for the appropriate females.

During the first months after capture, octopuses were hand-fed live crustaceans, molluscs removed from their shell, and offered amphipods *ad libitum*. Hand feeding consisted of bumping the food into the octopuses arms. Later, sand shrimp (*Crangon*) and brittle stars (Ophiuroids) were added in *ad libitum* quantities to the tank while

Figure 1. Average and standard deviation of temperatures at which brooding octopuses were kept. The number next to the average temperature is the sample size for that 60-day period. The horizontal lines show the brooding period for the two females (1 = first inversion, 2 = second inversion, F = first hatchling, M = median hatchling, L = last hatchling, D = death).



polychaetes, mussels, crabs, amphipods, and other small invertebrates were added as they became available.

Octopuses that laid eggs were observed and occasionally offered food by hand. However, brooding octopuses were disturbed as little as possible. Once the eggs started hatching, brooding octopuses were filmed with a 24-hour time-lapse VCR under red light.

To measure mantle length (ML), mantle width (MW), and inter-ocular width (IOW) of day-old octopuses, they were filmed and measurements made with an Optimas Video Analysis System. This system was used to reduce stress on the animals. Summers (1985) used a somewhat similar method and he briefly discusses the reliability of using photographic size determination.

Wet weights ± 1 mg of 15 hatchlings from each brood were obtained with a Mettler P163 scale. Members of the first batch of hatchlings were dried with a tissue to absorb excess water before being weighed. Many animals weighed with this method died; several of the dead hatchlings had tears in their skin. As this method damaged the hatchlings and caused mortality, it was abandoned. Individuals from the second batch were weighed in a weighing tray with a micron screen bottom. This tray was placed on a tissue before being weighed to absorb excess water. Preserved and recently dead juvenile *B. arcticus* were weighed with both methods to quantify the difference between the two methods. The second method produced results that were 10.1% (n=26) higher. Weights of octopuses in the first batch were converted so that they could be compared to those of the second batch.

Mating and hatching was filmed by Dave Gaudet (Halifax Cable) with a housed

Sony CCD 3-chip DX3 camera using high-8 format. The primary author induced several eggs to hatch by handling them and/or adding sugar to the water.

Results

Adult mortality was highest within the first few weeks of capture. By September, 1994, twelve of the eighteen octopuses collected during the previous summer were still alive. Of those, eight were still alive in September, 1995. Of the 18 *B. arcticus* collected in 1994, only one was male. However, in 1995 the sex ratio was 50/50 (n=32).

Average temperature during the brooding period was 7.3 °C for the first female and 7.8 °C for the second female (Figure 1). During the experiment the min/max thermometer recorded a minimum temperature of −1.8 °C on September 28, 1995. This drop in temperature was caused by a pump failure. A similar problem that dropped the temperature to 0.0 °C occurred in the fall of 1994. A maximum temperature of 16.7 °C was recorded by the min/max thermometer during July 17-24, 1995. These extreme temperatures probably lasted for only a short time.

On August 16, 1994, a female *B. arcticus* was discovered brooding at least 3 eggs which were laid the previous night. The female refused food. There were about 10 eggs on August 17. The female laid more eggs by August 22 and ate a few amphipods. Due to my effort to disturb the female as little as possible while she was laying eggs, eggs were not counted until several months later. Although precise observations could not be made, no noticeable additions of eggs took place after two weeks. To count the eggs, it was necessary to remove the female for a brief period. Fifty-five eggs were counted but only 54 were accounted for at hatching. This discrepancy is likely due to difficulties in counting the eggs while keeping the female away from them. Eggs were glued individually to the side of the tank by the female. The glue was initially clear but it turned green after a few days.

While brooding eggs the female occasionally ate food offered to her. She took fish pellets, amphipods, *Crangon*, and crushed mussels. Food was offered by hand-feeding as described above.

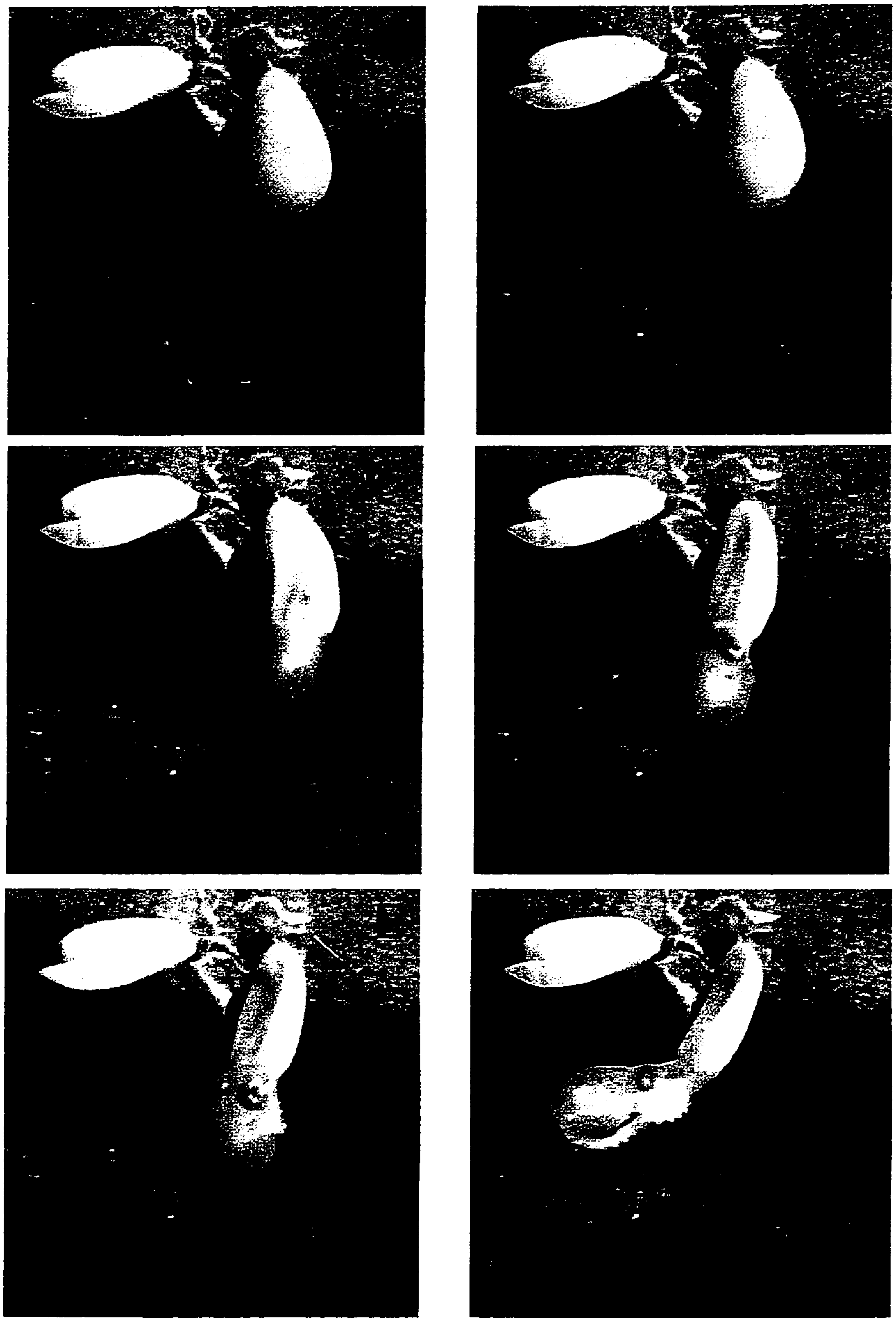
Brooding females would sit on their eggs and cover most of them with their web. They often directed their funnel down which provided a water current around the eggs. The video tapes of brooding females revealed that although they occasionally moved slightly away from the eggs, they would always keep a few arms on the eggs while exploring the perimeter around the brood with the other arms. When a probe was used to try to get a better look at the eggs, the female would actively defend them, sometimes by blasting jets of water at the probe and/or grabbing it.

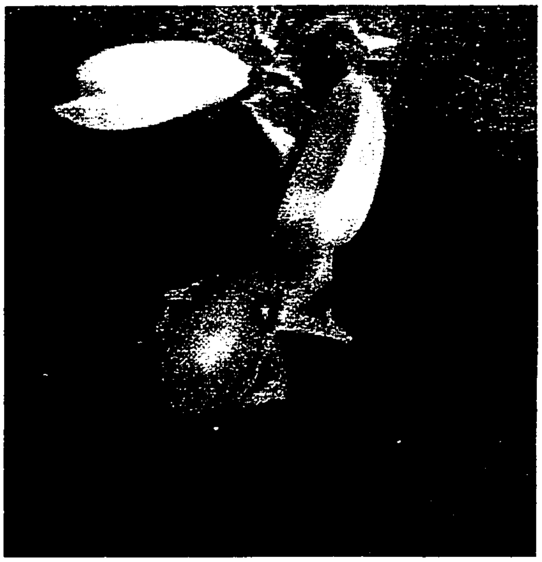
An egg was taken on January 20, 1995, 158 days after the first eggs were laid, to determine if they were fertilized. Incirrate embryos, except those produced by argonauts, flip position in the egg twice during development (Boletzky 1987b). The egg was viable and the embryo was in the first inversion. By July 31, 1995 (day 350), most of the embryos had flipped the second time. At least one egg had not flipped by August 8, 1995. On August 27, 1995 (day 377), the first octopus hatched. By October 23, 1995 (day 434), all the octopuses in the first batch had hatched although 6 of the last 7 were induced to hatch so they could be filmed (Figure 2).

Eggs were 11 mm long and 6 mm wide before hatching. Hatchlings (n=15) weighed 208 ± 17 mg (SD), had a mantle length of 7.71 ± 0.49 mm (SD), a mantle width of 7.14 ± 0.39 mm (SD), and an inter-ocular width of 2.30 ± 0.20 mm (SD). The median day of hatching was October 14, 1995 (day 425), and, assuming the median day of egg laying was 1 week after the first egg was laid, the average octopus in batch 1 took 419 days at an average temperature of 7.3 °C. This female died 3 days after the last egg in her brood hatched.

On October 30, 1994, a slightly larger female started laying viable eggs in the lower left corner of the same tank. Over 50% of these eggs flipped the second time by October 23, 1995 (day 359 from first laying of eggs). The first egg in the second batch hatched on or a few days before November 8, 1995 (day 375), and the last on December 30, 1995 (day 427) (Figure 1). The median day of hatching was December 17, 1995 (day 414). The average octopus in batch two took 407 days at 7.8 °C to develop. This batch, counted as they emerged, yielded 36 hatchlings with an average weight of 283 ± 20 mg (SD) (n=15), mantle length of 8.91 ± 0.43 mm (SD), mantle width of 8.60 ± 0.84 mm (SD), and an inter-ocular width of 2.84 ± 0.23 mm (SD). The second female died 2 days before her last egg hatched. Brooding behaviour was as noted for the first female except a few days before the second female died she left her eggs, at one point for several hours, and then returned to them. She was in very bad condition by this time and appeared to be having trouble breathing and orienting herself. Both females moved away from the eggs shortly before dying. Females were preserved in formalin after they died. The preserved

Figure 2. Hatching. A: Swollen egg just prior to hatching. B: During hatching, the pressure in the swollen egg pushes the tip of the octopus' mantle out. C: The octopus works his mantle out through the hole in the egg. D & E: The octopus uses his arms to help pull the rest of his body out. He pulls the rest of his arms out of the egg (F) and leaves the egg (G & H). I: The fully functional hatchling crawls away. Images from high-8 video by D. Gaudet.





specimens weighed 20.82 g and 17.55 g respectively. The two females weighed an estimated 30 - 40 g prior to laying eggs.

None of the hatchlings from either batch had any of the outer yolk sac remaining upon hatching. Video tapes revealed that hatchlings are able to hang upside down from the surface tension (see Marliave 1981 and Van Heukelem 1976). A variety of foods were offered to the first batch of hatchlings. They were initially hand-fed fresh mussel meat, and bits of gammaridean amphipods, mysid shrimp, and *Crangon septemspinosus*. Plankton and live gammaridean amphipods were offered to some of the hatchlings. Later they were offered *Corophium volutator ad libitum*; *C. volutator* is a small burrowing gammaridean amphipod. Hatchlings from the second batch were rarely hand-fed and were offered *C. volutator ad libitum* within a few days of hatching.

Several other females that were collected in 1994 laid eggs on the side of the tank or in a clay pipe. These eggs disappeared after a few months. An egg was removed from one of these females. When this egg was examined several months later it was found to be unfertilized. Presumably these batches were not viable and the females ate them. A single male was kept in the tank with the females.

Nine of the females that survived collection in 1995 were brooding eggs in May of 1996. Of these broods, 8 were fertile and 7 were able to be counted (the eighth octopus laid eggs in a plastic pipe). Therefore, the 9 *B. arcticus* in this study laid 12, 13, 18, 19, 36, 48, 54, 89, and 105 fertile eggs. However, I estimate that none of these cephalopods weighed 70 or more grams when they laid their eggs. Macalaster (1976) reports that 70 g is the average size of mature females throughout their range.

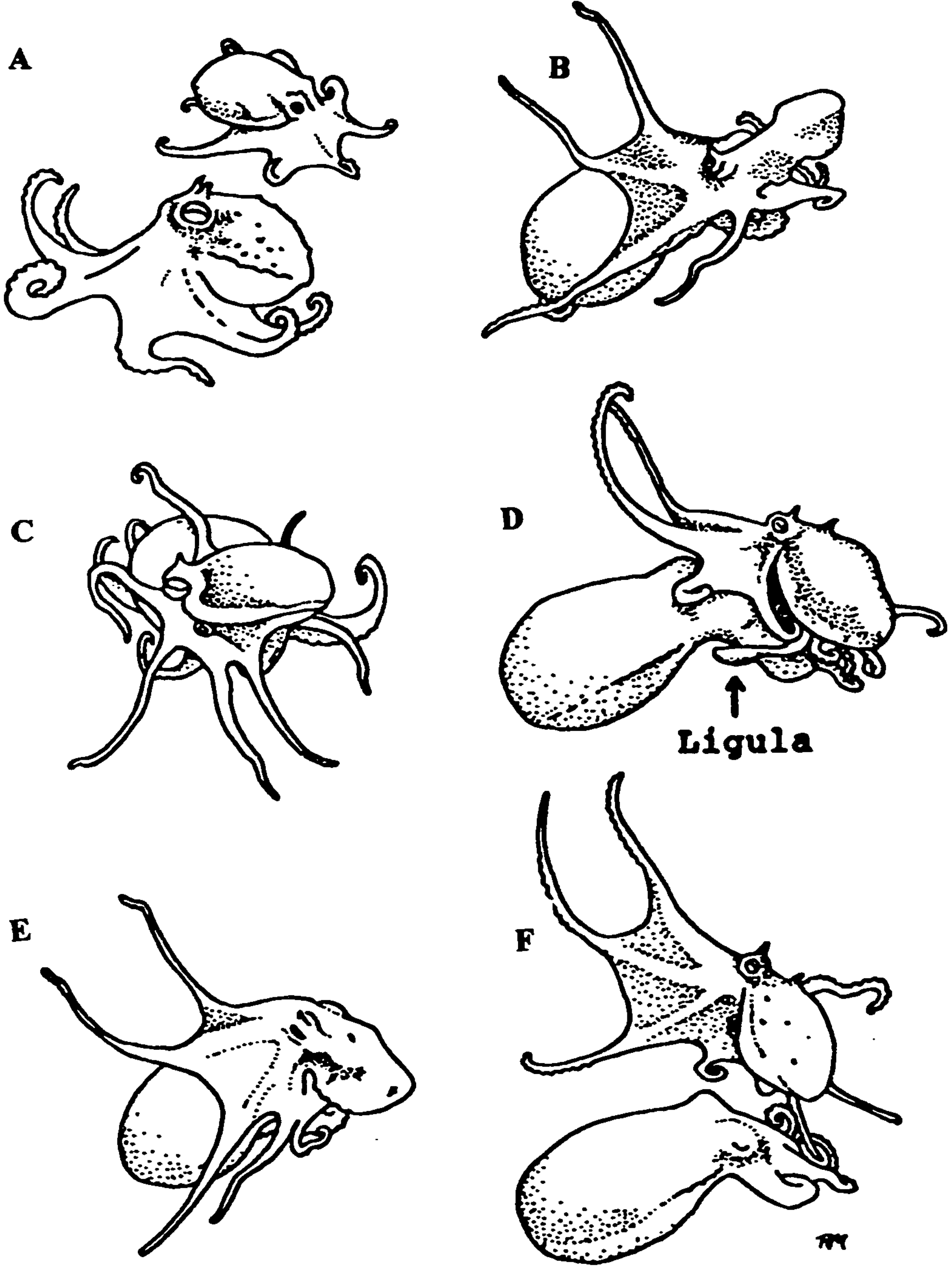
B. arcticus uses the mounting position to mate. The smaller male approached the female (Figure 3A), reached out an arm and touched her, and then immediately initiated mating by mounting her (Figure 3B). He enveloped her mantle in his web and inserted his hectocotylus into the mantle of the female (Figure 3C). Its folded shape suggests that it is actually inserted into the oviduct to open it. During mating the male contracts his mantle in a dramatic pumping arch motion (Figure 3D); this may help the large spermatophore pass to the funnel. I presume that the spermatophore is then passed from the funnel to the large grove on the hectocotylus and that through peristalsis it is transferred to the ligula which is in or on the oviduct. Figure 3E depicts the male withdrawing his ligula as he prepares to leave (Figure 3F). The female remains motionless during mating which lasted 140 seconds. *B. arcticus* mates readily - specimens collected the following year mated in the cooler that they were kept in while still at sea. These matings followed the pattern outlined above.

Discussion

This study presents the first detailed data on the brooding and development time for a deep-sea octopus. *Bathypolypus arcticus* brood eggs for over 400 days. This is longer than the life span of most near-shore octopuses! Additionally, this study describes for the first time the mating behavior of *B. arcticus*. Mating in *B. arcticus* is of interest as these little cephalopods have the largest ligula relative to body size of any octopus.

In this experiment brooding behaviour and developmental time was very consistent

Figure 3. Mating *Bathypolypus arcticus*. The male (foreground) and female octopus (A). He touches then pounces on her (B). C: He mounts her and inserts his ligula into her mantle cavity. D: The male stretches his mantle dramatically (this happened twice) presumably to help pump the spermatophores to his ligula. The male removes his ligula (E) and departs (F). Illustrations by Rebekah McClean.



between the two females despite differences in egg size, female size, and time of laying. This suggests that the length of the brooding period is relatively fixed at a given temperature.

Brooding *B. arcticus* in this experiment were kept at average temperatures of 7.3 and 7.8 °C, which are significantly higher that the 4 ± 2 °C SD that O'Dor and Macalaster (1983) report for wild specimens. Therefore, one might expect *B. arcticus* to brood eggs for even longer in nature as length of embryonic development has been shown to depend on temperature in cephalopods (Boletzky 1987b, 1994). Ken Drinkwater (personal communication) reports that average monthly bottom temperatures in the area where the octopuses were collected were as warm as 11-12 °C in August and September of 1994 and that the average annual temperature was 8.0 °C in 1994 - these temperatures are approximately 1.1 °C higher than usual. O'Dor and Macalaster state that laboratory specimens experienced mortality with only brief periods of temperatures from 10 °C to 12 °C while my specimens survived periods of weekly average temperatures in the 11 °C range. Although O'Dor and Macalaster (1983) found no evidence of migration, I point out that migration to warmer water to spawn has not been ruled out. Villanueva (1992) found evidence of up-slope ontogenic migration in *Bathypolypus sponsalis*.

Brooding *Bathypolypus arcticus* occasionally take food to offset the extremely high energetic cost of brooding eggs for over a year. The octopus O'Dor and Macalaster (1983) reported on may have eaten her own eggs to have sufficient energy to brood the remaining 4 through to hatching. If this is true, it shows that *B. arcticus* can brood for a year by eating its own eggs. The cost of such a protracted brooding period is a sharp decrease in fecundity. An alternative explanation is that this female only had a few viable sperm from her mating which occurred at least 5 months earlier. Perhaps she ate the other 36 eggs because they were not viable.

The long brooding period found in *B. arcticus* must have substantial costs of time, energy, and risk of predation. This period may limit *B. arcticus* to a semelparous strategy as the costs of brooding are not worth the effort unless the number of eggs and their chance of survival is sufficiently large. Historicity, or phylogenetic legacy (Williams 1992), may also limit many octopuses to a semelparous strategy.

Parental care is necessary for all incirrate octopus eggs as they lack a protective egg case. Without the mother caring for and defending her eggs, they would be rapidly suffocated by fouling organisms (Boletzky 1994). However, the length of this brooding period varies. *B. arcticus* benefit in several ways from their large young and long brooding period. Sibling competition favour smaller broods of larger eggs (Stearns 1992). Longer brooding periods may be selected for in *B. arcticus* as the egg stage is likely to have a high survival rate compared to hatchlings. Stearns (1992) notes that "selection should increase the proportion of time spent in the safest developmental stages". Yampolsky and Scheiner (1996) discuss demographic reasons that favour large offspring in cold environments for exothermic animals.

As *Bathypolypus arcticus* are not found in high densities (O'Dor and Macalaster 1983), chances to mate may be rare for these octopuses. The female's ability to store viable sperm for at least 5 months (often incorrectly cited as 10 months, see O'Dor and Macalaster 1983) and the male's large spermatophores and ligula are likely adaptations to maximize fitness under such conditions. It is possible that large ligula and spermatophores are selected for in sperm competition. Cigliano (1995) notes that octopuses meet the criteria for sperm precedence and that the spoon-shaped ligula could be used to scoop out competitors sperm. Another (non-exclusive) possibility is that the large ligula expands once in the oviduct to anchor the hectocotylus during mating. Further investigation will be needed to confirm or reject these theories.

The fact that a squishy, soft-bodied cephalopod can survive collection in a scallop trawl, days at sea, and acclimation to laboratory conditions, indicates the hardiness of *B. arcticus*. Less traumatic collection techniques that specifically target deep-sea cephalopods are allowing investigators to study more species of deep-sea octopus in the laboratory. Researchers at the Monterey Bay Aquarium and Monterey Bay Aquarium Research Institute used an ROV specifically to capture and film cirrate octopuses and they have been able to keep these octopuses alive for several months (Stein Hunt and Hochberg, personal communication).

Obviously, we are just beginning to understand the life history of deep-sea octopuses. Additional studies involving modern sampling techniques such as video monitoring from submersibles and remote operated vehicles (see Vecchione and Roper 1991) will continue to complete the picture of how deep-sea cephalopods behave in nature. However, as deep-sea cephalopods cannot always be found, workers must hope for chance encounters. Telemetry has provided more continuous records of the behaviour of *Loligo* and *Nautilus* (Carlson et al. 1984, O'Dor et al. 1993, O'Dor et al. 1994) and could be employed to study other deep-sea cephalopods as well. Cost and logistical problems limit the use of these techniques.

It is widely acknowledged that laboratory conditions can bias results. However, laboratory studies currently provide the best and most direct means of examining development time, life span, reproductive strategy, growth rates, and other processes that occur in individuals over time. Also, the effect of variables such as temperature, sex, and diet can be analyzed and separated. Laboratory conditions allow the experimenter to observe and manipulate behaviour such as mating or hatching. Further laboratory studies of hardy deep-sea species are desperately needed if we want to understand how these organisms live in their cold, dark world.

Chapter 5

Do larger cephalopods live longer? The effects of temperature and phylogeny on interspecific comparisons of age and size at maturity.

Abstract

The relationship between size and age at maturity in cephalopods is unresolved. The most recent interspecific comparison of size and age of cephalopods contradicts two previous studies by concluding that larger species do not live longer. This paper addresses the confounding effects of temperature and phylogeny while answering the question, "Do larger cephalopods live longer?" To test this hypothesis, life-history data from 18 species of cephalopods, from five orders, with sizes at maturity spanning five orders of magnitude, were obtained from the literature. Without temperature consideration and with *Nautilus* included in the sample, regression analysis suggests ($r^2 = 0.376$, $p = 0.007$) that larger cephalopods take longer to reach maturity. Once temperature was controlled by using physiological time (degree-days), the coleoid cephalopods moved closer to the best fit line and the genus *Nautilus* became an outlier. When *Nautilus* was removed and time measured in degree-days, the relationship was very strong ($r^2 = 0.785$, $p<0.001$). I conclude that coleoid cephalopods achieve larger size by delaying maturity and that temperature, as well as phylogeny, must be considered when making interspecific comparisons.

Introduction

Cephalopods are an ancient molluscan class notable for their active lifestyle and well-developed senses. They range in adult size from *Idiosepius*, an eight mm long sepiolid, to the giant squid, *Architeuthis,* which can grow to over fifteen m in length, making it the world's largest invertebrate. Size at maturity is a critical trait, as larger animals can produce more offspring and possibly provide better parental care (Stearns 1992). Cephalopods are found from the poles to the tropics and from the intertidal zone to the abyss. They are also important predators in the world's oceans, with high growth rates and wet weight food conversion efficiencies of 30 to 60% (Clarke 1996; Hanlon and Messenger 1996).

All extant cephalopods (including octopuses, squid, cuttlefish and sepiolids) except *Nautilus* species are classified in the subclass Coleoidea (Sweeney and Roper 1998). Members of the genus *Nautilus*, the only genus in the subclass Nautiloidea, are the only externally shelled cephalopods. They are known to have morphological, behavioral and life-history differences that easily distinguish them from all other cephalopods (Calow 1987; Hanlon and Messenger 1996; Sweeney and Roper 1998).

By comparing different species of cephalopods, several workers have suggested that a positive relationship exists between size and age at maturity. Forsythe (1984) compared six species of octopuses and concluded "that larger octopuses have longer life spans and smaller species such as *Octopus joubini* have shorter life spans." Van Heukelem

(1976) examined the life span of cephalopods from four orders and concluded that "in general, the larger species have longer life spans." However, Calow (1987) was the first and only researcher to statistically test this hypothesis. His results differed from the two previous studies. Calow quantified time to maturity and size at maturity for a broader sample of cephalopods from four orders. Unlike the previous two authors, his data set included *Nautilus* spp. and *Bathypolypus arcticus*.

Bathypolypus arcticus is a small octopus that is most common at depths of 200-600 m and at low temperatures of 4 ± 2 °C in the Atlantic Ocean (O'Dor and Macalaster 1983). Calow did not find a significant relationship between size at maturity and life span, and, therefore, concluded that species that are large at maturity achieve their larger size by growing faster. This conflict may have arisen because none of these authors made any attempt to normalize for temperature effects and because the anomalous *Nautilus* genus was not separated from the coleoid cephalopods.

Temperature is known to have a considerable effect on the metabolism and body size of ectotherms, which make up approximately 99% of the Earth's species (for a recent review of intraspecific effects of temperature see Atkinson and Sibly 1997). Ectotherms require a certain amount of time and temperature for growth to adult size (Begon et al. 1990). For example, *Octopus bimaculoides* kept at 23 °C had their life span shortened by about 20% but were three times larger when five months old compared to octopuses raised at 18 °C (Forsythe and Hanlon 1988).

In a changing environment, temperature variation is likely to affect the population biology of short-lived, semelparous cephalopods more than longer-lived, iteroparous fish

(Forsythe and Hanlon 1988). Forsythe (1993) developed a model based on laboratory growth rates of *Loligo forbesi* at known temperatures. This model demonstrated that even a 1 °C increase in temperature during the 90-day exponential growth phase causes a doubling of weight. A 2 °C increase in temperature predicted that animals grow five times larger!

This study investigates the effect temperature has on time to maturation by using physiological time, i.e. degree-days. Degree-days are calculated by multiplying the average daily temperature in degrees Celsius by the time in days (Begon et al. 1990). For example, *Illex illecebrosus* eggs take 16 days to develop at 13 °C (O'Dor and Dawe 1998). This yields 208 degree-days.

A complication, in addition to temperature, is the inclusion of a member of the subclass Nautiloidea, which represents fewer than 1% of all extant species of cephalopods and is very different from their modern cousins. Not only do these ectochochleates have an external shell, a primitive eye which is open to the sea and two pairs of gills, their life-history traits also reflect their distant relationship to the other cephalopods. Members of the genus *Nautilus* lay extremely large eggs, have long life spans and are iteroparous polycyclic spawners (Saunders and Landman 1987; Ward 1987). Although there is debate about when Nautiloidea split from the other cephalopods, the scheme presented by Hanlon and Messenger (1996) shows the subclasses Nautiloidea and Coleoidea diverging in the Ordovician period.

Another difficulty with interspecific comparisons of size and age at maturity in cephalopods is the large range of variation within a species. Differences in temperature as well as other abiotic and biotic factors can affect the exponential growth rates of young cephalopods and cause large differences in size and age at maturity (Forsythe and Hanlon 1988; Forsythe 1993). For this reason, I use average sizes at maturity from animals that live at known temperatures. I sought new data to include species that mature over a large range of sizes, which will further decrease the effect of intraspecific differences in size at maturity. Using species from a variety of taxa, as well as habitats, should best represent cephalopods as a group; I have used all available data.

The primary objective of this study was to compare size at maturity and life span of a variety of cephalopods with a modern data set to resolve the question, "Do larger cephalopods live longer?" The secondary objective was to identify confounding factors that can bias this type of interspecific comparison. I analyzed a data set which included temperature as a factor to test the hypothesis that larger cephalopods take longer to reach maturity. Both standard time (days) and physiological time (degree-days) were used in the analysis to examine the importance of controlling for the effect of temperature when studying life-history traits with a time component. As Calow's (1987) data included *Nautilus* spp., my analysis was undertaken both with and without the genus *Nautilus* to determine whether including this ancient group compounded the effect of ignoring the role of temperature in the life-history trends in modern cephalopods.

Materials and methods

Data from the Literature

Life-history data were collected from the literature (Table 1) on 18 representative species of cephalopods. The data set included species from five orders: Nautilida (*Nautilus*), Octopodida (octopuses), Sepiida (cuttlefish), Sepiolida (sepiolids) and Teuthida (squid), following Sweeney and Roper (1998). For consistency, all of the species used by Calow (1987) that data could be found for were retained, but in many cases more recent and accurate data were available and used. In some cases, only data from fishery estimates were available, but whenever possible data from animals that had been reared through their entire life cycle in the laboratory at constant temperature were used. This ensured more uniform adult sizes as well as increasing the accuracy of the age and temperature data used to calculate degree-days. When several references are summarized in a single value, a simple numerical average was taken across sources (e.g. values of 0.035, 0.04 and 0.045 g reported for hatchling *Octopus "joubini"* weights were averaged to 0.04 g). See Appendix 6 for specific details on where each number came from. There is some question as to the identity of the octopus that has been referred to as "*Octopus joubini*" in the literature (see Forsythe and Toll 1991).

Although there is considerable within-species variation in cephalopods (Yang et al. 1986), I used average size at maturity at a given temperature, as it best describes a typical member of that species. I avoided using range data, which are much more variable and may not reflect an "average" member of a species. Where published averages of the traits of interest do not exist, the midpoint of range data was used. Bell (1980) used a similar method to compare age at maturity in placental mammals, Squamata (reptiles), amphibians and freshwater fish. As the largest cephalopod my data set is 100,000 times larger in size

Table 1. Data set of hatchling size, size at maturity, age at maturity, average lifetime temperature, age at maturity, growth rate and physiological growth rate for 18 species of Cephalopods. Data from the laboratory (L) and from the field (F). (G= growth; T= temperature). Values used in the calculations can be obtained upon request from the author.

Taxon	Abbr.	Data	Hatchling Size (g)	Size at Maturity (g)	Age at maturity (days)	Avg. Lifetime Temperature (°C)	Age at Maturity (degree-days)	G	G/T
Subclass Nautiloidea									
Order Nautilida									
Family Nautilidae									
Nautilus belauensis	Nb	L,F	5.9[a]	1157[b]	3978[c]	13[d]	51714	0.13	0.01
Subclass Coleoidea									
Order Octopodida									
Family Octopodidae									
Bathypolypus arcticus	Ba	L,F	0.214[e,f]	70[f]	1400[e]	4.0[f]	5600	0.41	0.103
Octopus bimaculoides	Ob	L	0.07[g]	545[g]	341[g]	23.0[j]	7843	2.63	0.114
Octopus dofleini	Od	F	0.025[h,i]	16300[h,i]	1019[i,j]	10.7[h,i]	10903	1.31	0.123
Octopus sp. "*joubini*"	Oj	L	0.04[k,l,m]	30[k]	182[k]	25[k,l]	4550	3.64	0.145
Octopus maya	Om	L	0.093[n]	3262[n]	257[n]	25[n]	6425	4.07	0.163
Octopus vulgaris	Ov	L,F	0.0014[o]	2417[p]	435[p]	18.3[p]	7960	3.3	0.18
Order Sepiida									
Family Sepiidae									
Sepia officinalis	So	L	0.175[q]	1200[q]	335[q]	22.5[q]	7538	2.64	0.117
Sepia pharaonis	Sp	L	0.1[r]	84.1[r]	110[r]	30[r]	3300	6.12	0.204
Sepiella inermis	Si	L	0.01[r]	36.6[r]	90[r]	30[r]	2700	9.12	0.304

Order Sepiolida									
Family Idiosepiidae									
Idiosepius pygmaeus	Ip	L,F	0.00033[a]	0.175[s]	50[s]	25.2[s]	1260	12.55	0.498
Family Sepiolidae									
Euprymna scolopes	Es	L	0.005[t]	2.67[t]	80[t]	23[t]	1840	7.85	0.341
Order Teuthida									
Family Loliginidae									
Loligo forbesi	Lf	L,F	0.007[u]	910[v]	365[u]	14[a]	5110	3.23	0.23
Loligo opalescens	Lo	L	0.001[w]	21.2[w]	184[w]	16[w]	2944	5.41	0.338
Loligo vulgaris	Lv	F	0.003[x]	447[y]	245[y]	14[a]	3430	4.86	0.347
Sepioteuthis lessoniana	Sl	L	0.044[z]	122.7[r]	90[r]	30[r]	2700	8.81	0.294
Family Ommastrephidae									
Illex illecebrosus	Ii	L,F	0.00015[A]	400[B]	365[C]	12.6[B]	4599	4.05	0.322
Family									
Thysanoteuthis	Tr	F	0.002[a]	8966[D]	240[D]	24.5[D]	5880	6.38	0.26

Footnotes are as follows: a-see Materials Methods, b-Saunders and Spinosa 1978, c-Landman and Cochran 1987, d-Saunders and Ward 1987, e-Wood et al. 1998, f-O'Dor and Macalaster 1983, g-Forsythe and Hanlon 1988, h-Cosgrove 1993, i-Hartwick 1983, j-Cosgrove 1976, k-Hanlon 1983, l-Forsythe 1984, m-Forsythe and Hanlon 1981, n-Van Heukelem 1983, o-Villanueva 1995, p-Mangold 1983, q-Forsythe et al. 1994, r-Nabhitabhata 1995, s-Lewis and Choat 1993, t-Hanlon et al. 1997, u-Forsythe and Hanlon 1989, v-Martins 1982, w-Yang et al. 1986, x-Packard 1969, y-Coelho et al. 1994, z-Hanlon 1990, A-O'Dor et al. 1986, B-O'Dor 1983, C-O'Dor and Dawe 1998, D-Nigmatullin et al. 1995.

than the smallest, the methods outlined here should be robust enough to handle some error in size or age at maturity estimates, as such errors should be small relative to the total range.

Calculations

Life-history traits were quantified, and the following measurements were recorded: size at maturity (weight in grams of a mature female), age at maturity (time in days until first reproduction), physiological age at maturity (time in degree-days until first reproduction), and instantaneous relative growth rate (G) calculated by:

$$G = \frac{\ln W_2 - \ln W_1}{(t_2 - t_1)}$$

where W_2 is the weight at maturity, W_1 is the weight at hatching and $t_2 - t_1$ is the time between hatching and maturity (Forsythe and Van Heukelem 1987). Physiological instantaneous relative growth rate (G/T) was defined as simply the above, with time in degree-days.

Estimates

In a few cases, the required data had to be estimated. There are, for example, no published weights of hatchling *Nautilus* spp. The weight of a hatchling *Nautilus belauensis* was estimated using hatchling shell size and a regression analysis of the cubed shell diameter versus the weight of seven young *N. belauensis* that weighed less than 50 g (Carlson et al. 1992) in addition to a single hatchling *N. pompilius* that was weighed for this study on April 24, 1996, at the Waikiki Aquarium. The hatchling *N. pompilius* weighing 4.33 g, with a maximum shell diameter of 26.25 mm, fit a highly significant correlation ($r^2 = 0.990$, $p < 0.001$) between cubed shell diameter and weight, which indicates that a hatchling *N. belauensis* with a 30 mm shell diameter (Carlson et al. 1992) would weigh approximately 5.9 g.

Five preserved hatchling *Idiosepius pygmaeus* were weighed by G. Pecl (James Cook University, Townsville, Australia pers. comm.), and their average weight of 0.33 ± 0.06 mg used. There are no published hatchling weights for this species.

To estimate hatchling weight for *Thysanoteuthis rhombus*, a value of 80% of egg weight was used. *T. rhombus* egg weight was calculated from the egg diameters reported by Nigmatullin et al. (1995). I assumed these eggs were spherical (volume = $4/3\pi r^3$) and had a density close to water. *T. rhombus* hatchlings were estimated to weigh 0.002g. Error in this estimated hatchling weight would affect the comparison of growth and size at maturity (Table 2) but not the size and age at maturity (Figures 1, 2; Table 3).

Bathypolypus arcticus were included because they were used in Calow's (1987) analysis, and the authors have experience with this species. While O'Dor and Macalaster (1983) have published size at maturity of 70g, size at hatch of 0.15g, temperature of 4°C and estimated age at maturity of 2 years, recent rearing studies indicate that age of maturity in their study was significantly underestimated. The hatchlings have very slow growth rates. For example, one juvenile fed crustaceans *ad libitum* took 583 d to reach 3.8 g at 6°C (2215 degree-days). This predicts maturation at 1400d at 4°C when modeled conservatively. Other individuals grew at similar rates (Wood unpublished data).

Regional temperature for the Azorean *Loligo forbesi* was estimated by averaging

Table 2. Correlation between growth rate and size at maturity. Note the negative correlations between growth rate and size at maturity in all cases. (G= growth; T= temperature)

Comparison	Pearson Correlation
G vs. coleoid and nautiloid size at maturity	-0.663
G vs. coleoid size at maturity	-0.662
G/T vs. coleoid and nautiloid size at maturity	-0.624
G/T vs. coleoid size at maturity	-0.633

Figure 1: Log_{10} of weight at maturity versus $\log_{10}$ of age at maturity measured in days for 17 coleoid species and one nautiloid species. With *Nautilus* in the sample ($y=1.620x - 1.609$, $r^2 = 0.376$) as represented by the dashed line. The solid line shows the relationship when *Nautilus* is removed from the sample($y=2.228x - 2.979$, $r^2 = 0.451$). Full names of species are listed in Table 1.

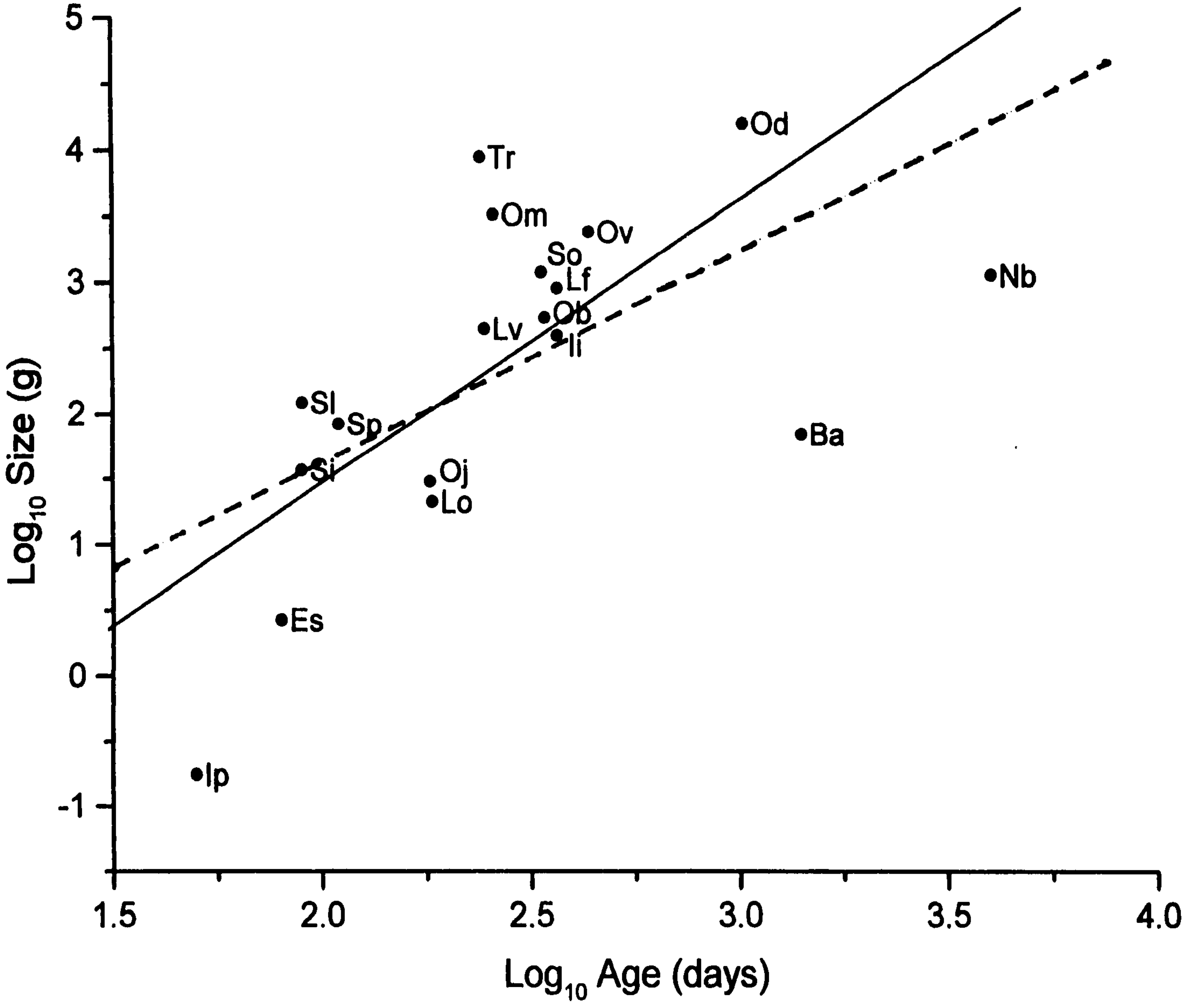

5
4
3
2
1
0
-1
Log$_{10}$ Size (g)
1.5
2.0
2.5
3.0
3.5
4.0
Log$_{10}$ Age (days)
Od
Tr
Om
Ov
So
Lf
Nb
Ob
Lv
Ii
Sl
Sp
Ba
Si
Oj
Lo
Es
Ip

Figure 1

Figure 2: Log_{10} of weight at maturity versus $\log_{10}$ of age at maturity in degree-days for 17 coleoid species and one nautiloid species. With *Nautilus* in the sample the equation of the line and r^2 are $y=2.526x - 7.001$ and 0.502 respectively as represented by the dashed line. The solid line shows the relationship when *Nautilus* is removed from the sample ($y=4.546x - 14.238$, $r^2 = 0.758$).

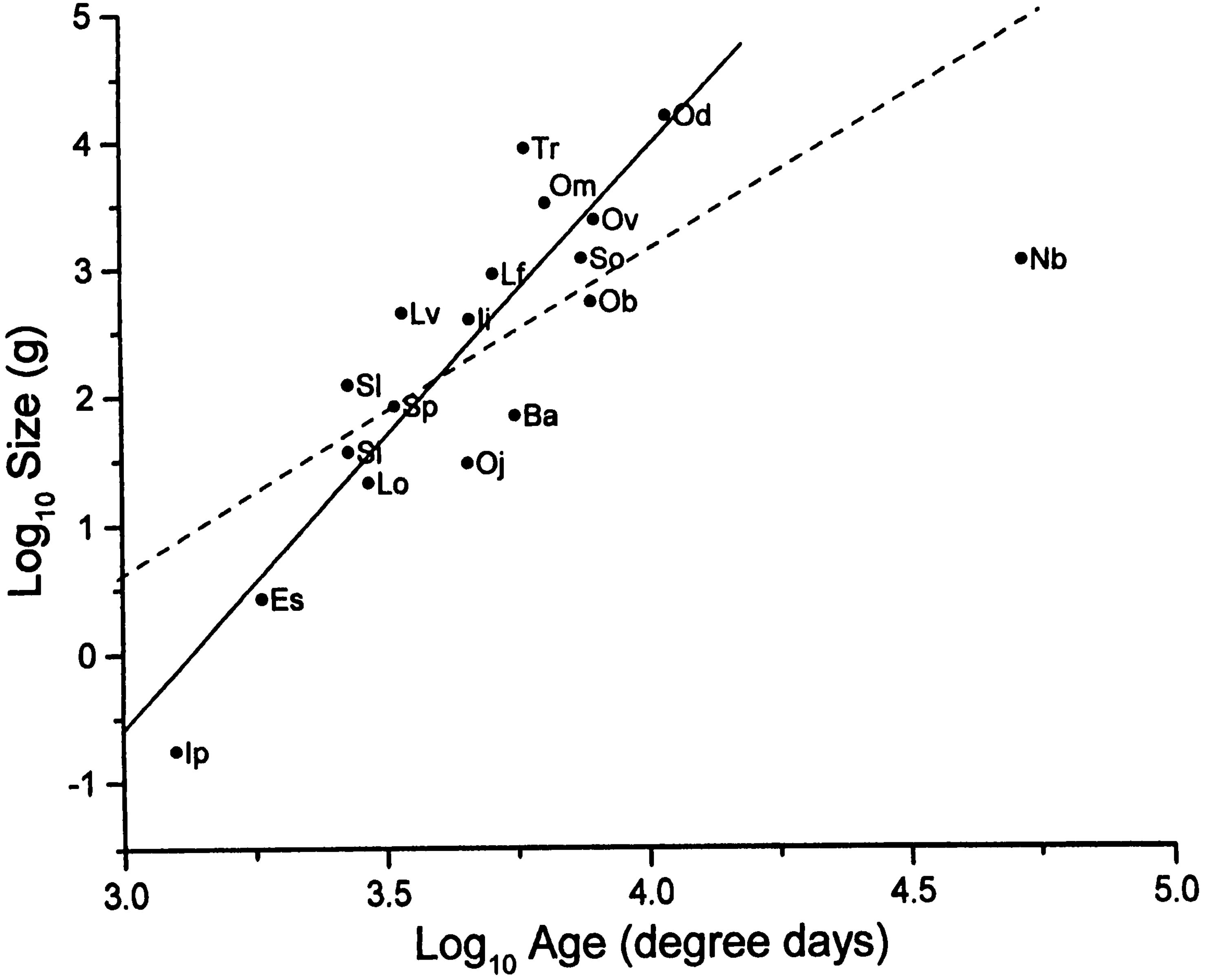

Log$_{10}$ Size (g)
5
4
3
2
1
0
-1
3.0
3.5
4.0
4.5
5.0
Log$_{10}$ Age (degree days)
Od
Tr
Om
Ov
So
Nb
Lf
Ob
Lv
Ii
Sl
Sp
Ba
Sl
Oj
Lo
Es
Ip

Figure 2

Table 3. Predicted size at maturity from age in days and age in degree-days for cephalopod groups including and excluding nautiloids. (H_o= null hypothesis)

Cephalopod group	R Squared	P-value	Conclusion
Age in days			
Coleoid and Nautiloid	0.376	$p=0.007$	Reject H_o
Coleoid only	0.451	$p=0.003$	Reject H_o
Age in degree-days			
Coleoid and Nautiloid	0.502	$p=0.001$	Reject H_o
Coleoid only	0.785	$p<0.001$	Reject H_o

temperatures at 200 m depth for 40°N; 28°W and 36°N; 28°W (Fuglister 1960). A depth of 200 m was used as that is where adults are fished and the depth where tagged specimens were observed (O'Dor et al. 1994). A similar estimate of local temperature was made for the *L. vulgaris* population used in the analysis.

Statistical analysis

Calow's (1987) hypothesis that larger cephalopods grow faster was investigated by examining the Pearson correlation of growth (G) and growth/temperature (G/T) on size at maturity (Table 2). This was done both with and without *Nautilus* spp. in the data I used for analysis. Life-history data were $\log_{10}$ transformed to prevent heterogeneity of variance.

The hypothesis that larger cephalopods live longer was tested with regression analysis using the Systat 7 statistical package (Table 3). A linear regression analysis was completed using data from Table 1. The analysis was repeated four times thus testing all combinations of time, measured in both days and degree-days in the presence or absence of *Nautilus* spp. Results of the various analyses were compared.

An additional regression analysis was done on the life-history traits of coleoid cephalopods at the taxonomic family level. This was done in order to determine if non-independent data from closely related species were responsible for the results. For example, my data set has six species of octopuses all in the same family, and five of these six are in the same genus. It could be argued that these five or six species should be treated as one data point (Harvey et al. 1995). When there was more than one species in a family I used the species that is first alphabetically.

Results

Using my data set, with *Nautilus* in the sample and time measured in calendar days, I found a significant positive relationship between age and size at maturity in cephalopods (r^2=0.376, p=0.007, Table 3). While this regression has a significant non-zero slope, Figure 1 shows a high degree of scatter. Both *N. belauensis* and *Bathypolypus arcticus* take more than 1000 days to reach maturity although they are not particularly large cephalopods.

When *Nautilus* was removed from the sample, the fit improved (r^2=0.451, p=0.003, Figure 1). *Bathypolypus arcticus* was an outlier in this comparison (studentized residual = −3.7). This may be due to the fact that *B. arcticus* lives at 4°C, while most of the other species live around 20°C.

With *Nautilus* back in the sample, time measured in degree-days improved the relationship (r^2=0.502, p=0.001, Figure 2). The use of degree-days brought the coleoid cephalopods including *B. arcticus* noticeably closer together, but *N. belauensis* was an outlier in this analysis (studentized residual = −4.5).

For coleoid cephalopods, there was a strong significant relationship between size at maturity and physiological time (r^2=0.785, $p<0.001$, Figure 2). A visual comparison of Figure 2 with Figure 1 confirms that there is much less variation in the data around the regression line. The equation of the solid line is $y= 4.546\pm0.615(x) - 14.238\pm2.240$, with the ± indicating the standard errors. Using this equation, five of the six squid are above the line while four of the six octopuses are below. The three cuttlefish are near the line while the two sepiolids are below and near the origin. Of the species for which laboratory data were used exclusively, four were above the line and five below. When both laboratory and field data were used, the split was even with two above and below the line. However, the three species from which data were obtained exclusively from the field (Lv, Tr, Od) were above the line. This relationship is still stong when analyzed at the family level (r^2=0.854, p=0.003). However, the sedentary deep-sea octopus *Bathypolypus arcticus* and the oceanic squid *Thysanoteuthis rhombus* were outliers with studentized residuals of −2.1 and 2.1, respectively (Figure 3).

I also examined the relationship between growth rate and size at maturity, both with and without *Nautilus* and with temperature in calender days and in degree-days. In all cases, there was a negative correlation between growth rate and size at maturity (Table 2).

Discussion and conclusions

Size and age at maturity

When degree-days are used, results indicate that larger size at maturity in coleoid cephalopods is a consequence of longer life spans rather than faster growth rates. As shown in Table 3, size at maturity is significantly and positively correlated to physiological age. Furthermore, no evidence that supports the hypothesis that larger cephalopods grow faster was found (Table 2). In all cases, the correlation between growth rate and size at maturity was negative in the range of −0.624 to −0.663. If anything, cephalopods that

Figure 3: Log_{10} of weight at maturity versus $\log_{10}$ of age at maturity in degree-days for representatives of seven families of coleoid cephalopods. $y = 5.262x - 16.876$, $r^2 = 0.854$.

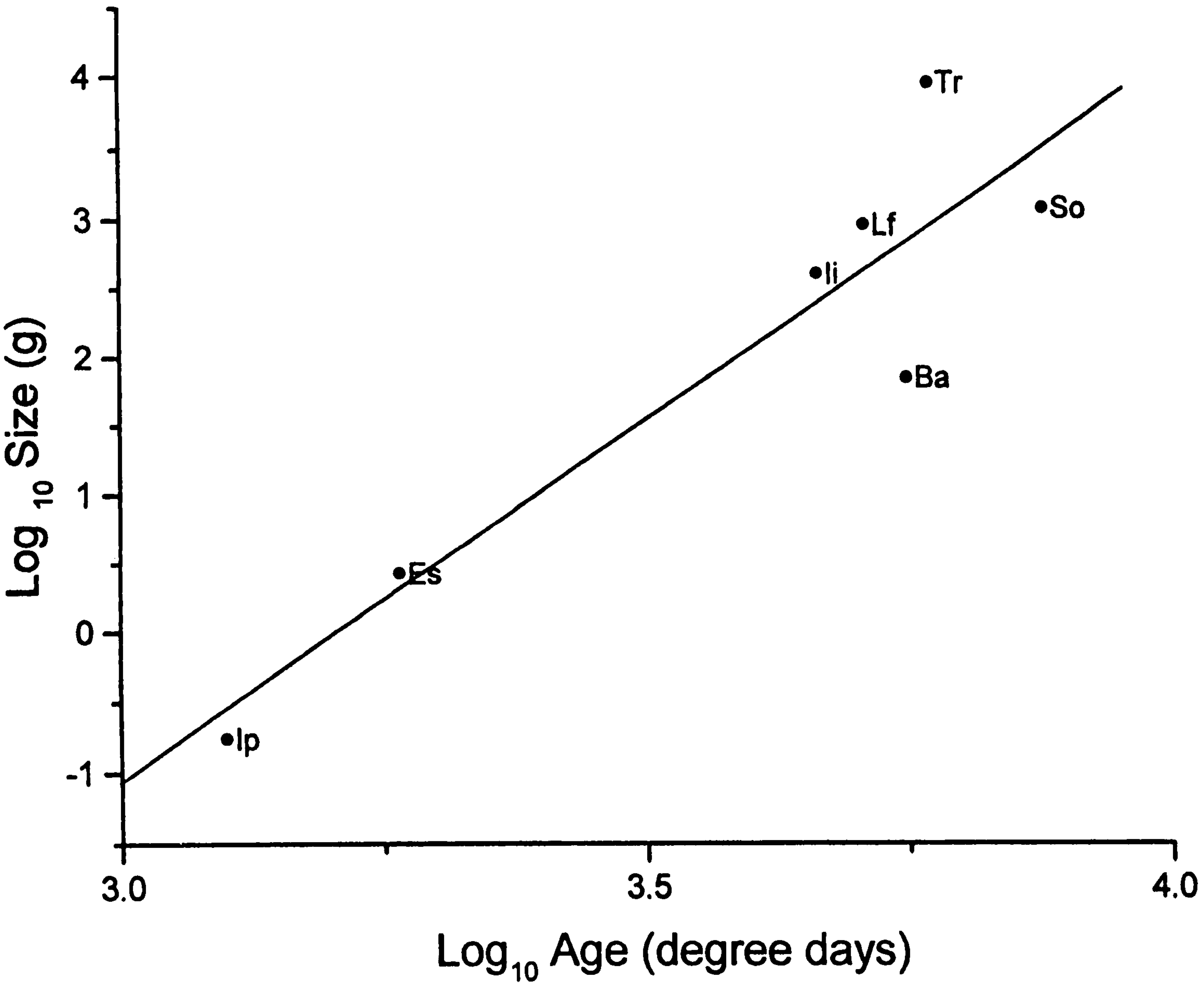

Log $_{10}$ Size (g)
4
3
2
1
0
-1
●Tr
●So
●Lf
●Ii
●Ba
●Es
●Ip
3.0
3.5
4.0
Log$_{10}$ Age (degree days)

Figure 3

mature at smaller sizes grow faster. I interpret these results as evidence for a life history trade off in coleoid cephalopods between maturing early at a smaller size and maturing later at a larger size. Organisms that mature earlier spend less time exposed to predators and therefore are more likely to survive to reproduce due to the shorter period between their birth and maturity. Additionally, organisms that mature earlier increase their fitness because the offspring are produced sooner, and these offspring also start reproducing earlier. Delaying maturity leads to higher fecundity, which also increases fitness (Stearns 1992). However, the risk of mortality before reproduction is expected to increase as age at maturity increases. For modern cephalopods, my results support Van Heukelem's (1976) and Forsythe's (1984) conclusions that larger cephalopods have longer life spans.

***Nautilus* as a confounding factor and lineage effects**

Members of the genus *Nautilus* represent fewer than 1% of extant cepahlopods. When making the generalization that cephalopods which live longer mature at a larger size, Van Heukelem (1976) likely meant modern cephalopods, as all his examples were coleoids. As shown in Table 3, removing *Nautilus* from the analysis improved the relationship between age at maturity and size at maturity.

The inclusion of Nautiloidea appears to mask the significant correlation in the rest of the group. Figure 2 graphs size versus age in degree-days. *Nautilus* is an outlier from the trend set by the other cephalopods and is thought to live and reproduce for years after reaching maturity (Saunders and Landman 1987; Hanlon and Messenger 1996). This is unlike coleoid cephalopods which have short life spans and are typically semelparous. Had life span been used instead of age at first maturity, the iteroparous *Nautilus* would have been even more of an outlier. Removing *Nautilus* makes biological as well as statistical sense.

Unlike *Nautilus*, the coleoid cephalopods do not noticeably deviate from my reported trend, nor do all the members of these groups cluster together. Even species in the same genus, such as *Octopus*, are not clustered. However, there may be a small lineage effect in growth rates between Octopodida and Teuthida. Most of the octopuses mature at a lower weight than predicted by the regression line in Figure 2 and have lower growth rates (Table 1), while five of the six squid have higher growth rates. The cuttlefish appeared between these two. These patterns should be explored as better data become available for a wider variety of species.

Some researchers argue that it is incorrect to apply phylogenetic correction techniques as proposed by Harvey et al. (1995) in all comparative analyses (Westoby et al. 1995). Although I am arguing for a lineage-specific relationship between size and age in coleoid cephalopods, Harvey et al. (1995) argues that species may not be the appropriate unit for comparison as they are not independent. What if one taxon, say the genus *Octopus* or the family Octopodidae, has a strong relationship between size and age at maturity due to lineage effects while the other groups do not? This would likely be noticed in Figure 2, but one can alternatively sacrifice sample size to increase the number of independent evolutionary events in the lineage (Stearns 1992).

To eliminate such biases, I used the first species alphabetically for the each of the seven coleoid families listed in Table 1 to test the regression of age on size at maturity. As the results (r^2=0.854, p=0.003) were similar to those found at the species level, the relationship that larger cephalopods live longer holds when the data set is analyzed at the family level.

The importance of temperature

Calow (1987) acknowledged that temperature may affect cephalopod life-history traits. Perhaps because the data were limited, Calow did not control for temperature. In this study, using degree-days to measure time increased the significant relationship between age and size at maturity. There will be some error in the effect of lifetime temperature estimates in environments where the temperature changes as the cephalopods mature (Forsythe 1993; Rodhouse 1998). This is because seasonal and other changes in temperature will have a greater effect on exponentially growing hatchlings than on older cephalopods. I found that once time was measured in degree-days, 78% of the variability of size at maturity in coleoid cephalopods is predicted by age at maturity. With better data and a more complicated and accurate model that examines age-specific effects of temperature, I expect a further reduction of the unaccounted variability in size at maturity.

Confounding factors can dramatically change results when testing life-history hypotheses or constructing phylogenetic trees based on life-history traits. Temperature affects growth rates in ectothermic animals such as cephalopods. Using physiological time (e.g. degree-days) is one way to test interspecific life-history hypotheses for ectotherms. For example, *Bathypolypus arcticus* is a small octopod that lives at 4 °C. This octopod takes much longer to mature than a similarly sized Florida Pygmy Octopus (*Octopus "joubini"*) which lives at 25 °C. I argue that, while there are significant behavioral, physiological, ecological and life-history differences between these two species, an eightfold difference in life span is not as significant as it first appears. Most, but not all, of the difference in life span is due to the effect of temperature, not any real differences in life-history traits. If temperature is not taken into account and the species examined come from a broad range of environments, an interspecific correlation between size at maturity and life span is primarily a correlation between size at maturity and the temperatures at which the different species live.

Although Forsythe (1984) and Van Heukelem (1976) did not explicitly control for temperature, the effect of temperature on growth rates and life spans of the species that they examined would have been much less. This is because the species that they examined did not live in as wide a range of temperatures as the species examined by Calow (1987) and this paper.

Environmental variables

At a given temperature, growth rate and size at maturity increase with resource availability (Atkinson and Sibley 1997). Differences in resource availability are expected to cause some variation in the data. However, cephalopods studied to date seem to be limited by predation, not food. For example, squid may rarely be out of food because, when their preferred foods such as fish and crustaceans are limited, squid will prey on other squid in their school (Dawe and Brodziak 1998). Octopuses spend very little time foraging, which may be optimal behavior if the octopuses are minimizing exposure to predators by limiting their hunting time and not actively defending territories (Mather and O'Dor 1991; Mather 1993). Aronson (1986) showed that the number of octopuses was inversely related to the number of predatory fish present. While food is an important biotic factor, these examples suggest that it is often predation risk, and not food that limits the growth of wild cephalopods.

Due to cephalopods' excellent senses, mobility and nocturnal habits, it is especially difficult to make direct observations of cephalopods in the field. Researchers depend largely on laboratory-based culture programs for cephalopod life-history data (Boletzky and Hanlon 1983). Although there are few studies comparing field and laboratory growth rates in cephalopods, much of the available information indicates that the high laboratory growth rates are not abnormal. Animals caught in the wild and transferred to the laboratory often do not feed as readily or grow as fast. "Wild caught cephalopods are particularly prone to mechanical damage," and this is especially true for squid (Boyle 1991). The squid *Loligo opalescens* grows faster in the field than those cultured in the laboratory (Jackson 1994). Pecl and Moltschaniwskyj (1999) found differences in cellular growth mechanisms between the sepiolid *Idiosepius pygmaeus* from the field and those collected and then kept in 30cm^3 glass aquaria for 7 days. However, many species of coastal cuttlefish and octopus thrive in the laboratory and have been kept for several generations. Van Heukelem (1976) found no difference in growth between branded wild *Octopus cyanea* and well-fed laboratory *O. cyanea*. It is possible that laboratory environments alter growth patterns in pelagic cephalopods like squid, but it is likely that benthic and epibenthic animals like octopuses and cuttlefish are less affected when kept under these conditions.

I found little evidence for differences between the laboratory and field data used in my analysis. Life-history data collected from the laboratory or the laboratory and field accounted for 14 of my 17 species, and both were distributed evenly about the regression line. In only three species were used field data used exclusively, two squid (Lv, Tr) and an octopus (Od). These appear above the line, although the octopus is almost on the line. By using field data, workers frequently underestimate the effect of temperature because of sparse information on what the fast growing planktonic phase is exposed to and for how long. Temperature data are often based solely on adults, even though influences on the fast-growing young may have a substantial impact on later life. These young may, for example, be found higher in the water column, in warmer waters, than the better-sampled adults.

Species are likely to adapt to the temperature and environment at which they live. According to Begon et al. (1990), "the effects of different temperatures on ectotherms follow a typical pattern, though there is variation from species to species." This study, like Calow's (1987), used animals that live at a wide range of average temperatures (4°C to 30°C in my case) and a variety of environments. No doubt some of the variation seen in Figures 1 to 3 is caused by adaptation to environmental conditions. Variation of size at maturity, temperature and phylogenetic differences all can confound the analysis, but once addressed, the "typical pattern" underlying the variation appears.

While the above factors are important, my approach is valid. The largest cephalopod in my data set is 100,000 times heavier at maturity than the smallest. Species from a variety of habitats such as open-ocean, deep-sea and coastal areas from five cephalopod orders were used. This broad sample of species with parameters that vary over six orders of magnitude is robust enough to handle the likely errors in age and size at maturity as well as differences in feeding, local conditions, etc. This process does not ignore adaptation; on the contrary, it helps to find it. Species or groups of species can be removed from the analysis if it makes both biological and statistical sense to do so (i.e. *Nautilus*). This method also points to new questions, such as "Why does *Thysanoteuthis rhombus* grow so fast for its size?"

Difficulties

One of the my primary challenges was collecting a data set that could be used for comparison between groups because workers do not measure size in each order in Cephalopoda with units of measurement that are comparable between or even within groups. For example, size in octopuses is usually measured in weight, while size in squid and cuttlefish is measured in mantle length and size in *Nautilus* is measured in shell diameter or shell aperture width. The mantle lengths of one species of squid are usually not comparable to other species of squid due to morphological differences. While there are conversions for many species from mantle length to weight, these conversions are almost always for maturing or mature animals, and I was not willing to extrapolate to hatchlings, as morphology (i.e. the ratio of mantle length to interocular width) often changes with age. Weight is the best measurement for interspecific comparisons of size in cephalopods, as it does not depend on the shape of the animal being measured. Directly measuring weight is the most accurate and sensitive measure of size, as length must be cubed to convert it into weight (Forsythe and Van Heukelem 1987). Locating published cephalopod hatchling weights in the literature was the major bottleneck that limited the number of species used in this analysis. In several cases, hatchling weights for well-studied species could not be found although published mantle lengths were abundant.

Another challenge was that ranges, sometimes over an order of magnitude, are often reported for various cephalopod life-history traits. Averages, preferably with standard deviations, are more useful for this type of analysis and were preferentially used. I encourage workers to publish average and standard deviation values accompanied with similar temperature data.

Future Directions

There is a need for more basic information for interspecific comparisons. Life-history data from deep-sea and open-ocean species are still rare. Size and age at maturity data are needed for investigating lineage effects between squid, cuttlefish and octopus. Life tables for species are essential so that the effect of age-specific mortality can be investigated. Understanding of basic cephalopod biology becomes more and more critical as cephalopods make up an increasing percentage of the world's fisheries (Pauly et al. 1998).

In focusing on the differences between cephalopods and fish (e.g. O'Dor and Webber 1986), cephalopod biologists may understate the differences among cephalopods themselves. For example, Rodhouse's (1998) comment that cephalopods other than *Nautilus* "are generally short lived (usually 1 year) and have monocyclic reproduction and a semelparous life history" is technically correct, but may mislead. Nonetheless, my analysis is consistent with suggestions (Pauly 1998; Rodhouse 1998) that the short, rapid-growth life histories of cephalopods illustrate physiological progenesis. This concept is attractive as it explains the differences between cephalopods and both fish and other molluscs (Bayne et al. 1976, Young and Thompson 1976), but my analyses make it clear that physiological progenesis proceeds on a physiological timescale. Thus, four years at 4°C is still a "short" life span for *Bathypolypus arcticus* in physiological time. The approach described here seems a powerful way of resolving apparent differences among cephalopods from diverse habitats, once we begin to record the right sorts of data.

Chapter 6

Summary of Results

Summary of new contributions of this thesis

1) The effect of different diets on hatchling deep-sea octopuses was investigated. Like near-shore octopuses, hatchling deep-sea octopuses do not grow on frozen food but do well when fed live crustacean diets. Hatchlings did not live on brittle star diets although brittle stars are a common prey of adults in the field. Possible reasons for adults eating foods with low energy per gram ingested such as brittle stars are discussed (Chapter 2).

2) This work presents the first growth rate data for deep-sea or cold-water cephalopods. Growth rates for octopuses fed live crustaceans *ad libitum* ranged 0.117 %/d at 2.4°C to 0.779 %/d at 9.9°C. These growth rates are very low, even when degree-days are used (Chapter 2 and 3).

3) This work presents various estimates of life span for *Bathypolypus arcticus*. For octopuses living at their natural temperatures of 4°C, a conservatively estimated minimum age at maturity of six years is proposed. This doubles the previous estimate of *B. arcticus* life span and suggests that other deep-sea cephalopods may also live much longer than previously thought (Chapter 3). Therefore, the "live fast and die young" paradigm discussed in Chapter 1 does not apply to all coleoid cephalopods.

4) For the first time, methods for rearing cephalopods at temperatures below 10°C for extended periods of time are presented (Chapter 3). One of the most critical aspects to

maintaining deep-sea life in the laboratory is the ability to keep constant low temperatures for long periods of time despite mechanical breakdowns and power outages. The use of multiple fail-safe mechanisms and backup plans is stressed.

5) Copulation of *B. arcticus* is observed and described for the first time. This behavior is of interest due to their large reproductive ligula, large spermatophores, low fecundity and large eggs. Mating is initiated by the usually smaller male mounting the female and inserting his ligula into her mantel cavity. He then transfers one or two large spermatophores in the 2 to 3 minute copulation. Although mating had not been observed until now, mature *B. arcticus* mate readily in the laboratory.

6) This thesis is the first to quantify brooding, in both time and temperature, for any species of deep-sea or cold-water cephalopod. *B. arcticus* take over 400 days to brood eggs at 7.5°C. This is the longest brooding period ever recorded for an octopus (Chapter 4).

7) This thesis is also the first to report fecundity based on actual eggs laid from more than one specimen of *Bathypolypus arcticus*. Octopuses laid 12, 13, 18, 19, 36, 48, 54, 89, and 105 fertile eggs. Hatchlings are about 0.25 g which is very large given the small size of their parents. These important life history traits are extremes compared with other octopuses. *B. arcticus* has among the fewest and largest offspring (relative to adult size) for any octopus species (Chapter 4).

8) This work clarifies a controversy in the literature by finding a positive correlation between size and age at maturity for a variety of cephalopod species including *B. arcticus*. This is done by identifying and controlling confounding factors of phylogenetic effects and temperature effects. Once this is done, there is strong evidence that larger cephalopods take longer to reach maturity (Chapter 5).

Summary and comparison of the life cycle and ecology of *B. arcticus*.

Coleoid cephalopods are generally characterized as having short life spans, a semelparous reproductive strategy and traits such as rapid growth to maturity. These traits are often associated with the juvenile stage of other molluscs (Pauly 1998, Rodhouse 1998). This is in contrast to many marine molluscs like bivalves and gastropods which typically spend a small part of their life as rapidly growing juveniles, have a long adult life span and are frequently iteroparous (Young and Thompson 1976, Chapter 1).

Eels, like octopuses, are typically semelparous (Merrett and Haedrich 1997, Rodhouse 1998). Like *B. arcticus*, the deep-sea eels *Coryphaenoides leptolepis*, *C. profundicolus* and *C. armatus* are thought to reproduce only once despite a long life span in some species. However, Merrett and Haedrich (1997) present evidence that iteroparity is at least possible in the deep-sea eel *Histiobranchus bathybius*. An ever increasing number of cephalopods have been found that reproduce more than once and several of the octopuses that do so are deep-sea species. However, unlike the loss of ink sacs which is common in deep-sea octopuses (Voss 1988a), semelparity still exists in a number of taxa of deep-sea animals.

Molluscs in deep-sea environments have lower fecundity and a shift away from smaller planktotropic offspring to lecithotrophy with a brief non-feeding planktonic stage (Allen 1983). Cephalopods, deep-sea or not, have well-developed offspring and do not have the planktonic trochophore and veliger larva forms typical of marine molluscs. Compared to other classes of molluscs, all of which include species that have larval forms that look very different from adults, all hatchling cephalopods are well developed and look similar to adults. The fecundity of cephalopods, while variable, is in general less than that of other molluscs (Boyle 1987). The fecundity of octopuses like *B. arcticus* that have direct development is especially low but hatchlings are correspondingly large (Sweeney et al. 1992).

B. arcticus hatch at average sizes of 0.20 g (Chapter 2) although there is both between and within brood variation. O'Dor and Macalaster (1983) report a hatchling weight of about 0.15 g based on a single octopus. I found weights at hatching of 0.208 ± 0.017 g and 0.283 ± 0.020 g for two broods (Chapter 3, 4) and an average weight and standard deviation of 0.196 ± 0.028 g for 173 octopuses from nine broods (Chapter 2). Given that the average size of adults is only 70 g, *B. arcticus* hatchlings are the largest relative to adult body size of any of the coleoid cephalopods listed in Chapter 5. Hatchlings are 0.306% of adult size. *Octopus* "joubini" which matures at 30 grams has the next highest hatchling to adult size size of 0.133%. The common octopus, *Octopus vulgaris*, has planktonic offspring that are 0.00006% of the size of adults. *B. arcticus* are at the coleoid cephalopod extreme of two related life-history traits; they are at the low extreme of fecundity and high extreme of relative hatchling size.

Growth rates of hatchling *B. arcticus* are dependent on temperature, but are very low (Chapter 3). A relative instantaneous growth rate of 0.221%/d is an average growth rate for hatchling octopuses at 6 °C while hatchlings of other species at temperatures of 18 to 25 °C had instantaneous relative growth rates of 2.76 to 7.05. Even when the effect of temperature was corrected by using degree-days, *B. arcticus* grow very slowly when compared with near-shore octopuses (Chapter 3). In addition to temperature, the growth rate of *B. arcticus* may be affected by other factors of deep-sea environments such as the availablity of food.

B. arcticus are not very active, especially when compared with near-shore species of octopuses. Their approach to obtaining food consists of a sit-and-wait predation strategy and a sift-through-the-substrate strategy. Deep-sea fish also have adaptations to minimize energy consumption. All have reduced metabolic rates and benthopelagic species have altered their skeletal and muscular systems to achieve near perfect neutral buoyancy which reduces the energetic costs of locomotion (Merrett and Haedrich 1997). The metabolism of pelagic cephalopods declines even more rapidly with depth than for fish. This is likely due to a switch from jetting to more efficient means of locomotion and a decreased reliance on locomotion in finding prey and escaping from predators (Seibel et al. 1997).

B. arcticus are low-energy predators that eat a variety of prey both in the field (Macalaster 1976) and in the laboratory (Chapter 2). The biomass of plankton, benthic megafaunal invertebrates and demersal fish decreases logarithmically with depth (Allen

1983, Merrett and Haedrich 1997). This leaves deep-sea octopuses in an environment with less prey which may force them to consume lower quality prey such as brittle stars that shallow-water species of octopuses do not ingest (Appendix 2).

In the laboratory, hatchlings readily accept live or frozen food, especially crustaceans, but like near-shore octopuses they only survive and grow on live food (Chapter 2). Frozen amphipods may have some value as short-term emergency rations. Brittle stars are not a typical component of the diet of near-shore species of cephalopods (Appendix 2). However, brittle stars were the most common prey ingested in the field by *B. arcticus* based on stomach contents, although Macalaster (1976) observed that they were not eaten in the laboratory. Brittle stars are extremely common on the continental slope (Packer et al. 1994) and are of low nutritional value (Chapter 2). In the laboratory I found that both adults and hatchlings will eat brittle stars although they seem to shift their diet away from this low-quality food if given better alternatives. Although adults eat brittle stars in the laboratory and field, hatchlings do not survive on an exclusive diet of these echinoderms. Ambrose (1984) found that *O. bimaculatus* strongly prefer crustaceans like crabs to all other foods but crustaceans only made up 5% of octopuses' diets in the wild due to their rarity. In other areas, crustaceans make up the majority of octopuses' diets (Van Heukelem 1976). Prey ingested was found to be a function of both octopuses' preferences and prey abundance. For example, relatively common animals that octopuses moderately preferred made up the bulk of their diet (Ambrose 1984). Similarly, I believe that *B. arcticus* in the wild will eat brittle stars, which are very abundant, when more suitable food is not available.

Laboratory experiments showed that the growth rates of near-shore octopuses fed diets other than live crustaceans were lower than the growth rates of octopuses raised on crustacean diets (DeRusha et al. 1989). I found this trend for *B. arcticus* as well. Animals in the field eat lower quality diets than the crustacean diets on which age at maturity estimates are based and also may not be eating *ad libitum*. Eating less and eating a lower quality diet is likely to reduce growth rates increasing the time needed for animals to reach a given size.

Based on a series of conservative estimates, the best projection is that *B. arcticus* at 4 °C takes at least six years to reach maturity. After reaching maturity, females take over 400 days to brood eggs. The life span of *B. arcticus*, already one of the longest for any species of coleoid cephalopod, may be even longer than estimated in Chapter 3. It is possible that these octopuses live for ten or more years in the wild. Some of this long life span is due to the low temperatures at which these octopuses live. However, even when degree-days are used, growth rates are still lower than for near-shore species (Chapter 3). Low amounts of prey, low quality diet, low amounts of energy spent searching for prey and differences in major life-history traits point to a strategy that diverges from the traditional cephalopod "live fast and die young" model. The behavior of *B. arcticus* also differs from that of near-shore species (Chapters 2, 3 and 4).

Mating of *B. arcticus* had never been previously observed (Mangold 1987) and is of interest due to the large reproductive arm and large spermatophores of males. In the laboratory, males are successful most of the time. Once the typically smaller male contacts a female with his arm he quicky tries to mount her. He then inserts his large ligula into the her mantle. The ligula could expand once inside the female in order to anchor the male to the female during mating or could be used to scoop out competitor's sperm. One or two spermatophores are transferred during the two and a half minute mating sequence (Chapter 4).

Due to the low mobility and low abundance of *B. arcticus*, there must be tremendous evolutionary pressure on them to mate when they encounter a mature member of the opposite sex. At a given time, males only have an average of 3-4 large spermatophores weighing 0.2 to 0.6 g each (O'Dor and Maclaster 1983) instead of the 200 or more spermatophores that many near-shore octopus species have (Mangold 1987). Males have occasionally been observed trying to mount other males. Male *B. arcticus* would frequently be spermatophore-limited if encounters with mature females were frequent and the production of new spermatophores slow.

Mature females from the lower Bay of Fundy lay 44 ± 34 (n=9) eggs. In captivity, the female attaches the eggs individually to a hard surface like the side of a fiberglass tank or inside a clay flowerpot or acrylic tube. At temperatures of 7.5 °C female octopuses spend over 400 days brooding their eggs (Chapter4). While brooding, they clean their eggs, oxygenate them and protect them from predators by blasting jets of water at foreign objects and researchers. This brooding period is the longest ever reported for any species of octopus and represents a tremendous cost in terms of energy and predation risk over time. During the first few months of brooding, females will occasionally take small amounts of food but for most of the brooding period they will not eat, and slowly waste away as they metabolize their own body to provide energy to brood their eggs.

Females typically die while the last of their brood is hatching. By this time they are in extremely poor condition. After they continuously brood their eggs for over 400 days they often leave their eggs a few days or a week before their death. They may do this to keep their decaying body from either fouling the eggs or attracting predators to the eggs.

Well-developed eggs will hatch early if stressed and the resulting hatchlings are viable. If the eggs are too premature, they will either not hatch or may hatch before all of the yolk sac is used up. Light, excessive handling, chemical changes in the water (adding a small amount of sugar for example) and being removed from the brood and placed on the bottom of the tank can cause hatching. This response is likely an adaption that provides the mobile hatchling the means to get away from localized stresses in the environment. Hatchlings are fully functional versions of their parents. They can walk, jet and even exhibit behaviors like cleaning immediately after hatching.

Based on the small numbers of eggs laid, the long time it takes *B. arcticus* to reach maturity and the tendency of *B. arcticus* to not hide in lairs, predation pressure must be extremely low for these octopuses. The low abundance of *B. arcticus* may help prevent predators from searching specifically for them.

The distribution of these octopuses may be restricted to deeper waters because of increased predation pressure in near-shore habitats. A slow moving octopus with a very limited range of body patterns, lacking the behavior of hiding in lairs and with no ability to produce ink decoys would likely not last long in environments with high numbers of visual predators. Another hypothesis that is not necessarily exclusive of the one mentioned above is that *B. arcticus* are limited to deep-water because the warmer and more variable temperatures found in shallow water are fatal to them.

Final thoughts on size at maturity and temperature.

Chapter 5 was the second chapter from this thesis to be published and at the time of publication, a detailed estimation of age at maturity of *B. arcticus* had not yet been undertaken. From the work presented in Chapter 3, it now appears that *B. arcticus* live much longer than previously published by O'Dor and Macalaster (1983) and longer than indicated in Chapter 5.

Using the new minimum estimated age at maturity of six years moves *B. arcticus* even further to the right, away from the trend shown by the regression line in Figure 2, Chapter 5. This, along with evidence presented above, reinforces the conclusion that the differences in age at maturity in *B. arcticus* cannot be explained by temperature alone. I suggested above that lower quality diet, low metabolism, a shift away from active hunting of prey and reduced predation pressure may be factors selecting for longer life spans.

There may be major differences in life-history traits such as life span between benthic deep-sea octopuses which have restricted mobility and their mid-water and more mobile relatives such as cirrate octopuses, squid, and vampyroteuthis. One cannot help but wonder how long larger species of deep-sea octopuses live.

Future directions

The sensory world of *B. arcticus* and other deep-sea octopuses has not been adequately explored. We know they have well developed, relatively large brains that are capable of large amounts of sensory input, but there is still much we do not know about the importance of each sense. Observations of these octopuses mating and feeding leaves one with a strong impression that their perception of their environment is primarily chemotactile. *B. arcticus* were typically caught at depths over 100 m (Chapter 1). Depths of 100 to 150m are the lower limit that solar energy can support photosynthesis in clear oceanic water (Merrett and Haedrich 1997). The depth at which light can support photosynthesis is likely much lower near the shore and in high current areas like the Bay of Fundy. Although *B. arcticus* react to strong light by jetting, it is not clear how good their visual acuity is due to reduction in the size of their optic lobes (Macalaster 1976). I have observed mature males and females wander around in the same small tank for some time without any apparent recognition until one of them physically touches the other. Only then is mating quickly initiated. If there was ever an animal in need of a dating service, *B. arcticus* would be it. One wonders how they ever find each other in the wild given the low abundance and limited mobility; perhaps chemosensory trails are released into water currents by mature individuals. Chemoreception and chemotaxis has been demonstrated in a variety of cephalopods in response to ink, prey, amino acids and predators (Lee 1992; O'Dor et al. 1993; Boal and Golden 1999).

Pheromones are used by other molluscs such as the predatory gastropod *Urosalpinx cinerea* which is attracted to well fed conspecifics but repulsed by starved individuals (Kohn 1983), and *Navanax*, another predatory gastropod that uses mucus secretions as a pheromone for sexual activity (Kandel 1979). The use of pheromone systems has been documented for pelagic and benthopelegic fish and is suspected in benthic deep-sea fish (Merrett and Haedrich 1997). The use of sexual pheromones from conspecifics had been proposed but not investigated for cephalopods (Hanlon and Messenger 1996).

Macalaster (1976) found that the inferior frontal lobe, which is responsible for chemotactile information from the octopuses' arms, of a single *B. arcticus* weighed 12.7% of the total weight of the brain. This part of the brain is only 5.4% of the brain's volume in the deep-sea *Bathypolypus sponsalis* and 4.0% or less in near-shore and intermediate depth octopuses such as *Octopus vulgaris*, *Octopus deflippi* and *Eledone cirrosa* (Macalaster 1976). "We need to know more about the different kinds of mechanical and chemical stimuli that may be influencing cephalopod behavior" (Hanlon and Messenger 1996). Based on the above, deep-sea octopuses are ideal candidates for investigations into the chemical and tactile world of cephalopods.

The cost of brooding, especially in energetic units, should also be explored. Even if hand fed, these octopuses do not eat, except occasionally at the very beginning of their brooding period. In nature, they probably do not eat at all while they brood eggs. The amount of energy put into eggs and into brooding eggs is of interest since *B. arcticus* seem to be the extreme, at least in terms of time invested and size of eggs relative to adult size (Chapter 4).

Rearing several *B. arcticus*, or any species of deep-sea cephalopod for that matter, from hatchling to maturity at the temperatures at which they naturally occur would be of considerable interest as it could confirm the extremely long life span estimates proposed here. Such work would take long term dedication, perhaps ten or more years of constantly cold water and more than a bit of luck. Perhaps even more challenging, but also needed, is direct investigation of the effect of pressure on life processes like growth rates and metabolism. With advancing technology such as ROV's it may actually be easier to develop tag and release studies in nature than to maintain *B. arcticus* in the laboratory.

Growth rate data for deep-sea octopuses should be easier to obtain than complete life span data as it will take approximately 6 months to get adequate increases in growth as opposed to many years to estimate life span. The entire life cycle of some small tropical octopuses takes about as long as simply getting a decent growth rate in deep-sea species. Ideally, growth experiments will be done on octopuses of a known age (i.e. hatchlings) so that rates can be compared without confounds of variable and unknown age. It would also be a tremendous advantage if techniques for aging octopuses collected in the field were developed.

Another area of cephalopod biology that warrants investigation is the body patterns of animals in low complexity habitats. Hanlon and Messenger (1996) propose that the number of body patterns a cephalopod species has correlates with habitat complexity. Animals that are diurnal and live in complex environments should be selected for well developed cryptic behavior in order to avoid vertebrate predators. Thus they are predicted to have more body patterns than those species that live in less visually complex environments. For example, cephalopods with a high diversity of body patterns, like *Sepioteuthis sepioidea* and *Octopus cyanea*, live in complex coral reef environments and are active during the day. *O. briareus* is mostly nocturnal although I have also observed it in predawn hours when there is enough light to see without a dive light. This octopus is capable of a number of body pattern changes but not as many as the diurnal species. All of these species are capable of much more visual complexity than *B. arcticus* which has a very limited bag of visual tricks. They are capable of becoming darker and lighter. The only other color pattern I have seen is one in which half of the mantle is light and the other half dark ("all dark unilateral" in Hanlon and Messenger 1996 terminology), yielding only three chromatic components. However, the preferred bottom type still is not known with certaintyclear for *B. arcticus.* A mud or sand bottom would presumably be less complex than rock bottoms, but in either case light levels are low. Detailed investigations of their, or any other deep-sea cephalopod, body patterning has not yet been done. Of the seven habitat types listed by Hanlon and Messenger (1996), the three low complexity habitats (midwater, open ocean, and deep or mud benthos) do not have even a single datum for body pattern complexity. Testing these three habitats is needed to support or refute Hanlon and Messenger's hypothesis.

Sperm competition and female choice would also be interesting areas to investigate with *Bathypolypus arcticus.* Although others have failed to get *B. arcticus* to mate in the laboratory, I found that they mate readily which makes them good candidates for this kind of work. Due to their low abundance (O'Dor and Macalaster1983) and limited mobility (Chapter 4), I believe that there is tremendous evolutionary pressure on octopuses to mate whenever they have the chance. In the laboratory, males will occasionally try to mount other males and they are quick to mate with females once they have contacted them with their arms. It should be possible to label their large spermatophores and track them inside the female. Females are typically larger than males and often seem to struggle when mating is initiated. I have video tape of one mating, after which the female reached into her mantle cavity, pulled out a spermatophore and then ate it. In other animals, this is a way females can determine the paternity of their offspring (Simmons 1987). Macalaster (1976) also found a female octopus that had a spermatophore in its stomach. For these reasons, I feel that sperm competition and female choice experiments on *B. arcticus* would be productive. Lack of sufficient numbers of animals prevented investigation into these areas.

Work on all aspects of ecology, behavior, life-cycle or physiology of *B. arcticus* or any species of deep-sea cephalopod is needed. At this point, it is very difficult to compare the research presented here with other deep-sea species as so little has been done with live animals.

Technical Note 1

Does weighing stress affect the growth rate of octopuses?

Abstract

Weight is the best measure of growth in cephalopods. However, cephalopods have delicate skin that damages easily and is prone to infection. Although weighing has been used in numerous studies of octopus growth in the laboratory, no one has ever investigated whether the stress and damage potentially caused during weighing influences octopus' growth rates. In this study, octopuses were randomly assigned to one of four treatments: those weighed three times a week, those weighed biweekly, those weighed biweekly but anaesthetized with $MgCl_2$ before they were weighed, and those weighed only at the beginning and end of the 92 day experiment. I did not find any effect of weighing stress on octopus growth. Since no effect of weighing stress was found, the data from the treatment that was weighed three times per week is ideal for modeling growth equations for young cephalopods. The use of exponential growth equations for young cephalopods is discussed.

Introduction

There have been numerous studies of the growth rates of cephalopods. *Octopus bimaculoides*, *Octopus joubini*, *Octopus briareus*, *Octopus maya* and *Octopus vulgaris* have been raised by Boletzky, Boyle, Forsythe, Hanlon, Villanueva and other workers in the laboratory. In order to produce accurate growth curves, it is necessary to weigh octopuses frequently so that there is adequate data to distinguish between different growth models. According to Forsythe and Van Heukelem (1987), octopuses should be weighed at least once every two weeks for small species and at least once every month for larger ones.

In cephalopods, weight is a more accurate measure of growth than mantle length (Forsythe 1984; Forsythe and Van Heukelem 1987). Octopuses lack hard body parts, therefore measurements of length are prone to error (Forsythe 1984). Mantle lengths can be converted to weight for species of cephalopods for which a conversion equation has been published, but the measurement error is cubed by this conversion (Forsythe and Van Heukelem 1987). Furthermore, even if conversion equations exist, they are seldom valid over a species' entire range in size, and are particularly inaccurate for hatchlings (Chapter 5). Also, weight is the best measure of size for interspecific comparisons. Such comparisons can not be made using length data as different species have different morphologies (Chapter 5).

One potential disadvantage of weighing octopuses and other cephalopods is that the very act of removing them from water and weighing them might damage or stress them thereby reducing growth rates. Cephalopods have delicate skin and are vulnerable to mechanical damage (Boyle 1991). Although octopuses are hardier than squid, they can develop ulcerations in their skin which can become fatally infected (Hanlon et al 1984; Boyle 1991). Ron O'Dor (pers. obs.) observed that adult wild caught *O. vulgaris* do not grow if captured and weighed every three days. Stress can be particularly disruptive in young, growing animals, as it can inhibit the amount and effectiveness of circulating growth hormones (Sapolsky 1992).

In contrast, Forsythe (1984) found that Florida pygmy octopuses anaesthetized with a 1.5% solution of ethyl carbamate (Urethane) and weighed would feed normally within 5 to 10 minutes of recovering. He has also observed *O. bimaculoides* and *Sepia officinalis* recover quickly, feed, and show no signs of stress after weighing with anaesthetic (Forsythe pers. comm.).

Octopus bimaculoides was chosen for this experiment because they have large hatchlings and have "superior qualities for laboratory culture" (Hanlon and Forsythe 1985). Also, Wood (pers. obs.) and Forsythe (pers. comm.) have noticed that this species is less likely to escape because they have a stronger aversion to being out of the water than most species. Due to this aversion, it was thought that the effect of weighing stress might be stronger and therefore more detectable in this species compared to other species.

Despite numerous studies of octopus growth rates, the superiority of weight to length data, and the necessity of frequent weighing when generating good mathematical descriptions of their growth rates, no published study has investigated whether weighing stress affects octopuses' growth rates.

Methods

Octopus hatchlings (*Octopus bimaculoides*) were randomly assigned to one of four treatments. The first treatment was weighed three times per week (n=6), the second was weighed once every two weeks (n=5), the third was weighed once every two weeks with $MgCl_2$ used as an anaesthetic prior to weighing (n=4), and the fourth treatment was weighed only at the start and end of the 92 day experiment (n=4). In most studies of growth, octopuses are weighed once every two weeks or less. Forsythe and Hanlon (1988) found that the exponential growth phase of *O. bimaculoides* reared at 23 °C was 140 days. The experimental duration of 92 days was chosen to obtain as many data points as possible while the octopuses were clearly in the early fast growing phase of their life cycle.

The wet weights of the hatchlings were measured to the nearest milligram using a Mettler P163 scale using a modified weighing boat (see Chapter 2). When octopuses were weighed, I recorded whether they inked as an indication of stress level. Octopuses treated with $MgCl_2$ were put in a weak concentration of the anaesthetic until they relaxed their grip on the side of the rearing chamber (usually a few minutes).

Husbandry

Octopuses were kept in individual rearing chambers as described by Wood and Wood 1998 and fed *Gammarus* sp. amphipods *ad libitum*. The chambers were kept in a closed quarantine system of the Aquatron Laboratory of Dalhousie University. A wet/dry filter, sponge filter and large protein skimmer filtered the water. See Moe (1992) for more information on maintaining closed marine systems. During the experiment, the biological load in the system was very low which made maintaining optimal water quality easier.

Temperature and salinity were recorded three times a week throughout the experiment. Once per week, after temperature and salinity were recorded, 25% of the water was changed. Freshwater was added as needed to lower the salinity. Also, a minimum/maximum thermometer was checked weekly. Min/max thermometers are an inexpensive way to document the most extreme temperatures during the experiment. These extremes would otherwise go unnoticed as researchers are unlikely to take the temperature at the exact moment when it is highest or lowest in the 92 day experiment.

Water samples were taken once every week and tested for nitrite using an Aquarium pharmaceuticals nitrite kit and for nitrate using Red sea fish pHarm's "Reef Lab" nitrate test.

Statistics

An analysis of variance was used to test whether the weight of octopuses in the four treatments differed. I expected that octopuses that were weighed three times per week, and possibly those that were weighed once every two weeks, would have reduced growth rates due to stress. I also expected that the use of mild amounts of anaesthetic might alleviate this stress. A one tailed t-test was used to test this hypothesis.

The weight data from the three times per week treatment was log transformed and a regression was performed to obtain a growth curve. The validity of using the exponential growth equation to describe juvenile growth was tested using the large amounts of data produced by weighing the octopuses three times a week.

Results

I failed to find an effect of weighing frequency on the weight of octopuses at the end of the experiment ($p=0.729$). In addition, weight provided no evidence that the use of $MgCl_2$ reduced stress during weighing ($p=0.237$). The weights of octopuses and their standard deviations are illustrated in Figure 1. For clarity, standard deviations for the most frequently weighed treatment are only displayed every two weeks. Also, when standard deviations were reported, the data was shifted in half day intervals so that the error bars would not obscure each other.

Octopuses in the $MgCl_2$ treatment never inked. There were five instances of inking observed in the other biweekly treatment. A total of 17 instances of inking occurred overall; all of them occurred during the first 41 days of the experiment.

The growth equation for the treatment weighed three times per week is plotted in Figure 2. The equation of the line is: $y=0.0140x + 2.0096$ ($p < 0.0001$, $r^2 = 0.989$, slope error = 0.0003, constant error = 0.0180).

During the experiment there were some instances of mortality due to autophagy. This was traditionally thought to be due to stress but Budelman (1998) believes that a bacteria or virus is responsible. My notes indicate that the instances of lesions and autophagy effected octopuses in all four treatments equally. Survival rates were good

Figure 1. Average weight and standard deviation for four treatments of *Octopus bimaculoides* during the 92 day experiment.

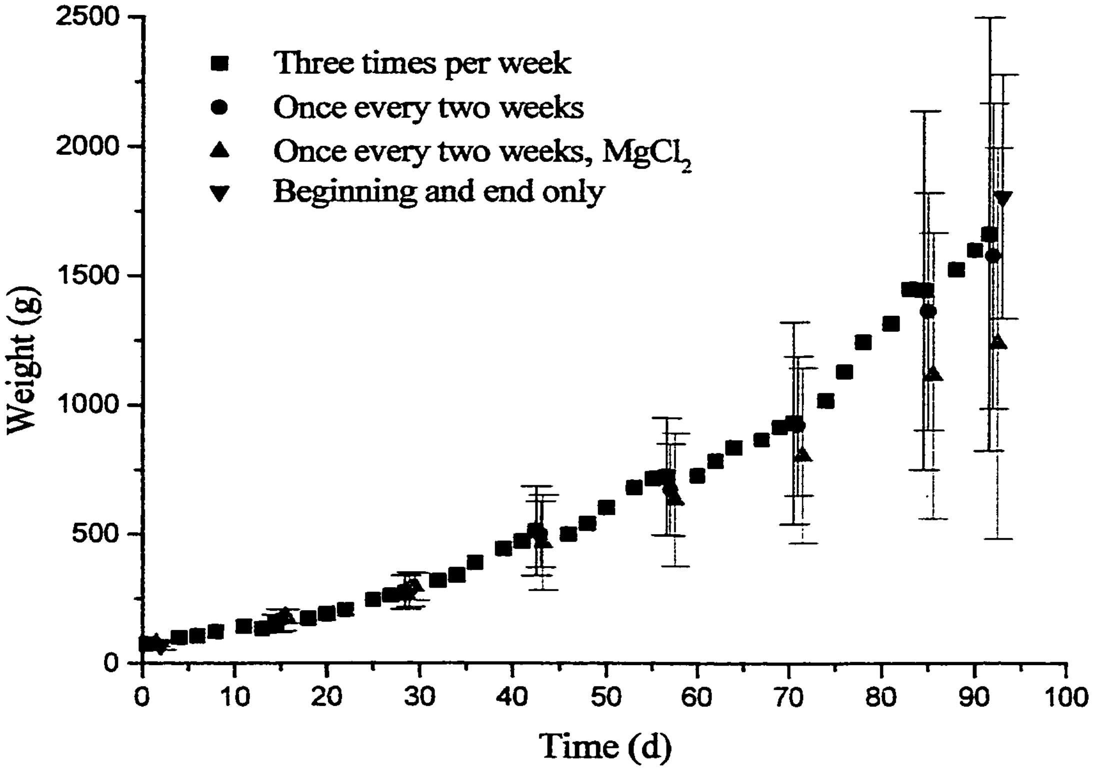
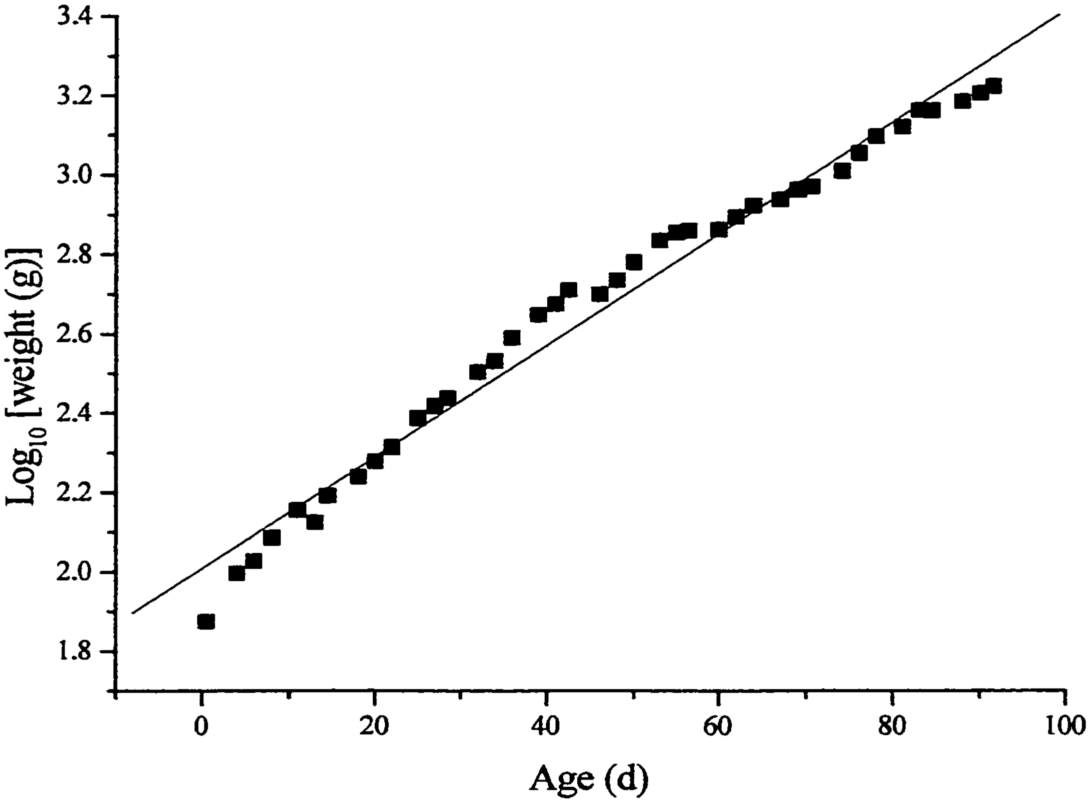


Figure 2. Regression of octopus age and Log_{10} (weight). $P < 0.0001$, $r^2 = 0.989$.



(89%), with 83%, 100%, 100% and 75% of the octopuses surviving in the three times-per-week, biweekly, biweekly with $MgCl_2$ and the start and end only treatments respectively.

During the experiment, the average and standard deviation of the temperature which was measured three times per week was 25.0 ± 0.6 °C and the salinity was 31.2 ± 0.5 PPT. The min/max thermometers recorded short term variations in the temperature. During the second week of the experiment, a minimum temperature of 12.3 °C was recorded. This was due to the addition of too much cold water during a water change. The next lowest temperature recorded was 19.6 °C from the first week. The highest temperature recorded during the 92 day experiment was 26.3 °C.

Water quality was very good throughout the experiment. Nitrite levels were below detectable limits in all fourteen samples. Hanlon and Forsythe (1985) recommend keeping nitrate below 100 ppm although they report that short term exposure of levels up to 500 ppm are not fatal. Nitrate levels were between 0 and 2.5 ppm (estimated 1.25 ppm) in eleven samples and undetectable in three samples.

Discussion

The effect of weighing stress on growth

I was surprised to find no evidence of weighing stress on the final weights of octopuses. Octopuses in all treatments except the $MgCl_2$ treatment became more difficult to capture for weighing as the experiment continued and they were very active when removed from the water. I believed that this increased activity was an indication of stress.

Octopuses in the three-times-per-week treatment were weighed at least six times more frequently than in standard experiments on octopus growth (Forsythe 1984; Forsythe and Hanlon 1988). Despite this, the average weight of octopuses in the four treatments was similar (Figure 1). These negative results are great news for the authors of octopus growth studies.

Octopuses in the $MgCl_2$ treatment were much easier to catch and weigh as they did not try to crawl out of the weighing dish during the procedure. I hypothesized this would reduce stress in this group. However, I failed to find an increase in final size in the treatment that was given $MgCl_2$ to alleviate the effect of weighing stress.

Cephalopods use ink to escape predators and it was thought that inking frequency would be a good measure of stress. Octopuses in the $MgCl_2$ treatment never inked although five instances of inking occurred in the other treatment that was weighed biweekly. Although octopuses exposed to $MgCl_2$ did not ink or try to escape from the weighing dish, the anesthetic itself may have stressed the octopuses.

The data in Figure 1 is an excellent example of the large variation in sizes found between siblings and of the heteroscedastic data that is common in cephalopod growth studies. In other words, the variance increases substantially as the animals grow. At the start of the experiment, octopuses weighed from 51 to 96 g. Ninety-two days later, the octopuses' weight ranged from 342.2 to 2598.7 g, a difference of almost an order of magnitude. The standard deviation of weight at the start of the experiment for the treatment that was weighed three times per week was 11 g. At the end of the experiment, this standard deviation was 836 g, seventy six times larger than it was at the start! This increasing variation with time violates the assumption of homogeneity of variance required by many statistical tests such as regression analysis (Zar 1984) and is why cephalopod growth data is frequently log transformed. Regression analyses are robust to violations of the assumptions if they are not too severe. After log transformation, the size of the standard deviation decreases from seventy six-fold to four-fold.

Modeling cephalopod growth

The growth equation for the treatment weighed three times per week had an r^2 of 0.989 ($p<0.0001$). Thus 98.9% of the variation in size is explained by age. The exponential growth model fits the first part of cephalopods' growth well and is simple. Other models used to describe the growth of cephalopods are either more complex or do not fit the data as well.

For example, Electronic LEngth Frequency ANalysis (ELEFAN) was developed to model growth parameters, total mortality, recruitment patterns and selection pressure of tropical species from length-frequency (L/F) and catch-at-length (C/L) data (Pauly 1994). A similar methodology has been applied to "animals as different from each other as penaeid shrimps and West African hake" (Pauly 1994). However, care must be taken when using mathematical models. Using *Idiosepius pygmaeus*, a tropical sepiolid, as an example, Jackson and Choat (1992) found that the ELEFAN generated growth curves are inappropriate for cephalopods. The ELEFAN software package produced lifespans from three to ten years (Jackson and Choat 1992), whereas both laboratory and field studies of *I. pygmaeus* show that maximum life span is only 80 days (Lewis and Choat 1993)!

Pauly and others (Caddy 1983) have used asymptotic growth equations to describe cephalopod growth. Pauly (1998) suggests that cephalopods are "paedomorphic" i.e. their life-long growth rates are similar to those found in juvenile stages of other molluscs and fish. He then ignores the later 90% of the fish model that does not describe cephalopod growth. If you throw away the upper 90% of a von Bertalanffy model, the small portion left is effectively an exponential growth curve. However, the rapid growth rates and short life spans of cephalopods when compared to fish and other molluscs is not a new finding (Boyle 1987). Forsythe and Van Heukelem (1987), Saville (1987), Forsythe and Hanlon (1989) and Jackson and Choat (1992) have all concluded that asymptotic growth curves are inappropriate for modeling cephalopod growth and life spans. Exponential models for the first part of a cephalopod life span followed by logarithmic models are preferred.

Pauly's (1998) 10% of the von Bertalanffy curve does contain a smooth transition from the exponential growth of cephalopods during the first third (Forsythe and Hanlon 1988) of their lifespan to the logarithmic growth during the rest of their lifespan. However, using the more complicated von Bertalanffy model and then throwing out 90% of the curve it generates is neither a more elegant model, nor is it significantly more accurate. Its predictive power for modeling growth parameters, total mortality, recruitment patterns and selection pressure have not been demonstrated for any species of cephalopod. Pauly's model is effectively the same as an exponential growth curve followed by logarithmic growth and still includes the assumption that each species has a maximum or asymptotic size. In theory, growth rapidly slows down as this is approached.

There is simply no evidence for this in any coleoid cephalopod (Forsythe and Van Heulelem 1987). Previous experiments with asymptotic models have not been good, i.e. Jackson and Choat (1992) found that they were off by over an order of magnitude!

Conclusion

Frequency of weighing had no effect on the weights of octopuses over a 92 day period. There is no evidence that the use of $MgCl_2$ prior to weighing causes octopuses to grow better. While I can not statistically conclude that there is no effect, the fact that none was found is good news for biologists who study the growth patterns of octopuses.

The data fit an exponential growth curve. More complicated asymptotic growth models have not demonstrated as a reliable predictive power for cephalopods. Pauly's (1998) recent suggestion of throwing out the top 90% of the von Bertalanffy's model that does not fit cephalopod growth works but is not an elegant solution and is not likely to be employed by other cephalopod workers.

Technical Note 2

First growth data and prey choice for a cold water octopus, *Bathypolypus arcticus*

Bathypolypus arcticus is a small octopus that weighs 70 g at maturity and lives in 4 ± 2 °C water, typically at a depth of 200 to 600 meters in the Atlantic Ocean (O'Dor and Macalaster 1983). While most octopuses live roughly a year, *B. arcticus* is thought to live for six or more years (Chapter 3). Wood et al. (1998) found that female *B. arcticus* brood their eggs for over 400 days at 7-8 °C. During this period, the octopuses usually refuse food and stay with their eggs so that they can guard, clean and oxygenate them.

The majority of research on cephalopods is based on near-shore and tropical species; consequently, there is very little known about deep-sea cephalopods (Forsythe and Van Heukelem 1987). Even near shore cephalopods are difficult to study in the field and much of our knowledge about cephalopods comes from laboratory experiments. As there currently is no reliable way to age octopuses, laboratory rearing and tag-recapture are the only methods to acquire reliable growth data. There is no published growth data for any species of cephalopod at temperatures below 10 °C.

Macalaster (1976) studied the stomach contents of 450 *B. arcticus* and brittlestars were the most common prey found, although she found no evidence that ophiuriods were eaten in the lab. Typically, crustaceans, fish and molluscs form the bulk of cephalopod diets with ophiuroids, polycheates, chaetognaths and siphonophores forming part of some species' diet (Nixon 1987). Although low in caloric value per weight, brittlestars are a common benthic group with densities of 240-970 m^2 at depths of 150-275 m in the Gulf of Maine (Packer et al. 1994).

The goals of this experiment are to quantify predation of *B. arcticus* given a choice of potential prey and to record the first growth data for a deep-sea cephalopod.

Methods

From October 25, 1994 to January 3, 1995 six *B. arcticus* were kept individually in 3 gallon plastic buckets. These octopuses were between 24 and 42 grams at the start of the experiment and had been caught during the summer and early fall of 1994 in the lower Bay of Fundy. Each bucket had half an inch of sand covering the bottom and its own supply of cold water.

Octopuses were offered one brittle star (*Ophiopholis aculeata*), a large sand shrimp (*Crangon* over a gram) a small sand shrimp (*Crangon* under 0.8 grams) and a small mussel (*Mytilus*). Each day during the forty day experiment the octopuses were checked and ingested food was replaced. From prior observations I learned that it was necessary to sketch the brittlestars in order to accurately determine if any of their arms had been eaten. The wet weight of all food was measured to the nearest milligram and recorded before it was added to the bucket.

Temperature was recorded daily in two of the containers. Octopuses were weighed to the nearest gram at the beginning of the experiment, on day 20, and at the end of the experiment (day 40). Relative instantaneous growth rate and gross growth efficiency for the animals that gained weight were calculated using the standard equations (Forsythe and Van Heukelem 1987). To allow comparisons with other octopuses raised at warmer temperatures, time in the growth rate equation was measured in degree-days.

Results and Discussion

Octopuses ate large shrimp, small shrimp and brittlestars during the experiment but did not eat any mussels. The amount of each type of food eaten is shown in Figure 1. The growth rates of the octopuses in the experiment are shown in Table 1. Only three of the six octopuses ate brittlestars and fewer brittlestars were eaten towards the end of the experiment. The three octopuses that ate brittlestars did not grow while all three octopuses that did not eat brittlestars had positive growth rates.

I hypothesize that brittlestars are not the preferred food of *Bathypolypus arcticus*. *B. arcticus* almost never ate the central disk of the brittlestars which may be the most nutritious part as it contains the gonads in mature animals (Packer et al. 1994). Although a common benthic macro invertebrate, brittlestars are low in caloric value (Chapter 2). Their ingestion may provide energy for metabolism but not provide enough for growth. The *B. arcticus* in this experiment appeared to switch to an almost all shrimp diet after the first 20 days.

The average relative instantaneous growth rate for all octopuses was 0.16 ± 0.32 %/d. The average growth rate for just the three octopuses that gained weight was

Figure 1. Food consumed by six *B. arcticus* in ten day intervals.

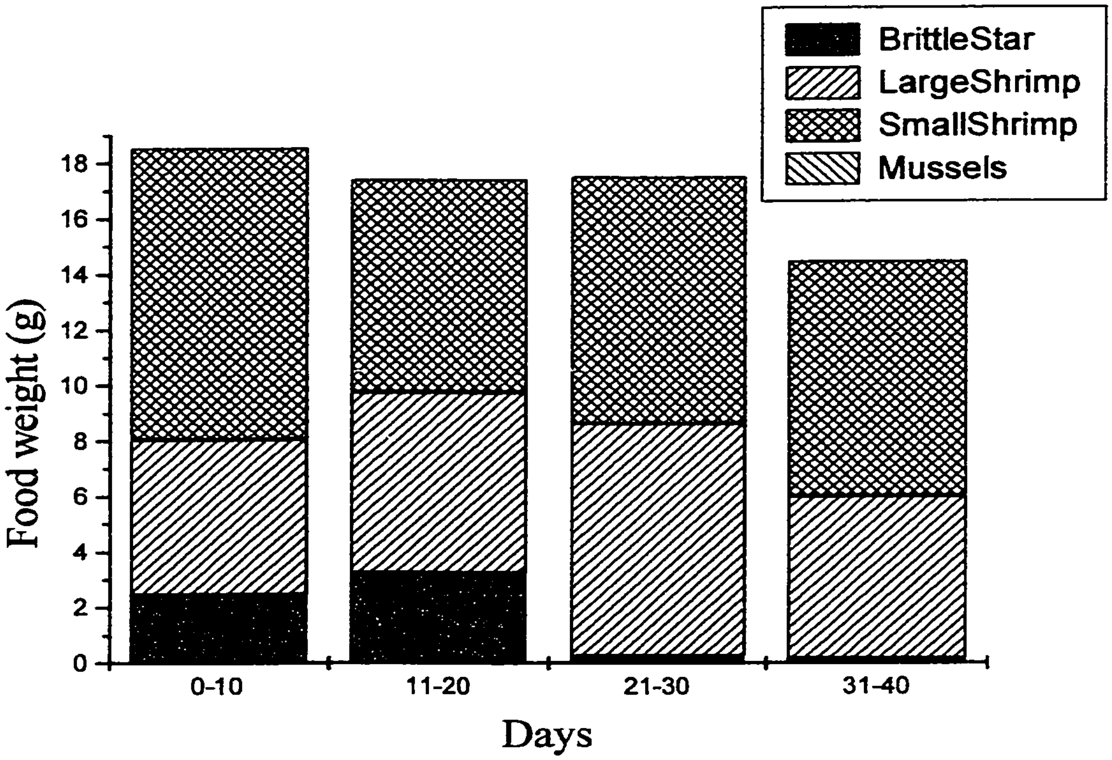


Table 1. Weight and growth rates of deep-sea octopuses over a 40 day period.

	Octopus weight			
	Day 1	Day 20	Day 40	G (%/day)
octo1	35	33	35	0.000
octo2	35	35	35	0.000
octo3	42	42	44	0.116
octo4	41	42	40	-0.062
ocot5	34	40	47	0.809
octo6	24	24	25	0.102

0.34 ± 0.40 %/d and the highest growth rate recorded was 0.80 %/d. These values are the first growth rate data ever measured for any deep-sea octopus. It is difficult to compare these growth rates to the growth rates of better studied warm water octopuses without taking temperature into account (Chapter 2 and 3).

The temperature in the two tanks in which it was recorded was 7.3 ± 1.0°C. This average drops to 7.2 ± 0.6°C if the anomaly on day 25, when the temperature warmed up to 12°C is removed.

Often, only animals that grow are used in calculating growth rate, and this is the value that should be compared to other species. The value of 0.34 %/d is quite low, and even when temperature is controlled *B. arcticus* appear to grow slower than their warm water relatives. *B. arcticus* grow slowly in part due to the temperatures in which they live but temperature does not completely explain their slow growth rates.

Octopuses collected in the wild are of unknown age. Some may have already laid eggs and thus are not feeding while others may be younger and growing rapidly. This causes large amounts of variation in the data and makes meaningful comparisons to other species difficult. All the octopuses in this experiment ate well suggesting that none of them had already laid eggs. The use of hatchlings, which are of known age, eliminates this problem but has not been done for any other species of deep-sea octopus.

There has been little laboratory work done on cold water and deep sea cephalopods. This preliminary experiment illuminates more questions than it answers. What is the role of brittlestars in the diets and energy budget of the deep-sea octopus *B. arcticus*? Are they consumed when nothing better is available? Can *B. arcticus* subsist on brittlestars? Most cephalopods live fast and die young - how long does a deep-sea octopus with very slow growth rates live? How does the behavior, energy budgets and life history of deep-sea octopuses compare to their better studied shallow water relatives?

Appendix 1

Cephalopod predators

Although all cephalopods are predators, they are never top predators. Cephalopods fall prey to birds, fish, marine mammals and other cephalopods. All of these predators have well developed senses and are relatively advanced animals.

This appendix lists predators of cephalopods. Records were only included when the cephalopod prey was identified to species level. This restriction improved the quality of the data set but limited its size since many papers simply list "squid" or "octopus". For example, Randall (1967) is frequently cited in many lists of cephalopod predators as he listed 29 species of fish that prey on cephalopods. However, only four of these records identify the species of cephalopod predated and therefore only four are included in the following list. Nevertheless, with over 300 entries, the following table is one of the most complete lists of cephalopod predators published.

This data set is from the November 11, 1999 version of CephBase (http://www.cephbase.dal.ca/ Wood et al. 1999). CephBase is a dynamic html relational database-driven interactive web page. The purpose of CephBase is to provide life history, distribution, catch and taxonomic data on all living species of cephalopods (octopus, squid, cuttlefish and nautilus). The use of online biological databases is still in its infancy but the field is growing rapidly. Information such as this can be used to explore the relationships between groups of animals and facilitate collaboration between specialists studying different groups of organisms.

Predator	Predator Sci. Name	Cephalopod Prey	Reference
Birds			
Albatross		*Moroteuthis robsoni*	Clarke 1983
Albatross		*Moroteuthis knipovitchi*	Clarke 1983
Albatross		*Lepidoteuthis grimaldii*	Clarke 1983
Albatross		*Ancistrocheirus lesueurii*	Clarke 1983
Albatross		*Gonatus antarcticus*	Clarke 1983
Albatross		*Mesonychoteuthis hamiltoni*	Clarke 1983
Albatross		*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Albatross		*Todarodes sagittatus*	Clarke 1983
Albatross		*Pholidoteuthis boschmai*	Clarke 1983
Antarctic prion (seabird)	*Pachyptila vittata desolata*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Black-browed albatross	*Diomedea melanophris*	*Histioteuthis eltaninae*	Croxall and Prince 1994
Black-browed albatross	*Diomedea melanophris*	*Kondakovia longimana*	Clarke 1983
Black-browed albatross	*Diomedea melanophris*	*Gonatus antarcticus*	Croxall and Prince 1994
Black-browed albatross	*Diomedea melanophris*	*Alluroteuthis antarctica*	Croxall and Prince 1994
Black-browed albatross	*Diomedea melanophris*	*Martialia hyadesi*	Croxall and Prince 1994
Black-browed albatross	*Diomedea melanophris*	*Illex argentinus*	Croxall and Prince 1994
Emperor penguin	*Aptenodytes forsteri*	*Gonatus antarcticus*	Piatkowski and Pütz 1994
Emperor penguin	*Aptenodytes forsteri*	*Kondakovia longimana*	Piatkowski and Pütz 1994
Emperor penguin	*Aptenodytes forsteri*	*Alluroteuthis antarctica*	Piatkowski and Pütz 1994
Emperor penguin	*Aptenodytes forsteri*	*Psychroteuthis glacialis*	Piatkowski and Pütz 1994
Gentoo penguin	*Pygoscelis papua*	*Gonatus antarcticus*	Thompson 1994
Grey-headed albatross	*Diomedea chrysostoma*	*Gonatus antarcticus*	Croxall and Prince 1994
Grey-headed albatross	*Diomedea chrysostoma*	*Kondakovia longimana*	Clarke 1983
Grey-headed albatross	*Diomedea chrysostoma*	*Illex argentinus*	Croxall and Prince 1994
Grey-headed albatross	*Diomedea chrysostoma*	*Martialia hyadesi*	Croxall and Prince 1994
Grey-headed albatross	*Diomedea chrysostoma*	*Alluroteuthis antarctica*	Croxall and Prince 1994
Grey-headed albatross	*Diomedea chrysostoma*	*Psychroteuthis glacialis*	Croxall and Prince 1994
Grey-headed albatross	*Diomedea chrysostoma*	*Histioteuthis eltaninae*	Croxall and Prince 1994
King penguin	*Aptenodytes patagonicus*	*Moroteuthis ingens*	Cherel et al. 1996
Light-mantled sooty albatross	*Phoebetria palpebrata*	*Histioteuthis eltaninae*	Croxall and Prince 1994
Light-mantled sooty albatross	*Phoebetria palpebrata*	*Psychroteuthis glacialis*	Croxall and Prince 1994
Light-mantled sooty albatross	*Phoebetria palpebrata*	*Gonatus antarcticus*	Croxall and Prince 1994

Light-mantled sooty albatross	*Phoebetria palpebrata*	*Kondakovia longimana*	Croxall and Prince 1994
Light-mantled sooty albatross	*Phoebetria palpebrata*	*Alluroteuthis antarctica*	Croxall and Prince 1994
Light-mantled sooty albatross	*Phoebetria palpebrata*	*Martialia hyadesi*	Croxall and Prince 1994
Light-mantled sooty albatross	*Phoebetria palpebrata*	*Illex argentinus*	Croxall and Prince 1994
Magellanic penguin	*Sphesniscus magellanicus*	*Illex argentinus*	Haimovici et al. 1998
Magellanic penguin	*Sphesniscus magellanicus*	*Gonatus antarcticus*	Thompson 1994
Northern fulmar	*Fulmaris glacialis*	*Gonatus fabricii*	Grieg 1930
Rockhopper penguin	*Eudyptes chrysocome*	*Gonatus antarcticus*	Thompson 1994
Seabirds		*Berryteuthis magister magister*	Nesis 1998
Thick-billed murre	*Uria lomvia*	*Berryteuthis magister magister*	Nesis 1998
Wandering albatross	*Diomedea exulans*	*Moroteuthis ingens*	Weimerskirch et al. 1997
Wandering albatross	*Diomedea exulans*	*Histioteuthis eltaninae*	Croxall and Prince 1994
Wandering albatross	*Diomedea exulans*	*Psychroteuthis glacialis*	Croxall and Prince 1994
Wandering albatross	*Diomedea exulans*	*Alluroteuthis antarctica*	Croxall and Prince 1994
Wandering albatross	*Diomedea exulans*	*Kondakovia longimana*	Clarke 1983
Wandering albatross	*Diomedea exulans*	*Martialia hyadesi*	Croxall and Prince 1994
Wandering albatross	*Diomedea exulans*	*Illex argentinus*	Croxall and Prince 1994
Wandering albatross	*Diomedea exulans*	*Taningia danae*	Roper and Vecchione 1993
Wandering albatross	*Diomedea exulans*	*Gonatus antarcticus*	Croxall and Prince 1994

Fish

	Coryphaenoides berglax	*Bathypolypus arcticus*	Grieg 1930
	Lophius upsicephalus	*Sepia australis*	Sánchez and Villanueva 1991
	Scyliorhinus capensis	*Sepia australis*	Sánchez and Villanueva 1991
	Raja wallacei	*Sepia australis*	Sánchez and Villanueva 1991
	Raja clavata	*Sepia australis*	Sánchez and Villanueva 1991
	Somniosus microcephalus	*Gonatus fabricii*	Grieg 1930
Alaska pollock	*Theragra chalcogramma*	*Berryteuthis magister magister*	Nesis 1998
Atlantic cod	*Gadus morhua*	*Eledone cirrhosa cirrhosa*	Royer et al. 1998
Barracuda		*Nototodarus gouldi*	Machida 1983
Bigeye tuna	*Thunnus obesus*	*Illex argentinus*	Haimovici et al. 1998
Bigeye tuna	*Thunnus obesus*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Blue ling		*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Blue marlin	*Makaira nigricans*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Blue shark	*Prionace glauca*	*Todarodes sagittatus*	Macnaughton et al. 1998

Blue shark	*Prionace glauca*	*Onychoteuthis banksii*	Macnaughton et al. 1998
Blue shark	*Prionace glauca*	*Gonatus steenstrupi*	Macnaughtonet al. 1998
Blue shark	*Prionace glauca*	*Histioteuthis hoylei*	Macnaughton et al. 1998
Blue shark	*Prionace glauca*	*Histioteuthis bonnellii*	Macnaughton et al. 1998
Blue shark	*Prionace glauca*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Blue shark	*Prionace glauca*	*Octopoteuthis rugosa*	Macnaughton et al. 1998
Blue shark	*Prionace glauca*	*Teuthowenia megalops*	Macnaughton et al. 1998
Blue shark	*Prionace glauca*	*Chiroteuthis imperator*	Macnaughton et al. 1998
Blue shark	*Prionace glauca*	*Histioteuthis reversa*	Macnaughton et al. 1998
Chinook salmon	*Oncorhynchus tschawytscha*	*Berryteuthis magister magister*	Nesis 1998
Chum salmon	*Oncorhynchus keta*	*Berryteuthis magister magister*	Nesis 1998
Cod	*Gadus aeglefinus*	*Rossia moelleri*	Grieg 1930
Cod		*Bathypolypus arcticus*	Macalaster 1976
Cod	*Gadus callarias*	*Todarodes sagittatus*	Grieg 1933
Cod	*Gadus aeglefinus*	*Bathypolypus arcticus*	Grieg 1930
Cod	*Gadus callarias*	*Bathypolypus arcticus*	Grieg 1930
Cod	*Gadus callarias*	*Benthoctopus piscatorum*	Grieg 1930
Cod	*Gadus callarias*	*Rossia moelleri*	Grieg 1930
Cod	*Gadus aeglefinus*	*Gonatus fabricii*	Grieg 1930
Cod	*Gadus* sp.	*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Cod	*Gadus virens*	*Gonatus fabricii*	Grieg 1930
Cod	*Gadus aeglefinus*	*Benthoctopus piscatorum*	Grieg 1930
Cod	*Gadus callarias*	*Gonatus fabricii*	Grieg 1930
Coho salmon	*Oncorhynchus kisutsch*	*Berryteuthis magister magister*	Nesis 1998
Deep sea shark	*Centroscymnus coelolepis*	*Taningia danae*	Roper and Vecchione 1993
Deep sea shark	*Centroscymnus coelolepis*	*Heteroteuthis dispar*	Villaneuva 1992
Deep sea shark	*Centroscymnus coelolepis*	*Ommastrephes bartramii*	Villaneuva 1992
Demersal pelagic fish	*Pagrus pagrus*	*Illex argentinus*	Haimovici et al. 1998
Demersal pelagic fish	*Trichiurus lepturus*	*Illex argentinus*	Haimovici et al. 1998
Demersal pelagic fish	*Pomatomus saltatrix*	*Illex argentinus*	Haimovici et al. 1998
Demersal pelagic fish	*Polyprion americanus*	*Illex argentinus*	Haimovici et al. 1998
Dog snapper	*Lutjanus jocu*	*Octopus vulgaris*	Randall 1967
Dolphin fish	*Coryphaena hippurus*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Elasmobranch	*Centrophorus squamosus*	*Octopus magnificus*	Villanueva 1993

Elasmobranch	*Galeus melastomus*	*Bathypolypus sponsalis*	Villaneuva 1992
Elasmobranch	*Galeus melastomus*	*Todarodes sagittatus*	Villaneuva 1992
Fish		*Histioteuthis bonnellii*	Clarke 1983
Fish		*Octopoteuthis rugosa*	Clarke 1983
Fish		*Gonatus fabricii*	Clarke 1983
Fish	*Merluccius capensis*	*Sepia australis*	Sánchez and Villanueva 1991
Fish	*Argyrosomus hololepidotus*	*Loligo vulgaris reynaudi*	Smale and Bruton 1985
Flathead		*Nototodarus gouldi*	Machida 1983
Greenland halibut		*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Greenland halibut	*Reinhardtius hippoglossoides matsuurae*	*Berryteuthis magister magister*	Nesis 1998
Greenland shark		*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Grenadiers		*Berryteuthis magister magister*	Nesis 1998
Grenadiers	*Macrurus* sp.	*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Gurnard		*Nototodarus gouldi*	Machida 1983
Gurnard	*Chelidonichthys capensis*	*Sepia australis*	Sánchez and Villanueva 1991
Gurnard perch		*Nototodarus gouldi*	Machida 1983
Haddock	*Melanogrammus aeglefinus*	*Sepiola atlantica*	Royer et al. 1998
Haddock		*Bathypolypus arcticus*	Macalaster 1976
Hake		*Bathypolypus arcticus*	Macalaster 1976
Hake		*Loligo subulata*	Royer et al. 1998
Hake	*Merluccius hubbsi*	*Illex argentinus*	Haimovici et al. 1998
Halibut		*Bathypolypus arcticus*	Macalaster 1976
Izak catshark	*Holohalaelurus regani*	*Sepia australis*	Sánchez and Villanueva 1991
Lancetfish	*Alepisaurus ferox*	*Taningia danae*	Roper and Vecchione 1993
Lancetfish	*Alepisaurus ferox*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Lancetfish	*Alepisaurus ferox*	*Ocythoe tuberculata*	Roper and Sweeney 1975
Lancetfish	*Alepisaurus borealis*	*Ocythoe tuberculata*	Roper and Sweeney 1975
Lancetfish	*Alepisaurus ferox*	*Onykia carriboea*	Tsuchiya and Okutani 1991
Large fish		*Sepia officinalis*	Le-Mao 1985
Ling		*Eledone cirrhosa cirrhosa*	Royer et al. 1998
Liparid	*Polypera simushirae*	*Berryteuthis magister magister*	Nesis 1998
Little tuna	*Euthynnus alletteratus*	*Loligo plei*	Randall 1967
Monkfish		*Eledone cirrhosa cirrhosa*	Royer et al. 1998

Monkfish		*Sepia officinalis*	Royer et al. 1998
Monkfish		*Undetermined forbesii*	Royer et al. 1998
Needlefish	*Tylosurus* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Northern bluefish tuna	*Thunnus thunnus*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Northern bluefish tuna	*Thunnus thunnus*	*Ocythoe tuberculata*	Roper and Sweeney 1975
Pacific cod	*Gadus macrocephalus*	*Berryteuthis magister magister*	Nesis 1998
Pacific pomfret	*Brama japonica*	*Berryteuthis magister magister*	Nesis 1998
Pelagic fish	*Isurus oxyrinchus*	*Illex argentinus*	Haimovici et al. 1998
Pink salmon	*Oncorhynchus gorbuscha*	*Berryteuthis magister magister*	Nesis 1998
Pollock	*Pollachius virens*	*Illex illecebrosus*	Dexter 1969
Red hind	*Epinephelus guttatus*	*Octopus briareus*	Randall 1967
Red salmon	*Oncorhynchus nerka*	*Berryteuthis magister magister*	Nesis 1998
Red snapper		*Nototodarus gouldi*	Machida 1983
Redfish	*Sebastes marinus*	*Gonatus fabricii*	Grieg 1930
Redfish	*Sebastes marinus*	*Bathypolypus arcticus*	Dexter 1969
Sablefish	*Anoplopoma fimbria*	*Berryteuthis magister magister*	Nesis 1998
Sailfish	*Istiophorus platyptrus*	*Illex illecebrosus*	Voss and Brakoniecki 1985
Saithe sea perch		*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Scad		*Nototodarus gouldi*	Machida 1983
Shark	*Sphyrna zygaena*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Shark	*Sphyrna lewini*	*Illex argentinus*	Haimovici et al. 1998
Shark		*Sepietta oweniana*	Villaneuva 1995
Shark		*Nototodarus gouldi*	Machida 1983
Skipjack tuna	*Katsuwonus pelamis*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin1998
Swordfish	*Xiphias gladius*	*Illex argentinus*	Haimovici et al. 1998
Swordfish	*Xiphias gladius*	*Illex illecebrosus*	Voss and Brakoniecki 1985
Swordfish	*Xiphias gladius*	*Bathypolypus arcticus*	Scott and Tibbo 1968
Swordfish	*Xiphias gladius*	*Histioteuthis bonnellii*	Bello 1991
Swordfish	*Xiphias gladius*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Swordfish	*Xiphias gladius*	*Heteroteuthis dispar*	Villaneuva 1995
Swordfish	*Xiphias gladius*	*Todarodes sagittatus*	Bello 1991
Swordfish	*Xiphias gladius*	*Ocythoe tuberculata*	Bello 1991
Swordfish	*Xiphias gladius*	*Argonauta argo*	Bello 1991
Swordfish	*Xiphias gladius*	*Ancistrocheirus lesueurii*	Bello 1991

Swordfish	*Xiphias gladius*	*Histioteuthis reversa*	Bello 1991
Swordfish	*Xiphias gladius*	*Ancistroteuthis lichtensteinii*	Bello 1991
Swordfish	*Xiphias gladius*	*Ommastrephes bartramii*	Bello 1991
Tuna	*Thunnus alalunga*	*Illex argentinus*	Haimovici et al. 1998
Tuna		*Taningia danae*	Roper and Vecchione 1993
Tuna	*Thunnus alalunga*	*Ocythoe tuberculata*	Roper and Sweeney 1975
Tuna	*Germon germon*	*Ocythoe tuberculata*	Roper and Sweeney 1975
Whiting	*Merluccius bilinearis*	*Illex illecebrosus*	Dexter 1969
Wolffish	*Anarhichas lupus*	*Bathypolypus arcticus*	Grieg 1930
Yellowfin grouper	*Mycteroperca venenosa*	*Sepioteuthis sepioidea*	Randall 1967
Yellowfin tuna	*Thunnus albacores*	*Illex argentinus*	Haimovici et al. 1998
Yellowfin tuna	*Thunnus albacores*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998

Marine Mammals

Baird's beaked whale	*Berardius bairdi*	*Berryteuthis magister magister*	Nesis 1998
Beaked whales		*Kondakovia longimana*	Clarke et al. 1998
Beaked whales		*Mesonychoteuthis hamiltoni*	Clarke et al. 1998
Blue whale		*Moroteuthis knipovitchi*	Clarke 1983
Bottlenose whale	*Hyperoodon planifrons*	*Alluroteuthis antarctica*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon rostratus*	*Gonatus fabricii*	Grieg 1930
Bottlenose whale	*Hyperoodon planifrons*	*Martialia hyadesi*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Moroteuthis ingens*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Moroteuthis knipovitchi*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Kondakovia longimana*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Brachioteuthis riisei*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Discoteuthis laciniosa*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Taningia danae*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Bathyteuthis abyssicola*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Histioteuthis eltaninae*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Batoteuthis skolops*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Chiroteuthis veranii*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Mesonychoteuthis hamiltoni*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Taonius pavo*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Teuthowenia pellucida*	Clarke and Goodall 1994
Bottlenose whale	*Hyperoodon planifrons*	*Galiteuthis armata*	Clarke and Goodall 1994

Bottlenose whale	*Hyperoodon planifrons*	*Gonatus antarcticus*	Clarke and Goodall 1994
Commerson's dolphin	*Cephalorhynchus commersonii*	*Loligo gahi*	Clarke and Goodall 1994
Common dolphin	*Delphinus delphis*	*Illex coindetii*	Clarke et al. 1998
Dall's porpoise	*Phocoenoides dalli*	*Berryteuthis magister magister*	Nesis 1998
Dolphin		*Nototodarus gouldi*	Machida 1983
Elephant seal	*Mirounga angustirostris*	*Taningia danae*	Roper and Vecchione 1993
False killer whale	*Pseudorca crassidens*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fin whale		*Moroteuthis knipovitchi*	Clarke 1983
Fur seal		*Nototodarus gouldi*	Machida 1983
Fur seal		*Kondakovia longimana*	Clarke 1983
Fur seal	*Arctocephalus tropicalis*	*Illex argentinus*	Haimovici et al. 1998
Greenland seal		*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Harp seal	*Phoca groenlandica*	*Todarodes sagittatus*	Hauksson and Bogason 1995
Hooded seal	*Cystophora cristata*	*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Hooded seal	*Cystophora cristata*	*Gonatus fabricii*	Grieg 1930
Leopard seal		*Kondakovia longimana*	Clarke 1983
Long-finned pilot whale	*Globicephala melas*	*Todarodes sagittatus*	Bjørke and Gjøsæter 1998
Long-finned pilot whale	*Globicephala melas*	*Selenoteuthis scintillans*	Gannon et al. 1997
Long-finned pilot whale	*Globicephala melas*	*Chiroteuthis veranii*	Gannon et al. 1997
Long-finned pilot whale	*Globicephala melas*	*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Long-finned pilot whale	*Globicephala melas*	*Histioteuthis reversa*	Gannon et al. 1997
Long-finned pilot whale	*Globicephala melas*	*Loligo pealeii*	Gannon et al. 1997
Marine mammal	*Kogia breviceps*	*Illex argentinus*	Haimovici et al. 1998
Narwhale		*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Northern bottlenose whale	*Hyperoodon ampullatus*	*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Northern bottlenose whale	*Hyperoodon ampullatus*	*Gonatus fabricii*	Lick and Piatkowski 1998
Northern fur seal	*Callorhynus ursinus*	*Berryteuthis magister magister*	Nesis 1998
Odontocete		*Gonatus fabricii*	Clarke 1983
Odontocete		*Todarodes sagittatus*	Clarke 1983
Pilot whale	*Globicephala malaena*	*Galiteuthis armata*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Moroteuthis ingens*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Brachioteuthis riisei*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Loligo gahi*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Illex illecebrosus*	Clarke and Goodall 1994

Pilot whale	*Globicephala malaena*	*Todarodes sagittatus*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Teuthowenia megalops*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Mesonychoteuthis hamiltoni*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Alluroteuthis antarctica*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Histioteuthis eltaninae*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Gonatus antarcticus*	Clarke and Goodall 1994
Pilot whale	*Globicephala malaena*	*Chiroteuthis veranii*	Clarke and Goodall 1994
Risso's dolphin	*Grampus griseus*	*Heteroteuthis dispar*	Villaneuva 1995
Risso's dolphin	*Grampus griseus*	*Ocythoe tuberculata*	Roper and Sweeney 1975
Ross seal		*Kondakovia longimana*	Clarke 1983
Rough-toothed dolphin	*Steno bredanensis*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Sea otter	*Enhydra lutris*	*Octopus dofleini dofleini*	Vincent et al. 1998
Seal		*Kondakovia longimana*	Clarke 1983
Seal		*Moroteuthis knipovitchi*	Clarke 1983
Seal		*Todarodes sagittatus*	Clarke 1983
Seal		*Gonatus antarcticus*	Clarke 1983
Seal		*Moroteuthis knipovitchi*	Clarke 1983
Seals		*Eledone cirrhosa cirrhosa*	Boyle and Pierce 1994
Seals		*Todarodes sagittatus*	Boyle and Pierce 1994
Short-finned pilot whale	*Globicephala macrorhynchus*	*Berryteuthis magister magister*	Nesis 1998
South African fur seal	*Arctocephalus pusillus pusillus*	*Octopus magnificus*	Villanueva 1993
Southern elephant seal	*Mirounga leonina*	*Martialia hyadesi*	Croxall and Prince 1994
Southern elephant seal	*Mirounga leonina*	*Kondakovia longimana*	Clarke et al. 1998
Southern elephant seal	*Mirounga leonina*	*Mesonychoteuthis hamiltoni*	Clarke et al. 1998
Sowerby's whale		*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Sperm whale	*Physeter catodon*	*Taningia danae*	Clarke 1983
Sperm whale	*Physeter macrocephalus*	*Todarodes sagittatus*	Clarke et al. 1998
Sperm whale	*Physeter macrocephalus*	*Teuthowenia megalops*	Clarke et al. 1998
Sperm whale	*Physeter catodon*	*Moroteuthis robsoni*	Clarke 1983
Sperm whale	*Physeter macrocephalus*	*Undetermined forbesii*	Clarke et al. 1998
Sperm whale	*Physeter macrocephalus*	*Histioteuthis bonnellii*	Clarke et al. 1998
Sperm whale	*Physeter macrocephalus*	*Eledone cirrhosa cirrhosa*	Clarke et al. 1998
Sperm whale	*Physeter macrocephalus*	*Haliphron atlanticus*	Clarke et al. 1998
Sperm whale	*Physeter catodon*	*Taningia danae*	Clarke 1983

Sperm whale	*Physeter catodon*	*Vampyroteuthis infernalis*	Clarke 1983
Sperm whale	*Physeter macrocephalus*	*Kondakovia longimana*	Clarke et al. 1998
Sperm whale	*Physeter catodon*	*Todarodes sagittatus*	Clarke 1983
Sperm whale	*Physeter catodon*	*Histioteuthis atlantica*	Clarke 1983
Sperm whale	*Physeter catodon*	*Histioteuthis miranda*	Clarke 1983
Sperm whale	*Physeter catodon*	*Histioteuthis hoylei*	Clarke 1983
Sperm whale	*Physeter catodon*	*Cycloteuthis akimushkini*	Clarke 1983
Sperm whale	*Physeter catodon*	*Lepidoteuthis grimaldii*	Clarke 1983
Sperm whale	*Physeter macrocephalus*	*Mesonychoteuthis hamiltoni*	Clarke et al. 1998
Sperm whale	*Physeter catodon*	*Kondakovia longimana*	Clarke 1983
Sperm whale	*Physeter catodon*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Sperm whale	*Physeter catodon*	*Octopoteuthis rugosa*	Clarke 1983
Sperm whale	*Physeter macrocephalus*	*Gonatus fabricii*	Bjørke and Gjøsæter 1998
Sperm whale	*Physeter catodon*	*Ancistrocheirus lesueurii*	Clarke 1983
Sperm whale	*Physeter catodon*	*Pholidoteuthis boschmai*	Clarke 1983
Sperm whale	*Physeter catodon*	*Pholidoteuthis boschmai*	Clarke 1983
Sperm whale	*Physeter catodon*	*Pholidoteuthis boschmai*	Clarke 1983
Sperm whale	*Physeter catodon*	*Gonatus antarcticus*	Clarke 1983
Sperm whale	*Physeter catodon*	*Gonatus antarcticus*	Clarke 1983
Sperm whale	*Physeter catodon*	*Gonatus fabricii*	Clarke 1983
Sperm whale	*Physeter catodon*	*Moroteuthis knipovitchi*	Clarke 1983
Sperm whale	*Physeter catodon*	*Berryteuthis magister magister*	Clarke 1983
Sperm whale	*Physeter catodon*	*Todarodes sagittatus*	Clarke 1983
Sperm whale	*Physeter catodon*	*Moroteuthis robsoni*	Clarke 1983
Sperm whale	*Physeter catodon*	*Todarodes sagittatus*	Clarke 1983
Sperm whale	*Physeter catodon*	*Dosidicus gigas*	Clarke 1983
Sperm whale	*Physeter catodon*	*Moroteuthis ingens*	Clarke 1983
Sperm whale	*Physeter catodon*	*Berryteuthis magister magister*	Clarke 1983
Sperm whale	*Physeter catodon*	*Moroteuthis robsoni*	Clarke 1983
Sperm whale	*Physeter catodon*	*Kondakovia longimana*	Clarke 1983
Sperm whale	*Physeter catodon*	*Moroteuthis robsoni*	Clarke 1983
Sperm whale	*Physeter catodon*	*Moroteuthis robsoni*	Clarke 1983
Sperm whale	*Physeter catodon*	*Moroteuthis robusta*	Clarke 1983
Sperm whale	*Physeter catodon*	*Moroteuthis robusta*	Clarke 1983

Sperm whale	*Physeter catodon*	*Moroteuthis knipovitchi*	Clarke 1983
Sperm whale	*Physeter macrocephalus*	*Illex argentinus*	Haimovici et al. 1998
Sperm whale	*Physeter catodon*	*Moroteuthis robsoni*	Clarke 1983
Spotted dolphin	*Stenella attenuata*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Walrus		*Benthoctopus sibiricus*	Robson 1931
Whale	*Berardius* sp.	*Gonatus fabricii*	Bjørke Gjøsæter 1998
Whales		*Sepia officinalis*	Le-Mao 1985
White Whale		*Gonatus fabricii*	Bjørke Gjøsæter 1998

Mollusca

Commander squid	*Berryteuthis magister magister*	*Berryteuthis magister magister*	Nesis 1998
Cuttlefish	*Sepia australis*	*Sepia australis*	Sánchez and Villanueva 1991
Giant squid	*Architeuthis dux*	*Eledone cirrhosa cirrhosa*	Lordan et al. 1998
Nautilus	*Nautilus pompilius pompilius*	*Nautilus pompilius pompilius*	Carlson 1987
Octopus	*Octopus* sp.	*Nautilus scrobiculatus*	Saunders et al. 1991
Octopus	*Octopus* sp.	*Nautilus pompilius pompilius*	Saunders et al. 1991
Octopus	*Octopus bimaculatus*	*Octopus bimaculatus*	Ambrose 1984
Ommastrephid squid	*Dosidicus gigas*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Ommastrephid squid	*Sthenoteuthis oualaniensis*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Ommastrephid squid	*Sthenoteuthis pteropus*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Ommastrephid squid	*Hyaloteuthis pelagica*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998

Appendix 2

Cephalopod prey

All cephalopods are active predators that primarily feed on crustaceans, fish and molluscs (Nixon 1987, Hanlon and Messenger 1996). Prey in these three groups are over 94% of the records listed in this appendix. Major groups of consumed crustaceans include: amphipods, crabs (many anomuran crabs but primary brachyuran species), copepods, euphausids (krill), mysid shrimp and decapod shrimp. Isopods, lobsters and mantis shrimp are also taken but less frequently. Almost all of the fish ingested are teleost fish. Some of these fish such as Hake, Redfish and Whiting appear on both the prey list and on the list of cephalopod predators. Predation from and competition with vertebrates such as fish are thought to have been major forces in the evolution of cephalopods (Chapter 1). Other molluscs round out the major prey of cephalopods. Major groups of prey within the phylum include: bivalves (clams, mussels, scallops), cephalopods (primarily squid eaten by other squid), gastropods (abalone, limpets and various other species are typically taken by octopuses). Chitons and tourists are also ingested, but much less frequently.

This appendix also lists prey groups less frequently eaten by cephalopods. All of these minor prey groups combined represent less than 6% of the records. However, these species may make up the bulk of a cephalopods diet. For example, brittle stars are though to be the major prey of *Bathypolypus arcticus* (Chapter 2). Chaetognaths were taken by six species of squid, two species of octopus predated echinoderms, polychaetes were ingested by a number of squid, cuttlefish and octopuses and foraminiferans were ingested by three species of deep-sea octopus including *B. arcticus*.

The miscellaneous category contains prey that there were not enough records to create a new heading for or items that are sufficiency vague, such as plankton, that don't fit into any of the taxa headings. Some of the prey, such as algae and bryozoans, are likely unintentional by-catch but could contain some limiting factor that is not otherwise provided in the animals diet. Other records in this category are simply cephalopod species that live in environments that we do not sample often and thus have fewer entries for. Examples are the deep-sea octopus *Bathypolypus arcticus* and the open ocean argonaut *Argonauta argo*.

This data is from the December 1st 1999 version of CephBase (http://www.cephbase.dal.ca/ Wood et al. 1999).

PreyCommonName	PreyName	Cephalopod Predator	Reference
Chaetognaths			
Chaetognath	*Sagitta elegans*	*Todarodes sagittatus*	Breiby and Jobling 1984
Chaetognaths		*Illex coindetii*	Sánchez et al.1998
Chaetognaths		*Illex argentinus*	Haimovici et al.1998
Chaetognaths		*Mesonychoteuthis hamiltoni*	Lu and Williams1994
Chaetognaths		*Loligo pealeii*	Boletzky and Hanlon 1983
Chaetognaths		*Illex illecebrosus*	Mangold 1983
Crustaceans			
Amphipod	*Leucothoe* sp.	*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Amphipod	*Primno macropa*	*Illex argentinus*	Ivanovic and Brunetti 1994
Amphipod	*Vibilia armata*	*Illex coindetii*	Sánchez et al.1998
Amphipod	*Cyllopus magellanicus*	*Illex argentinus*	Ivanovic and Brunetti 1994
Amphipod	*Monoculodes* sp.	*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Amphipod	*Orchomene* sp.	*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Amphipod	*Aora* sp.	*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Amphipod	*Hyperoche medusarum*	*Illex argentinus*	Ivanovic and Brunetti 1994
Amphipod	*Themisto gaudichaudii*	*Illex argentinus*	Ivanovic and Brunetti 1994
Amphipod	*Orchomene* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Amphipod	*Monoculodes* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Amphipods		*Octopus briareus*	Boletzky and Hanlon 1983
Amphipods		*Octopus australis*	Boletzky and Hanlon 1983
Amphipods	*Gammarus* spp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Amphipods		*Octopus vulgaris*	Boletzky and Hanlon 1983
Anomuran crab	*Pisida longicornis*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Anomuran crab	*Anapagurus laevis*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Anomuran crab	*Galathea* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Antarctic krill	*Euphausia superba*	*Martialia hyadesi*	González and Rodhouse 1998
Antarctic krill	*Euphausia superba*	*Psychroteuthis glacialis*	Lu and Williams 1994
Barnacle	*Semibalanus cariosus*	*Octopus dofleini dofleini*	Vincent et al. 1998
Blue crab	*Callinectes sapidus*	*Octopus briareus*	Boletzky and Hanlon 1983
Blue crab	*Callinectes ornatus*	*Octopus briareus*	Boletzky and Hanlon 1983
Brachiopod	*Terebratalia* sp.	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983

Brachyuran crab	*Gonoplax rhomoides*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Macropipus depurator*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Pisa armata*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Inachus* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Calappa granulata*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Dorippe lanata*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Ethusa mascarone*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Dromia personata*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Calappa flammea*	*Octopus briareus*	Boletzky and Hanlon 1983
Brachyuran crab	*Macropipus corrugatus*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Calappa* sp.	*Octopus cyanea*	Boletzky and Hanlon 1983
Brachyuran crab	*Pisa nodipes*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Brachyuran crab	*Medeus conchii*	*Eledone cirrhosa cirrhosa*	Boletzky and Hanlon 1983
Brachyuran crab	*Gonoplax* sp.	*Eledone cirrhosa cirrhosa*	Boletzky and Hanlon 1983
Brachyuran crabs		*Nautilus macromphalus*	Boletzky and Hanlon 1983
Brachyuran crabs		(Undetermined) *forbesii*	Guerra and Rocha 1994
Brine shrimp	*Artemia* sp.	*Octopus briareus*	Boletzky and Hanlon 1983
Calico crab	*Hepatus* sp.	*Octopus burryi*	Boletzky and Hanlon 1983
Copepod	*Candacia* sp.	*Loligo pealeii*	Maurer and Bowman 1984
Copepod	*Undinopsis* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Copepod	*Undinopsis* sp.	*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Copepod	*Stephos* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Copepod	*Ateidopsis* sp.	*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Copepod	*Aegisthus* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Copepod	*Oncaea* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Copepod	*Oothrix* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Copepod	*Oncaea media*	*Illex argentinus*	Haimovici et al. 1998
Copepod	*Calanus propinquus*	*Illex argentinus*	Ivanovic and Brunetti 1994
Copepod	*Calanoida* sp.	*Todarodes sagittatus*	Breiby and Jobling 1984
Copepod	*Oncaea* sp.	*Illex coindetii*	Sánchez et al. 1998
Copepods		*Sepia officinalis*	Boletzky and Hanlon 1983
Copepods		(Undetermined) *forbesii*	Guerra and Rocha 1994
Crab	*Acantholithodes hispidus*	*Octopus dofleini dofleini*	Vincent et al. 1998
Crab	*Libinia erinacea*	*Octopus briareus*	Boletzky and Hanlon 1983

Crab	*Ocypode albicans*	*Octopus briareus*	Boletzky and Hanlon 1983
Crab	*Paraxanthias taylori*	*Octopus bimaculatus*	Ambrose 1984
Crab	*Mursia cristimanus*	*Octopus magnificus*	Villanueva 1993
Crab	*Corystoides chilensis*	*Octopus tehuelchus*	Iribarne et al. 1991
Crab	*Pugettia gracilis*	*Octopus dofleini dofleini*	Vincent et al. 1998
Crab	*Portunus* spp.	*Octopus briareus*	Boletzky and Hanlon 1983
Crab	*Cancer gracilis*	*Octopus dofleini dofleini*	Vincent et al. 1998
Crab	*Chasmognathus granulata*	*Octopus tehuelchus*	Iribarne et al. 1991
Crab	*Cancer antennarius*	*Octopus bimaculatus*	Ambrose 1984
Crab	*Libinia spinosa*	*Octopus tehuelchus*	Iribarne et al. 1991
Crab	*Rochinia gracilipes*	*Octopus tehuelchus*	Iribarne et al. 1991
Crab	*Carcinus mediterraneus*	*Eledone cirrhosa cirrhosa*	Mangold 1983
Crab	*Pilumnus reticulatus*	*Octopus tehuelchus*	Iribarne et al. 1991
Crab	*Leurocyclus tuberculosus*	*Octopus tehuelchus*	Iribarne et al. 1991
Crab	*Cancer oregonensis*	*Octopus dofleini dofleini*	Vincent et al. 1998
Crab	*Lophopanopeus bellus*	*Octopus dofleini dofleini*	Vincent et al. 1998
Crab	*Chaceon* sp.	*Octopus magnificus*	Villanueva 1993
Crab	*Telmessus cheiragonus*	*Octopus dofleini dofleini*	Vincent et al. 1998
Crab	*Carcinus* sp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Crab megalopa larvae		*Nototodarus gouldi*	Machida 1983
Crabs		*Eledone cirrhosa cirrhosa*	Mangold 1983
Crabs		*Octopus magnificus*	Villanueva 1993
Crabs		*Uroteuthis edulis*	Natsukari and Tashiro 1991
Crabs		*Rossia pacifica pacifica*	Boletzky and Hanlon 1983
Crangonid prawn	*Philocheras* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Crustacean	*Grapsus grapsus*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Cardisoma guanhumi*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Gecarcinus lateralis*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Parathemisto* sp.	*Ommastrephes bartramii*	Araya 1983
Crustacean	*Calastacus longispinis*	*Octopus magnificus*	Villanueva 1993
Crustacean	*Mennippe mercenaria*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Hemisquilla ensigera*	*Octopus bimaculatus*	Ambrose 1984
Crustacean	*Astacus leptodactylus*	*Sepia officinalis*	Boletzky and Hanlon 1983
Crustacean	*Gonodactylus* sp.	*Octopus briareus*	Boletzky and Hanlon 1983

Crustacean	*Daphnia* sp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Crustacean	*Hippolyte* sp.	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Synalpheus brevicarpus*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Hippolyte* sp.	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Loxorhynchus* sp.	*Octopus bimaculatus*	Ambrose 1984
Crustacean	*Tozeuma carolinense*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Latreutes fucorum*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Stenorhynchus seticornis*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Coenobita clypeatus*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Aratus pisonii*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Oplophorus gracilirostris*	*Sthenoteuthis oualaniensis*	Young and Hirota 1998
Crustacean	*Dardanaus venosus*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Petrochirus digenes*	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Macrocoeloma* sp.	*Octopus briareus*	Boletzky and Hanlon 1983
Crustacean	*Parathemisto abyssorum*	*Todarodes sagittatus*	Breiby and Jobling 1984
Crustaceans		*Berryteuthis magister magister*	Nesis 1998
Crustaceans		*Sepia officinalis*	Le-Mao 1985
Crustaceans		*Bathypolypus arcticus*	Boletzky and Hanlon 1983
Crustaceans		*Illex argentinus*	Ivanovic and Brunetti 1994
Crustaceans		*Psychroteuthis glacialis*	Lu and Williams 1994
Crustaceans		*Loligo vulgaris reynaudi*	Augustyn 1990
Crustaceans		*Rossia pacifica pacifica*	Mangold 1983
Crustaceans		*Sthenoteuthis pteropus*	Boletzky and Hanlon 1983
Crustaceans		*Sepia australis*	Sánchez and Villanueva 1991
Crustaceans		*Sepietta oweniana*	Mangold 1983
Crustaceans		*Mastigoteuthis agassizii*	Verrill 1881
Crustaceans		*Sthenoteuthis oualaniensis*	Young and Hirota 1998
Crustaceans		*Loligo pealeii*	Boletzky and Hanlon 1983
Cumaceans		*Bathypolypus arcticus*	Boletzky and Hanlon 1983
Cumaceans		*Octopus australis*	Boletzky and Hanlon 1983
Decapod	*Merhippolyte agulhasensis*	*Octopus magnificus*	Villanueva 1993
Decapod	*Pontophilus gracilis*	*Octopus magnificus*	Villanueva 1993
Decapod	*Pontophilus* sp.	*Octopus magnificus*	Villanueva 1993
Decapod crustacean	*Pasiphaea longirostris*	*Illex coindetii*	Sánchez et al. 1998

Decapod crustacean	*Oregonia gracilis*	*Octopus dofleini dofleini*	Vincent et al. 1998
Decapod crustacean	*Pasiphaea sivado*	*Illex coindetii*	Sánchez et al. 1998
Decapod crustacean	*Hyas lyratus*	*Octopus dofleini dofleini*	Vincent et al. 1998
Decapod crustacean	*Pasiphaea multidentata*	*Illex coindetii*	Sánchez et al. 1998
Decapod crustacean	*Phyllolithodes papillosus*	*Octopus dofleini dofleini*	Vincent et al. 1998
Decapod crustacean	*Munida gregaria*	*Illex argentinus*	Haimovici et al. 1998
Decapod crustacean	*Munidopsis* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Decapod crustacean	*Pontophylus* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Decapod crustacean	*Pasiphaea heterocarpus*	*Illex coindetii*	Sánchez et al. 1998
Decapod crustacean	*Pontophylus* sp.	*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Decapod crustacean	*Plesionica* sp.	*Illex coindetii*	Sánchez et al. 1998
Decapod crustaceans		*Loligo vulgaris vulgaris*	Coelho et al. 1997
Decapod shrimp		*Loligo pealeii*	Maurer and Bowman 1984
Deep-sea portunid crab	*Bathynectes piperitus*	*Octopus magnificus*	Villanueva 1993
Euphausid shrimp		*Loligo vulgaris vulgaris*	Coelho et al. 1997
Euphausids		(Undetermined) *forbesii*	Guerra and Rocha 1994
Euphausids		*Sepia australis*	Sánchez and Villanueva 1991
Euphausiid	*Meganectiphanes norvegica*	*Illex coindetii*	Sánchez et al. 1998
Euphausiid	*Thysanoessa gregaria*	*Illex argentinus*	Ivanovic and Brunetti 1994
Euphausiid	*Meganyctiphanes norvegica*	*Todarodes sagittatus*	Breiby and Jobling 1984
Euphausiid	*Euphausia lucens*	*Illex argentinus*	Ivanovic and Brunetti 1994
Euphausiid	*Nematoscelis megalops*	*Illex argentinus*	Ivanovic and Brunetti 1994
Euphausiid shrimp	*Nyctiphanes couchi*	*Illex coindetii*	Sánchez et al. 1998
Euphausiid shrimp	*Euphasia* sp.	*Illex coindetii*	Sánchez et al. 1998
Euphausiid shrimps		*Ommastrephes bartramii*	Araya 1983
Euphausiids		*Illex illecebrosus*	Maurer and Bowman 1984
Euphausiids		*Loligo pealeii*	Maurer and Bowman 1984
Euphausiids		*Berryteuthis magister magister*	Nesis 1998
Fiddler crab	*Uca pugilator*	*Octopus briareus*	Boletzky and Hanlon 1983
Fiddler crab	*Uca* sp.	*Octopus burryi*	Boletzky and Hanlon 1983
Gammarid amphipod	*Eusirus minutus*	*Octopus magnificus*	Villanueva 1993
Gammarid amphipod	*Rhachotropsis* sp.	*Octopus magnificus*	Villanueva 1993
Gammarid amphipods		*Loligo pealeii*	Maurer and Bowman 1984
Gammarid amphipods		*Illex illecebrosus*	Maurer and Bowman 1984

Gammarid shrimp		*Loligo vulgaris vulgaris*	Coelho et al. 1997
Grass shrimp	*Palaemonetes pugio*	*Loligo plei*	Boletzky and Hanlon 1983
Hermit crab	*Clibanarius tricolor*	*Octopus briareus*	Boletzky and Hanlon 1983
Hermit crab	*Clibanarius vittatus*	*Octopus briareus*	Boletzky and Hanlon 1983
Hermit crab	*Aniculus aniculus*	*Nautilus macromphalus*	Boletzky and Hanlon 1983
Hermit crab	*Phyllopagurus californica*	*Octopus bimaculatus*	Ambrose 1984
Hermit crab	*Pagurus* sp.	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Hermit crab	*Pagurus* sp.	*Octopus tehuelchus*	Iribarne et al. 1993
Hermit crab	*Parapagurus dimorphus*	*Octopus magnificus*	Villanueva 1993
Hermit crabs		*Octopus vulgaris*	Villanueva 1993
Hermit crabs		*Octopus rubescens*	Villanueva 1993
Hermit crabs		*Octopus bimaculoides*	Villanueva 1993
Hermit crabs		*Octopus macropus*	Boletzky and Hanlon 1983
Hermit crabs		*Octopus maya*	Villanueva 1993
Hyperiid amphipods		*Berryteuthis magister magister*	Nesis 1998
Isopod	*Haploniscus* sp.	*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Isopod	*Cymodocea* sp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Isopod	*Sphaeroma* sp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Isopod	*Aegea gracilis*	*Octopus magnificus*	Villanueva 1993
Isopods		*Octopus australis*	Boletzky and Hanlon 1983
Isopods		*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Isopods		*Octopus cyanea*	Boletzky and Hanlon 1983
Isopods		*Loligo vulgaris vulgaris*	Coelho et al. 1997
Kelp crab	*Pugettia producta*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Kelp crabs		*Octopus briareus*	Boletzky and Hanlon 1983
Krill		*Alluroteuthis antarctica*	Lu and Williams 1994
Krill	*Euphausia* sp.	*Loligo gahi*	Guerra et al. 1991
Lobster	*Panulirus longipes*	*Nautilus macromphalus*	Boletzky and Hanlon 1983
Lobster	*Panulirus interruptus*	*Octopus bimaculatus*	Ambrose 1984
Lobster	*Panulirus argus*	*Octopus briareus*	Boletzky and Hanlon 1983
Lobster	*Stereomastis shumi*	*Octopus magnificus*	Villanueva 1993
Lobsters		*Octopus bimaculatus*	Ambrose 1984
Lobsters		*Eledone cirrhosa cirrhosa*	Mangold 1983
Mantis shrimp	*Squilla* sp.	*Sepia officinalis*	Boletzky and Hanlon 1983

Mantis shrimp	*Squilla mantis*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Mantis shrimp	*Squilla armata*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Mysid	*Mysis* sp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Mysid	*Neomysis japonica*	*Sepia esculenta*	Boletzky and Hanlon 1983
Mysid	*Neomysis japonica*	*Sepia aculeata*	Boletzky and Hanlon 1983
Mysid	*Praunus* sp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Mysid	*Metamysidopsis elongata*	*Loligo opalescens*	Boletzky and Hanlon 1983
Mysid shrimp		*Illex coindetii*	Sánchez et al. 1998
Mysid shrimp		(Undetermined) *forbesii*	Guerra and Rocha 1994
Mysids		*Octopus australis*	Boletzky and Hanlon 1983
Mysids		*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Mysids		*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Mysids		*Rossia pacifica pacifica*	Boletzky and Hanlon 1983
Norway lobster	*Nephrops norvegicus*	*Architeuthis dux*	Lordan et al. 1998
Ostracod crustaceans		*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Ostracod crustaceans		*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Pelagic red crab	*Pleuroncodes planipes*	*Loligo opalescens*	Boletzky and Hanlon 1983
Pelagic red crab	*Pleuroncodes planipes*	*Dosidicus gigas*	Mangold 1983
Penaeid shrimps (post larval)		*Dosidicus gigas*	Boletzky and Hanlon 1983
Pink shrimp	*Penaeus duorarum*	*Octopus briareus*	Boletzky and Hanlon 1983
Portunid crabs		*Octopus cyanea*	Boletzky and Hanlon 1983
Prawn	*Palaemon* sp.	*Sepia latimanus*	Boletzky and Hanlon 1983
Prawn	*Crangon* spp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Prawn	*Leander serrifer*	*Sepia aculeata*	Boletzky and Hanlon 1983
Prawn	*Leander serrifer*	*Sepia esculenta*	Boletzky and Hanlon 1983
Rock crab	*Cancer productus*	*Octopus dofleini dofleini*	Vincent et al. 1998
Rock lobster	*Panulirus homarus*	*Octopus vulgaris*	Mangold 1983
Sand crab	*Emerita talpoida*	*Octopus briareus*	Boletzky and Hanlon 1983
Shore crab	*Pachygrapsus transversus*	*Octopus vulgaris*	Mather 1991
Shore crab	*Mithrax forceps*	*Octopus vulgaris*	Mather 1991
Shore crab	*Sesarma cinereum*	*Octopus briareus*	Boletzky and Hanlon 1983
Shore crab	*Sesarma* sp.	*Octopus burryi*	Boletzky and Hanlon 1983
Shore crab	*Pachygrapsus crassipes*	*Octopus bimaculoides*	Boletzky and Hanlon 1983

Shore crab	*Pachygrapsus transversus*	*Octopus briareus*	Boletzky and Hanlon 1983
Shore crab	*Mithrax hispidus*	*Octopus briareus*	Boletzky and Hanlon 1983
Shore crab	*Carcinus maenas*	*Octopus joubini*	Boletzky and Hanlon 1983
Shore crab	*Pachygrapsus crassipes*	*Octopus bimaculatus*	Ambrose 1984
Shrimp		*Nototodarus gouldi*	Machida 1983
Shrimp	*Pasiphaea* sp.	*Todarodes sagittatus*	Breiby and Jobling 1984
Shrimp	*Alpheus glaber*	*Illex coindetii*	Sánchez et al. 1998
Shrimp	*Palaemon* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Shrimp	*Leander* sp.	*Octopus briareus*	Boletzky and Hanlon 1983
Shrimp	*Alpheus* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Shrimp		*Octopus dofleini dofleini*	Vincent et al. 1998
Shrimp	*Pandalina brevirostris*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Shrimp	*Pontocaris catafracta*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Shrimp	*Crangon crangon*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Shrimp	*Alpheus formosus*	*Octopus briareus*	Boletzky and Hanlon 1983
Shrimp		*Rossia pacifica pacifica*	Boletzky and Hanlon 1983
Shrimp	*Crangon* spp.	*Illex illecebrosus*	Mangold 1983
Shrimp	*Penaeus* sp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Shrimp		*Sepia latimanus*	Boletzky and Hanlon 1983
Shrimp	*Halocaridina rubra*	*Euprymna scolopes*	Shears 1988
Shrimp	*Palaemon debilis*	*Euprymna scolopes*	Shears 1988
Shrimp	*Solenocera membranacea*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Shrimp	*Palaemon pacificus*	*Euprymna scolopes*	Shears 1988
Shrimp	*Penaeus kerathurus*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Shrimp	*Xiphopenaeus riveti*	*Lolliguncula panamensis*	Boletzky and Hanlon 1983
Shrimp	*Alpheus glaber*	*Eledone cirrhosa cirrhosa*	Boletzky and Hanlon 1983
Shrimp		*Eledone cirrhosa cirrhosa*	Mangold 1983
Shrimp		*Octopus bimaculatus*	Ambrose 1984
Stomatopod	*Pterygosquilla armata capensis*	*Octopus magnificus*	Villanueva 1993
Stomatopods		*Octopus cyanea*	Boletzky and Hanlon 1983
Turtle crab	*Cryptolithodes sitchensis*	*Octopus dofleini dofleini*	Vincent et al. 1998
White shrimp	*Penaeus setiferus*	*Loligo plei*	Boletzky and Hanlon 1983
Xanthid crab	*Panopeus herbstii*	*Octopus briareus*	Boletzky and Hanlon 1983
Xanthid crabs		*Octopus cyanea*	Boletzky and Hanlon 1983

Echinoderms

Ophiurids		*Bathypolypus arcticus*	Boletzky and Hanlon 1983
Sea urchin	*Strongylocentrotus droebachiensis*	*Octopus dofleini dofleini*	Vincent et al. 1998

Fish

Ammodytid fish		(Undetermined) *forbesii*	Guerra and Rocha 1994
Anchovies		*Dosidicus gigas*	Boletzky and Hanlon 1983
Anchovy	*Engraulis encrasicolus*	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Anchovy	*Engraulis capensis*	*Loligo vulgaris reynaudi*	Augustyn 1990
Anchovy	*Engraulis encrasicolus*	*Illex coindetii*	Sánchez et al. 1998
Anchovy	*Engraulis anchoita*	*Illex argentinus*	Ivanovic and Brunetti 1994
Antarctic silverfish	*Pleuragramma antarcticum*	*Alluroteuthis antarctica*	Lu and Williams 1994
Antarctic silverfish	*Pleuragramma antarcticum*	*Psychroteuthis glacialis*	Lu and Williams 1994
Benthic cottoid fishes		*Berryteuthis magister magister*	Nesis 1998
Blenny	*Hypsoblennius gilberti*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Blenny	*Hypsoblennius gentilis*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Blue sprat	*Spratelloides gracilis*	*Uroteuthis edulis*	Natsukari and Tashiro 1991
Boar fish	*Capros aper*	(Undetermined) *forbesii*	Boyle and Pierce 1994
Bony fishes		*Sepia officinalis*	Boletzky and Hanlon 1983
Capelin	*Mallotus villosus*	*Todarodes sagittatus*	Breiby and Jobling 1984
Capelin	*Mallotus villosus*	*Illex illecebrosus*	Mangold 1983
Carangid fishes		*Dosidicus gigas*	Boletzky and Hanlon 1983
Chub mackerel	*Scomber japonicus*	*Dosidicus gigas*	Boletzky and Hanlon 1983
Cod	*Gadus morhua*	*Todarodes sagittatus*	Breiby and Jobling 1984
Eel		*Eledone cirrhosa cirrhosa*	Boletzky and Hanlon 1983
Engraulid fishes		*Dosidicus gigas*	Boletzky and Hanlon 1983
Estuarine fish	*Adeinia xenica*	*Loligo plei*	Boletzky and Hanlon 1983
Estuarine fish	*Fundulus similis*	*Loligo plei*	Boletzky and Hanlon 1983
Estuarine fish	*Fundulus* spp.	*Illex illecebrosus*	Mangold 1983
Estuarine fish	*Menidia beryllina*	*Loligo plei*	Boletzky and Hanlon 1983
Estuarine fish	*Fundulus grandis*	*Loligo plei*	Boletzky and Hanlon 1983
Estuarine fish	*Cyprinodon variegatus*	*Loligo plei*	Boletzky and Hanlon 1983
Estuarine fish	*Gambusia affinis*	*Loligo plei*	Boletzky and Hanlon 1983
Estuarine fish	*Mugil* spp.	*Loligo plei*	Boletzky and Hanlon 1983

Fish	*Notoscopelus elongatus*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Merluccius hubbsi*	*Illex argentinus*	Haimovici et al. 1998
Fish	*Ceratoscopelus maderensis*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Myctophum punctatum*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Sudis hyalina*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Synagrops* sp.	*Illex coindetii*	Sánchez et al. 1998
Fish	*Protomyctophum bolini*	*Martialia hyadesi*	González and Rodhouse 1998
Fish	*Krefftichthys anderssoni*	*Martialia hyadesi*	González and Rodhouse 1998
Fish	*Micromesisteus potassou*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Protomyctophum choriodon*	*Martialia hyadesi*	González and Rodhouse 1998
Fish	*Scomber colia*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Chlorophtalmus atlantica*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Epigonus telescopus*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Gadiculus argenteus*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Antonogadus megalokynodum*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Sardina pilchardus*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Opsanus beta*	*Octopus briareus*	Boletzky and Hanlon 1983
Fish	*Diaphus dumerilii*	*Illex argentinus*	Haimovici et al. 1998
Fish	*Cepola rubescens*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Fish	*Symbolophorus* sp.	*Octopus magnificus*	Villanueva 1993
Fish	*Coelorinchus* sp.	*Octopus magnificus*	Villanueva 1993
Fish	*Helicolenus dactylopterus*	*Octopus magnificus*	Villanueva 1993
Fish	*Paracallionymus costatus*	*Octopus magnificus*	Villanueva 1993
Fish	*Sufflogobius bibarbatus*	*Octopus magnificus*	Villanueva 1993
Fish	*Paralabrax clathratus*	*Octopus bimaculatus*	Ambrose 1984
Fish	*Myliobatis californica*	*Octopus bimaculatus*	Ambrose 1984
Fish	*Atherina* sp.	*Loligo vulgaris vulgaris*	Boletzky and Hanlon 1983
Fish	*Cetengraulis* spp.	*Lolliguncula panamensis*	Boletzky and Hanlon 1983
Fish	*Hippocampus erectus*	*Octopus briareus*	Boletzky and Hanlon 1983
Fish		*Sthenoteuthis pteropus*	Boletzky and Hanlon 1983
Fish	*Pollachius virens*	*Todarodes sagittatus*	Breiby and Jobling 1984
Fish	*Uranoscopus* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Fish	*Scorpaena brasiliensis*	*Octopus briareus*	Boletzky and Hanlon 1983
Fish	*Acanthostracion quadricornis*	*Octopus briareus*	Boletzky and Hanlon 1983

Fish	*Lampanyctus crocodrilus*	*Illex coindetii*	Sánchez et al. 1998
Fish	*Trachinotus carolinus*	*Octopus briareus*	Boletzky and Hanlon 1983
Fish	*Sebastes* sp.	*Todarodes sagittatus*	Breiby and Jobling 1984
Fish	*Melanogrammus aeglefinus*	*Todarodes sagittatus*	Breiby and Jobling 1984
Fish	*Micromesistius poutassou*	*Todarodes sagittatus*	Breiby and Jobling 1984
Fish	*Trisopterus esmarkii*	*Todarodes sagittatus*	Breiby and Jobling 1984
Fish	*Leptochinus maculatus*	*Todarodes sagittatus*	Breiby and Jobling 1984
Fish	*Opisthonema* spp.	*Lolliguncula panamensis*	Boletzky and Hanlon 1983
Fish	*Gymnammodytes semisquamatus*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Pomatoschistus minutus*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Austroglossus microlepis*	*Sepia australis*	Sánchez and Villanueva 1991
Fish	*Diaphus dumerilii*	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Fish	*Argentina sphyraena*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Trachurus trachurus*	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Fish	*Gyrnnocephalus cernuus*	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Fish	*Paleomonas* sp.	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Fish	*Microchirus boscanion*	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Fish	*Chelidonichthys* sp.	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Fish	*Sprattus sprattus*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Argentina sphyraena*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Micromesistius poutassou*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Gadiculus argenteus*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Trisopterus* spp.	(Undetermined) *forbesii*	Boyle and Pierce 1994
Fish	*Globiusculus flavescens*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Sardina pilchardus*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Atherina presbyter*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Blennius ocellaris*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Callionymus reticulatus*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Merluccius merluccius*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Cepola macrophtalma*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Trisopterus* sp.	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Aphia minuta*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Aphia minuta*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Cepola macrophtalma*	(Undetermined) *forbesii*	Guerra and Rocha 1994

Fish	*Hyperoplus lanceolatus*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Trachurus trachurus*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Gymnammodytes semisquamatus*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Fish	*Sardina pilchardus*	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Fish	*Micromesistius poutassou*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Nemichthes* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish	*Lipolagus ochotensis*	*Berryteuthis magister magister*	Nesis 1998
Fish	*Maurolicus japonicus*	*Berryteuthis magister magister*	Nesis 1998
Fish	*Atherina* sp.	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Stomias* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish	*Diplospinus* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish	*Callionymus lyra*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Leuroglossus schmidti*	*Berryteuthis magister magister*	Nesis 1998
Fish	*Cubiceps* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish	*Hygophum* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish	*Diaphus* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish	*Crystalogobius* sp.	(Undetermined) *forbesii*	Guerra and Rocha 1994
Fish	*Myctophum* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish	*Paralepis* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish	*Chauliodus sloani*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish	*Cyclothone* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fish (juveniles)	*Theragra chalcogramma*	*Berryteuthis magister magister*	Nesis 1998
Fish fry	*Mugil* sp.	*Sepia officinalis*	Boletzky and Hanlon 1983
Fishes		*Lolliguncula panamensis*	Mangold 1983
Fishes		*Loligo pealeii*	Boletzky and Hanlon 1983
Fishes		*Octopus cyanea*	Boletzky and Hanlon 1983
Fishes		*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Fishes		*Octopus rubescens*	Boletzky and Hanlon 1983
Fishes		*Lolliguncula brevis*	Boletzky and Hanlon 1983
Fishes		*Sthenoteuthis oualaniensis*	Young and Hirota 1998
Fishes		*Loligo vulgaris reynaudi*	Augustyn 1990
Fishes		*Sepia australis*	Sánchez and Villanueva 1991
Fishes		*Sepia officinalis*	Le-Mao 1985
Fishes		*Octopus vulgaris*	Villanueva 1993

Fishes		*Ommastrephes bartramii*	Mangold 1983
Fishes		*Nautilus macromphalus*	Boletzky and Hanlon 1983
Fishes		*Psychroteuthis glacialis*	Lu and Williams 1994
Flat fish		*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Flatfish		*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Gadid fish	*Micromesistius poutassou*	*Architeuthis dux*	Lordan et al. 1998
Gadid fish		(Undetermined) *forbesii*	Guerra and Rocha 1994
Garfishes		*Nototodarus gouldi*	Machida 1983
Gobiid fish		(Undetermined) *forbesii*	Guerra and Rocha 1994
Grunions		*Dosidicus gigas*	Boletzky and Hanlon 1983
Hagfish	*Myxine capensis*	*Octopus magnificus*	Villanueva 1993
Hake	*Merluccius capensis*	*Loligo vulgaris reynaudi*	Augustyn 1990
Hake	*Merluccius paradoxus*	*Octopus magnificus*	Villanueva 1993
Hatchetfish	*Argyropelecus affinis*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Herring	*Clupea harengus*	*Illex illecebrosus*	Boletzky and Hanlon 1983
Herring	*Clupea harengus*	*Todarodes sagittatus*	Breiby and Jobling 1984
Herring-like fishes		*Nototodarus gouldi*	Machida 1983
Horse mackerel		*Uroteuthis edulis*	Natsukari and Tashiro 1991
Horse mackerel	*Trachurus trachurus*	*Architeuthis dux*	Lordan et al. 1998
Juvenile barracudas		*Nototodarus gouldi*	Machida 1983
Latern fish		*Ommastrephes bartramii*	Araya 1983
Laternfish	*Lampanyctodes hectoris*	*Octopus magnificus*	Villanueva 1993
Lightfish	*Maurolicus mülleri*	*Octopus magnificus*	Villanueva 1993
Lightfish	*Maurolicus muelleri*	*Illex coindetii*	Sánchez et al. 1998
Lightfish	*Maurolicus muelleri*	*Illex argentinus*	Haimovici et al. 1998
Lightfish	*Maurolicus mülleri*	*Todarodes sagittatus*	Breiby and Jobling 1984
Mackerel	*Scomber scombrus*	*Illex illecebrosus*	Boletzky and Hanlon 1983
Mackerel	*Scomber scombrus*	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Mackerel		*Uroteuthis edulis*	Natsukari and Tashiro 1991
Mackerel (larvae)		*Ommastrephes bartramii*	Araya 1983
Moray eel		*Octopus cyanea*	Boletzky and Hanlon 1983
Myctophid fish	*Gymnoscopelus nicholsi*	*Martialia hyadesi*	González and Rodhouse 1998
Myctophid fish	*Gymnoscopelus nicholsi*	*Illex argentinus*	Ivanovic and Brunetti 1994
Myctophid fish	*Lampichthys procerus*	*Illex argentinus*	Ivanovic and Brunetti 1994

Myctophid fish	*Protomyctophum tenisoni*	*Illex argentinus*	Ivanovic and Brunetti 1994
Myctophid fish		*Berryteuthis magister magister*	Nesis 1998
Myctophid fish		*Illex coindetii*	Sánchez et al. 1998
Myctophid fishes		*Dosidicus gigas*	Boletzky and Hanlon 1983
Myctophid fishes		*Psychroteuthis glacialis*	Lu and Williams 1994
Pelagic fishes		*Dosidicus gigas*	Mangold 1983
Pilchard	*Sardinops ocellatus*	*Loligo vulgaris reynaudi*	Augustyn 1990
Redfish	*Sebastes marinus*	*Gonatus fabricii*	Lick and Piatkowski 1998
Rockfishes		*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Sailfin molly	*Poecilia latipinna*	*Loligo plei*	Boletzky and Hanlon 1983
Salmon smelt	*Salmo salar*	*Illex illecebrosus*	Boletzky and Hanlon 1983
Sandeel	*Ammodytes tobianus*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Sandeel	*Ammodytes tobianus*	*Todarodes sagittatus*	Breiby and Jobling 1984
Sandeel	*Ammodytes* spp.	(Undetermined) *forbesii*	Boyle and Pierce 1994
Sandtrout	*Leiostomus xanthurus*	*Loligo plei*	Boletzky and Hanlon 1983
Sardine	*Sardinops sagax caeruleus*	*Dosidicus gigas*	Boletzky and Hanlon 1983
Sardines		*Uroteuthis edulis*	Natsukari and Tashiro 1991
Sardines		*Ommastrephes bartramii*	Araya 1983
Saury		*Dosidicus gigas*	Boletzky and Hanlon 1983
Saury	*Sardinops sagax melanosticta*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Saury (larvae)		*Ommastrephes bartramii*	Araya 1983
Scad	*Trachurus picturatus*	(Undetermined) *forbesii*	Boyle and Pierce 1994
Sculpin		*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Skipper		*Dosidicus gigas*	Boletzky and Hanlon 1983
Small fish		*Onychoteuthis borealijaponica*	Okutani and Murata 1983
Small fishes		*Rossia pacifica pacifica*	Boletzky and Hanlon 1983
Smelt	*Osmerus mordax*	*Illex illecebrosus*	Boletzky and Hanlon 1983
Whiting	*Merlangius merlangus*	(Undetermined) *forbesii*	Boyle and Pierce 1994

Miscellaneous

Algae		*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Algae		*Sepia officinalis*	Boletzky and Hanlon 1983
Bryozoans		*Illex argentinus*	Ivanovic and Brunetti 1994
Hydroids		*Octopus magnificus*	Villanueva 1993
Nemerteans		*Sepia officinalis*	Boletzky and Hanlon 1983

Plankton		*Dosidicus gigas*	Boletzky and Hanlon 1983
Scyphozoan jellyfish	*Phyllorhiza punctata*	*Argonauta argo*	Heeger et al. 1992
Sipunculids (peanut worms)		*Bathypolypus arcticus*	Boletzky and Hanlon 1983
Tunicates		*Illex coindetii*	Sánchez et al. 1998
Zooplankton		*Uroteuthis edulis*	Natsukari and Tashiro 1991

Molluscs

Abalone	*Haliotis corrugata*	*Octopus bimaculatus*	Ambrose 1984
Abalone	*Haliotis kamchatkana*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Abalone	*Haliotis rufescens*	*Octopus bimaculatus*	Ambrose 1984
Abalone	*Haliotis fulgens*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Abalone	*Haliotis rufescens*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Abalone	*Haliotis fulgens*	*Octopus bimaculatus*	Ambrose 1984
Abalone	*Haliotis corrugata*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Archaeogastropods		*Octopus bimaculatus*	Ambrose 1984
Argentine shortfin squid	*Illex argentinus*	*Illex argentinus*	Ivanovic and Brunetti 1994
Bivalve	*Gari californica*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Modiolus rectus*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Chione undatella*	*Octopus bimaculatus*	Ambrose 1984
Bivalve	*Saxidomus giganteus*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Hinnites giganteus*	*Octopus bimaculatus*	Ambrose 1984
Bivalve	*Humilaria kennerleyi*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Macoma* sp.	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Diplodonta orbella*	*Octopus bimaculatus*	Ambrose 1984
Bivalve	*Hinnites giganteus*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Leptopecten montimerus*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Bivalve	*Anadara notabilis*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Bivalve	*Artigona rigida*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Bivalve	*Arcopagia fausta*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Bivalve	*Laevicardium laevigatum*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Bivalve	*Glycymeris pectinata*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Bivalve	*Semele rubropicta*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Chione undatella*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Bivalve	*Ventricolaria fordii*	*Octopus bimaculatus*	Ambrose 1984

Bivalve	*Argopecten aequisulcatus*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Bivalve	*Tresus* sp.	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Solen sicarius*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Mya truncata*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Limopsis* sp.	*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Bivalve	*Glycymeris subobsoleta*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Bivalve	*Glycymeris decussata*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Bivalve	*Semele proficua*	*Octopus vulgaris*	Mather 1991
Bivalve	*Epilucina californica*	*Octopus bimaculatus*	Ambrose 1984
Bivalve	*Pitar newcombianus*	*Octopus bimaculatus*	Ambrose 1984
Bivalve	*Pododesmus macroschisma*	*Octopus dofleini dofleini*	Vincent et al. 1998
Bivalve	*Arca zebra*	*Octopus vulgaris*	Mather 1991
Bivalve	*Americardia biangulata*	*Octopus bimaculatus*	Ambrose 1984
Bivalve	*Semele decisa*	*Octopus bimaculatus*	Ambrose 1984
Bivalve	*Macoma nasuta*	*Octopus dofleini dofleini*	Vincent et al. 1998
Bivalve	*Semele rupicola*	*Octopus bimaculatus*	Ambrose 1984
Bivalve	*Macoma inquinata*	*Octopus dofleini dofleini*	Vincent et al. 1998
Bivalve	*Tellina carpenteri*	*Octopus dofleini dofleini*	Vincent et al. 1998
Bivalve	*Tagelus californianus*	*Octopus bimaculatus*	Ambrose 1984
Bivalves		(Undetermined) *forbesii*	Guerra and Rocha 1994
Bivalves		*Octopus australis*	Boletzky and Hanlon 1983
Bivalves		*Bathypolypus arcticus*	Boletzky and Hanlon 1983
Bivalves		*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Black abalone	*Haliotis cracherodii*	*Octopus bimaculatus*	Ambrose 1984
Black abalone	*Haliotis cracherodii*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Blue mussel	*Mytilus edulis*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Blue mussel	*Mytilus edulis*	*Octopus dofleini dofleini*	Vincent et al. 1998
Bobtail squid	*Rondeletiola minor*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Bobtail squid	*Sepietta* sp.	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Broadtail shortfin squid	*Illex coindetii*	*Illex coindetii*	Sánchez et al. 1998
Cephalopod	*Spirula spirula*	*Illex argentinus*	Haimovici et al. 1998
Cephalopod		*Rossia pacifica pacifica*	Boletzky and Hanlon 1983
Cephalopod	*Argonauta* sp.	*Illex argentinus*	Haimovici et al. 1998
Cephalopods		*Dosidicus gigas*	Boletzky and Hanlon 1983

Cephalopods		*Sepia australis*	Sánchez and Villanueva 1991
Chiton		*Octopus dofleini dofleini*	Vincent et al. 1998
Chiton	*Stenoplax conspicua*	*Octopus bimaculatus*	Ambrose 1984
Chiton	*Callistochiton* sp.	*Octopus bimaculatus*	Ambrose 1984
Chiton	*Tonicella lineata*	*Octopus dofleini dofleini*	Vincent et al. 1998
Chiton	*Chiton tuberculatis*	*Octopus vulgaris*	Mather 1991
Clam	*Protothaca staminea*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Clam	*Lima hemphilli*	*Octopus bimaculatus*	Ambrose 1984
Clam	*Protothaca staminea*	*Octopus dofleini dofleini*	Vincent et al. 1998
Clam	*Protothaca staminea*	*Octopus bimaculatus*	Ambrose 1984
Clam	*Lima pellucida*	*Octopus vulgaris*	Mather 1991
Clam	*Protothaca* sp.	*Octopus dofleini dofleini*	Mather 1991
Clams		*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Cockle	*Clinocardium* sp.	*Octopus dofleini dofleini*	Mather 1991
Commander squid	*Berryteuthis magister magister*	*Berryteuthis magister magister*	Nesis 1998
Common octopus	*Octopus vulgaris*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Cranchiid squid		*Loligo vulgaris vulgaris*	Coelho et al. 1997
Cuttlefish	*Sepia* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Cuttlefish	*Sepia australis*	*Sepia australis*	Sánchez and Villanueva 1991
Cuttlefish	*Sepia elegans*	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Decapod cephalopod		*Sepia officinalis*	Boletzky and Hanlon 1983
European common squid	*Alloteuthis subulata*	(Undetermined) *forbesii*	Guerra and Rocha 1994
European squid	*Loligo vulgaris*	*Loligo vulgaris vulgaris*	Coelho et al. 1997
European squid	*Loligo vulgaris*	(Undetermined) *forbesii*	Guerra and Rocha 1994
Flying squid	*Todarodes sagittatus*	*Todarodes sagittatus*	Breiby and Jobling 1984
Gastropod	*Strombus gigas*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Gastropod	*Strombus costatus*	*Octopus vulgaris*	Boletzky and Hanlon 1983
Gastropod	*Polinices* sp.	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Trivia californiana*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Conus* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Gastropod	*Cypraea spadicea*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Trivia solandri*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Buccinanops gradatum*	*Octopus tehuelchus*	Iribarne et al. 1991
Gastropod	*Littorina sitkana*	*Octopus dofleini dofleini*	Vincent et al. 1998

Gastropod	*Crepipatella lingulata*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Tegula funebralis*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Tegula regina*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Hipponix tumens*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Calliostoma* sp.	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Tegula patagonica*	*Octopus tehuelchus*	Iribarne et al. 1993
Gastropod	*Tegula gallina*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Bursa californica*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Tegula aureotincta*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Megathura crenulata*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Tegula eiseni*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Columbella mercatoria*	*Octopus vulgaris*	Mather 1991
Gastropod	*Littorina* sp.	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Astraea undosa*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Acmaea* sp.	*Octopus dofleini dofleini*	Vincent et al. 1998
Gastropod	*Trichotropis cancellata*	*Octopus dofleini dofleini*	Vincent et al. 1998
Gastropod		*Octopus dofleini dofleini*	Vincent et al. 1998
Gastropod	*Astraea gibberosa*	*Octopus bimaculatus*	Ambrose 1984
Gastropod	*Tegula funebralis*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Gastropod	*Tegula gallina*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Gastropod	*Astraea undosa*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Gastropods		*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Gastropods		*Opisthoteuthis agassizii*	Villanueva and Guerra 1991
Gastropods		*Illex illecebrosus*	Mangold 1983
Gastropods		*Octopus australis*	Boletzky and Hanlon 1983
Gastropods	*Crepidula* spp.	*Octopus bimaculatus*	Ambrose 1984
Gastropods		*Bathypolypus arcticus*	Boletzky and Hanlon 1983
Gastropods		*Octopus magnificus*	Villanueva 1993
Gastropods		*Sepia officinalis*	Boletzky and Hanlon 1983
Glacial squid	*Psychroteuthis glacialis*	*Martialia hyadesi*	González and Rodhouse 1998
Glacial squid	*Psychroteuthis glacialis*	*Psychroteuthis glacialis*	Lu and Williams 1994
Glacial squid	*Psychroteuthis glacialis*	*Alluroteuthis antarctica*	Lu and Williams 1994
Heterpod molluscs		*Dosidicus gigas*	Boletzky and Hanlon 1983
Horned octopus	*Eledone cirrhosa*	(Undetermined) *forbesii*	Guerra and Rocha 1994

Jumbo flying squid	*Dosidicus gigas*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Jumbo flying squid	*Dosidicus gigas*	*Sthenoteuthis oualaniensis*	Young and Hirota 1998
Juvenile squids		*Nototodarus gouldi*	Machida 1983
Lamellibranchs		*Octopus vulgaris*	Boletzky and Hanlon 1983
Lamellibranchs		*Sepia officinalis*	Boletzky and Hanlon 1983
Limpet	*Collisella limatula*	*Octopus bimaculatus*	Ambrose 1984
Limpet	*Fissurella barbadensis*	*Octopus vulgaris*	Mather 1991
Limpet	*Collisella scabra*	*Octopus bimaculatus*	Ambrose 1984
Limpet	*Notoacmea* sp.	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Limpet	*Collisella* sp.	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Mesogastropods		*Octopus bimaculatus*	Ambrose 1984
Mollusc	*Turritella* sp.	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Mollusc	*Limacina retroversa*	*Todarodes sagittatus*	Breiby and Jobling 1984
Moon snail	*Polinices lewiss*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Moon snails		*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Mussel	*Brachiodontes rodriguezi*	*Octopus tehuelchus*	Iribarne et al. 1991
Mussel	*Brachiodontes dominguensis*	*Octopus vulgaris*	Mather 1991
Mussel	*Perna perna*	*Octopus vulgaris*	Mangold 1983
Mussel	*Mytilus californianus*	*Octopus bimaculatus*	Ambrose 1984
Mussel	*Mytilus californianus*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Neogastropod	*Olivella biplicata*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Neogastropod	*Kelletia kelletii*	*Octopus bimaculatus*	Ambrose 1984
Neogastropod	*Bulla gouldiana*	*Octopus bimaculatus*	Ambrose 1984
Neogastropod	*Olivella biplicata*	*Octopus bimaculatus*	Ambrose 1984
Neogastropod	*Nassarius tegula*	*Octopus bimaculatus*	Ambrose 1984
Neogastropod	*Kelletia kelletii*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Octopods		*Sepia officinalis*	Boletzky and Hanlon 1983
Octopods		*Octopus australis*	Boletzky and Hanlon 1983
Octopus	*Octopus* sp.	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Octopus		*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Octopus	*Eledone cirrhosa*	*Architeuthis dux*	Lordan et al. 1998
Octopus	*Japatella diaphana*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Octopus	*Octopus* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Octopus	*Octopus bimaculatus*	*Octopus bimaculatus*	Ambrose 1984

Octopus	*Ocythoe tuberculata*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Octopus	*Octopus dofleini*	*Octopus dofleini dofleini*	Vincent et al. 1998
Octopus	*Octopus* sp.	*Octopus magnificus*	Villanueva 1993
Ostracods		*Sepia officinalis*	Boletzky and Hanlon 1983
Pelagic molluscs		*Dosidicus gigas*	Mangold 1983
Pteropod molluscs		*Dosidicus gigas*	Boletzky and Hanlon 1983
Pteropods		*Sepia officinalis*	Boletzky and Hanlon 1983
Pteropods		*Illex illecebrosus*	Mangold 1983
Scallop	*Chlamys rubida*	*Octopus dofleini dofleini*	Vincent et al. 1998
Scallop	*Chlamys hastata*	*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Scallops		*Octopus dofleini dofleini*	Boletzky and Hanlon 1983
Snail	*Norrisia norrisi*	*Octopus bimaculatus*	Ambrose 1984
Snail	*Norrisia norrisi*	*Octopus bimaculoides*	Boletzky and Hanlon 1983
Squid	*Loligo* sp.	*Illex coindetii*	Sánchez et al. 1998
Squid	*Histioteuthis* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Squid		*Illex illecebrosus*	Mangold 1983
Squid	*Todarodes pacificus*	*Uroteuthis edulis*	Natsukari and Tashiro 1991
Squid		*Octopus magnificus*	Villanueva 1993
Squid		*Dosidicus gigas*	Mangold 1983
Squid	*Loligo vulgaris reynaudii*	*Loligo vulgaris reynaudi*	Augustyn 1990
Squid	*Sthenoteuthis oualaniensis*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Squid	*Liocranchia* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Squid	*Loligo gahi*	*Illex argentinus*	Ivanovic and Brunetti 1994
Squid		*Loligo pealeii*	Boletzky and Hanlon 1983
Squid		*Lolliguncula brevis*	Boletzky and Hanlon 1983
Squid		*Sthenoteuthis pteropus*	Boletzky and Hanlon 1983
Squid	*Watasenia scintillans*	*Ommastrephes bartramii*	Araya 1983
Squid	*Onychoteuthis borealijaponica*	*Ommastrephes bartramii*	Araya 1983
Squid	*Ommastrephes* spp.	*Ommastrephes bartramii*	Araya 1983
Squid	*Loligo vulgaris* sp.	*Octopus vulgaris*	Boletzky and Hanlon 1983
Squid	*Todarodes pacificus*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Squid	*Onychoteuthis borealijaponica*	*Onychoteuthis borealijaponica*	Okutani and Murata 1983
Squid	*Ctenopteryx* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Squid	*Loligo sanpaulensis*	*Illex argentinus*	Ivanovic and Brunetti 1994

Squid	*Martialia hyadesi*	*Martialia hyadesi*	González and Rodhouse 1998
Squid	*Gonatus antarcticus*	*Martialia hyadesi*	González and Rodhouse 1998
Squid	*Todaropsis eblanae*	*Illex coindetii*	Sánchez et al. 1998
Squid	*Ornithoteuthis volatilis*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Squid	*Abraliopsis* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Squid	*Octopoteuthis* sp.	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Squid	*Onychoteuthis banksi*	*Thysanoteuthis rhombus*	Nigmatullin and Arkhipkin 1998
Squid		*Mesonychoteuthis hamiltoni*	Lu and Williams 1994
Squids		*Berryteuthis magister magister*	Nesis 1998
Veined squid	*Loligo forbesi*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Veined squid	*Loligo forbesi*	(Undetermined) *forbesii*	Guerra and Rocha 1994

Polychaetes

Polychaete	*Nereis divesicolor*	*Loligo vulgaris vulgaris*	Guerra and Rocha 1994
Polychaete	*Nephthys* sp.	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Polychaete	*Nereis* sp.	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Polychaete	*Eunice* sp.	*Todarodes sagittatus*	Breiby and Jobling 1984
Polychaete	*Nereis pelagica*	*Todarodes sagittatus*	Breiby and Jobling 1984
Polychaete	*Choloeia inermis*	*Octopus magnificus*	Villanueva 1993
Polychaete	*Chaetopterus variopedatus*	*Octopus briareus*	Boletzky and Hanlon 1983
Polychaete	*Onuphia magna*	*Octopus briareus*	Boletzky and Hanlon 1983
Polychaete worm	*Perinereis* sp.	*Loligo vulgaris vulgaris*	Coelho et al. 1997
Polychaete worms		*Octopus australis*	Boletzky and Hanlon 1983
Polychaetes		*Illex illecebrosus*	Maurer and Bowman 1984
Polychaetes		*Sepia officinalis*	Boletzky and Hanlon 1983
Polychaetes		*Loligo opalescens*	Boletzky and Hanlon 1983
Polychaetes		*Eledone cirrhosa cirrhosa*	Boletzky and Hanlon 1983
Polychaetes		*Bathypolypus arcticus*	Boletzky and Hanlon 1983
Polychaetes		*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Polychaetes		(Undetermined) *forbesii*	Guerra and Rocha 1994
Polychaetes		*Loligo pealeii*	Maurer and Bowman 1984
Polychaetes		*Opisthoteuthis agassizii*	Villanueva and Guerra 1991

Protozoa

Foraminifers		*Opisthoteuthis vossi*	Villanueva and Guerra 1991
Foraminifers		*Opisthoteuthis agassizii*	Villanueva and Guerra 1991

Foraminifers		*Bathypolypus arcticus*	Boletzky and Hanlon 1983

Appendix 3

Cephalopod specimens donated to museum collections

With few hard parts for a fossil record, cephalopod phylogeny is still being worked out. This is especially true for deep-sea species. For more than half of the deep-sea species of octopuses there are only 2-3 preserved specimens world wide (Voss 1988a). This makes studying their evolution and systematics even more difficult. Therefore, it is likely that there will be many future revisions as more information becomes available. It is important to leave a record of voucher specimens for any work done on deep-sea octopuses. This appendix is a list of specimens I have donated to museum collections.

Bathypolypus arcticus

Kumpf (1958) and Macalaster (1976) agree that there is only one species of *Bathypolypus arcticus* in the Northeast Atlantic. However, these octopuses have a large range (O'Dor and Macalaster 1983) and are not very mobile so there is a possibility of variation between populations although Macalaster (1976) did not find any evidence of this.

The following specimens are important as there are no specimens of hatchling or very young *B. arcticus*. Furthermore, the age, diet and temperature at which these animals were maintained is known. These animals are from the diet experiment (Chapter 2). The letters identify the octopus (see Appendix 4)

18 specimens donated to the Canadian Museum of Nature

Contact: Dr. Jean-Marc Gagnon
Chief Collection Manager / Gestionnaire en Chef des collections
Invertebrate Collections / Collection des invertébrés
Canadian Museum of Nature / Musée canadien de la nature
P.O. Box 3443, Sta. D / Casier postal 3443, succ. D
Ottawa, Ontario K1P 6P4

Octopuses:

Brittlestar arms: DG, CE and BH
Frozen Haddock: CQ, EN, BJ and AP
Live *Corophium*: BE, CK, CV and DA
Frozen *Gammarus*: BP, DC, CB and Z
Live *Gammarus*: H, DZ and ER

10 specimens donated to the Natural History Museum, London, UK.

Contact: Mr. Fred Naggs and Richard Williams
Senior Curator of Recent Molluscs and Assistant Curator
Department of Zoology

Mollusca Section
Natural History Museum
Cromwell Road, South Kensington
London, UK SW7 5BD

The numbers in parentheses are the museum's registration numbers.

Brittlestar arms: CR (19991565)
Frozen Haddock: DP (19991569) and CW (19991571)
Live *Corophium*: BK (19991563) and DU (19991562)
Frozen *Gammarus*: CX (19991567) and EK (19991568)
Live *Gammarus*: BL (19991566) and CC (19991564)

Live Worms: EY (19991570)

6 specimens donated to the National Museum of Natural History

Various video tapes of *B. arcticus* mating and hatching have also been donated.

Contact: Dr. Mike Vecchione
NMFS Systematics Lab.
National Museum of Natural
History, Washington, DC
20560 USA

Frozen Fish: EC, EH
Frozen *Gammarus*: BA
Live *Gammarus*: CO, DN
Live *Corophium*: EU

5 specimens donated to the Huntsman Marine Science Centre

Contact: Dr. Gerhard Pohle
Curator, Invertebrates Atlantic Reference Centre
Huntsman Marine Science Centre
1 Lower Campus Road
St. Andrews N.B. E5B 2L7

Brittlestar arms: AF
Frozen Haddock: AH
Live *Corophium*: AN
Frozen *Gammarus*: ED
Live *Gammarus*: AD

Graneledone verrucosa

Contact: Dr. Janet Voight
Associate Curator
Dept. of Zoology
The Field Museum of Natural History
Roosevelt Rd at Lake Shore Dr.
Chicago, IL USA

Twenty-two specimens of *Graneledone verrucosa* including tissue samples were donated. Two specimens were purchased at Sobeys, a local supermarket, by Dr. Ellen Kenchington on 3/25/1996. I purchased the remaining 20 from National Sea Products on 6/26/1995 for a few dollars a pound. These 20 were collected at 42 39N and 63 30W at a depth of 600-800 fathoms using a 170 foot high lift Engle (net) from the Cap Chidley (ship) which was fishing for Grenadier. Octopuses were preserved in 10% formalin. Papillae, an important taxonomic feature of this species, were not evident until after preservation. The spermatophores of the 1.150 kg (wet weight) male that Ellen purchased were 15.8, 13.8, 13.9 and 11.7 cm in length. The catalogue numbers of the first two specimens are FMNH 286458, the catalogue numbers for the remaining 20 is FMNH 286457. Earl Dawe sent a single specimen from off Newfoundland (53 deg 11.82'N 51 deg 56.82'W 1145 m) and its catalogue number is 286459.

Other detail is as follows:

Octopus	**Date**	**Sex**	**Preserved Weight**	**Wet weight**
1	3/25/1996	F	918.9 g	1150 g
2	3/25/1996	F	753.1	1013
3	6/26/1995	F	745.0	931
4	6/26/1995	M	433.8	521
5	6/26/1995	F	454.0	570
6	6/26/1995	M	422.7	507

7	6/26/1995	F	586.2	705
8	6/26/1995	F	464.0	595
9	6/26/1995	F	697.4	844
10	6/26/1995	F	717.0	892
11	6/26/1995	F	515.7	655
12	6/26/1995	F	545.2	653
13	6/26/1995	F	1181.7	1525
14	6/26/1995	F	209.2	267
15	6/26/1995	F	641.6	820
16	6/26/1995	F	398.4	497
17	6/26/1995	M	723.5	870
18	6/26/1995	F	307.3	400
19	6/26/1995	F	223.1	285
20	6/26/1995	F	959.0	1227
21	6/26/1995	F	397.2	489
22	6/26/1995	F	1016.5	1287

Appendix 4

Growth rates of hatchling *Bathypolypus arcticus* fed five different foods

This appendix contains the growth data from Chapter 2 plus data from three octopuses that were fed worms and fifteen octopuses that were starved. The data are recorded here so that others may re-analyze it in the future.

In this appendix, age is reported in days; weight is in grams. Occasionally an octopus would disappear (escape) - these octopuses are noted with the abbreviation MIA. Other than their weights at hatching, these octopuses were not used.

On the final pages of this appendix, summary data are presented: "Avg" is the average weight in a given treatment at a given time, "Std" is the standard deviation, and "n" refers to the sample size. "NA" is not applicable and is used is several places such as for the standard deviation when there is only one octopus alive in the treatment, when the octopuses are not yet born (starvation treatment) or when data needed for a calculation are missing.

	Date hatched	Death	Age at Death	Survivors G	Age	Weight	Age (d)	Weight (g)	Age (d)	Weight (g)
				%/day	days	grams	9/28/1996	9/28/1996	10/27/1996	10/27/1996
Brittle stars	Orange Treatment	n=29								
E	8/15/1996	10/3/1996	49		1	0.209	44	0.207		
F	8/17/1996	9/27/1996	41		1	0.209				
J	8/18/1996	10/27/1996	70		1	0.192	41	0.183	70	0.163
L	8/22/1996	11/4/1996	74		1	0.214	37	0.199	66	0.194
N	8/22/1996	10/8/1996	47		1	0.208	37	0.172		
P	8/22/1996	9/27/1996	36		1	0.211				
T	8/22/1996	10/27/1996	66		1	0.188	37	0.165	66	0.170
W	8/22/1996	10/8/1996	47		1	0.164	37	0.170		
AF	8/22/1996	10/14/1996	53		1	0.176	37	0.157		
AG	8/22/1996	10/28/1996	67		1	0.208	37	0.208	66	0.223
AS	8/30/1996	11/13/1996	75		1	0.199	29	0.199	58	0.193
AU	8/30/1996	10/6/1996	37		1	0.215	29	0.231		
BC	8/30/1996	9/28/1996	29		1	0.191	29	0.165		
BD	8/31/1996	10/6/1996	36		1	0.129	28	0.122		
BH	9/4/1996	10/6/1996	32		1	0.213	24	0.200		
BN	9/4/1996	10/16/1996	42		1	0.202	24	0.197		
BO	9/4/1996	10/30/1996	56		1	0.206	24	0.184	53	0.125
BT	9/4/1996	10/30/1996	56		1	0.246	24	0.247	53	0.220
CE	9/4/1996	11/1/1996	58		1	0.195	24	0.202	53	0.165
CJ	9/4/1996	10/6/1996	32		1	0.210	24	0.184		
CN	9/4/1996	10/3/1996	29		1	0.225	24	0.193		
CR	9/4/1996	11/11/1996	68		1	0.204	24	0.177	53	0.153
DG	9/5/1996	11/13/1996	69		1	0.220	23	0.220	52	0.182
DQ	9/5/1996	10/21/1996	46		1	0.224	23	0.200		
DY	9/5/1996	10/8/1996	33		1	0.214	23	0.190		
EE	9/5/1996	11/1/1996	57		1	0.179	23	0.172	52	0.149
EQ	9/11/1996	10/28/1996	47		1	0.181	17	0.149	46	0.130
EX	9/12/1996	10/10/1996	28		1	0.159	16	0.192		
EZ	9/12/1996	11/4/1996	53		1	0.187	16	0.182	45	0.147

	Date hatched	Death	Age at Death	Survivors G	Age	Weight	Age (d)	Weight (g)	Age (d)	Weight (g)
				%/day	days	grams	9/28/1996	9/28/1996	10/27/1996	10/27/1996
Corophium	Blue Treatment	n=39								
B	8/14/1996	8/28/1996	14		1	0.191				
I	8/18/1996	5/5/1997	260	0.531	1	0.202	41	0.264	70	0.314
K	8/22/1996	11/18/1996	88		1	0.214	37	0.256	66	0.238
Q	8/22/1996	11/15/1996	85		1	0.196	37	0.273	66	0.289
S	8/22/1996	12/13/1996	113		1	0.213	37	0.274	66	0.278
V	8/22/1996	10/8/1997	412	0.811	1	0.206	37	0.324	66	0.375
Y	8/22/1996	1/8/1997	139		1	0.203	37	0.276	66	0.358
AB	8/22/1996	12/2/1996	102		1	0.189	37	0.268	66	0.300
AE	8/22/1996	11/11/1996	81		1	0.210	37	0.277	66	0.299
AJ	8/26/1996	11/18/1996	84		1	0.179	33	0.216	62	0.218
AK	8/28/1996	6/16/1997	292	0.703	1	0.178	31	0.289	60	0.343
AN	8/28/1996	11/4/1996	68		1	0.189	31	0.300	60	0.353
AO	8/29/1996	5/29/1997	273	0.622	1	0.195	30	0.262	59	0.316
AQ	8/29/1996	1/13/1997	137		1	0.210	30	0.264	59	0.306
AT	8/30/1996	1/22/1997	145		1	0.169	29	0.280	58	0.268
AZ (MIA)	8/30/1996	MIA-10/12?			1	0.158	29	0.150		
BB	8/30/1996	2/20/1997	174		1	0.186	29	0.288	58	0.314
BE	9/1/1996	1/20/1997	141		1	0.213	27	0.269	56	0.318
BG	9/2/1996	6/25/1997	296	0.523	1	0.185	26	0.263	55	0.323
BK	9/4/1996	10/8/1996	34		1	0.213	24	0.245		
BR	9/4/1996	12/18/1996	105		1	0.184	24	0.250	53	0.260
BZ	9/4/1996	2/12/1997	161		1	0.165	24	0.199	53	0.188
CH	9/4/1996	6/23/1997	292	0.542	1	0.216	24	0.246	53	0.302
CI	9/4/1996	12/16/1996	103		1	0.215	24	0.276	53	0.315
CK	9/4/1996	10/14/1996	40		1	0.194	24	0.268		
CV	9/4/1996	5/28/1997	266	0.765	1	0.240	24	0.280	53	0.352
DA	9/5/1996	12/20/1996	106		1	0.223	23	0.251	52	0.279
DB	9/5/1996	5/14/1997	251	0.506	1	0.240	23	0.290	52	0.309
DD	9/5/1996	12/15/1996	101		1	0.168	23	0.220	52	0.174

Corophium										
	Date hatched	Death	Age at Death	Survivors G	Age	Weight	Age (d)	Weight (g)	Age (d)	Weight (g)
				%/day	days	grams	9/28/1996	9/28/1996	10/27/1996	10/27/1996
DK	9/5/1996	5/28/1997	265	0.599	1	0.224	23	0.274	52	0.305
DU	9/5/1996	12/15/1996	101		1	0.231	23	0.299	52	0.345
DW	9/5/1996	10/6/1996	31		1	0.110	23	0.122		
EA	9/5/1996	12/24/1996	110		1	0.243	23	0.291	52	0.347
EF	9/5/1996	12/2/1996	88		1	0.158	23	0.219	52	0.227
EJ	9/10/1996			0.669	1	0.159	18	0.222	47	0.200
EP	9/11/1996	10/6/1996	25		1	0.172	17	0.226		
EU	9/12/1996	3/28/1997	197	0.622	1	0.205	16	0.279	45	0.314
FA	9/12/1996	10/21/1996	39		1	0.191	16	0.266		
FB	9/12/1996	10/21/1996	39		1	0.209	16	0.299		

	Age (d)	Weight (g)	Age (d)	Weight (g)	Age (d)	Weight (g)	Age (d)	Weight (g)	Age (d)	Weight (g)
	11/24/1996	11/24/1996	12/15/1996	12/15/1996	1/8/1997	1/8/1997	2/5/1997	2/5/1997	2/28/1997	2/28/1997
Corophium										
B										
I	98	0.375	119	0.426	143	0.458	171	0.566	194	0.634
K										
Q										
S	94	0.278								
V	94	0.461	115	0.578	139	0.761	167	0.961	190	1.187
Y	94	0.386	115	0.387						
AB	94	0.266								
AE										
AJ										
AK	88	0.424	109	0.482	133	0.567	161	0.649	184	0.663
AN										
AO	87	0.340	108	0.386	132	0.451	160	0.609	183	0.700
AQ	87	0.400	108	0.426	132	0.329				
AT	86	0.379	107	0.413	131	0.419				
AZ (MIA)										
BB	86	0.419	107	0.431	131	0.540	159	0.552		
BE	84	0.348	105	0.358	129	0.329				
BG	83	0.411	104	0.411	128	0.448	156	0.472	179	0.529
BK										
BR	81	0.278	102	0.233						
BZ	81	0.262	102	0.277	126	0.257	154	0.255		
CH	81	0.344	102	0.360	126	0.434	154	0.564	177	0.704
CI	81	0.394	102	0.304						
CK										
CV	81	0.484	102	0.585	126	0.719	154	0.930	177	1.001
DA	80	0.276	101	0.320						
DB	80	0.390	101	0.415	125	0.456	153	0.585	176	0.633
DD	80	0.298	101	0.238						

Corophium										
	Age (d)	**Weight (g)**	**Age (d)**	**Weight (g)**	**Age (d)**	**Weight (g)**	**Age (d)**	**Weight (g)**	**Age (d)**	**Weight (g)**
	11/24/1996	**11/24/1996**	**12/15/1996**	**12/15/1996**	**1/8/1997**	**1/8/1997**	**2/5/1997**	**2/5/1997**	**2/28/1997**	**2/28/1997**
DK	80	0.411	101	0.491	125	0.537	153	0.643	176	0.745
DU	80	0.423								
DW										
EA	80	0.408	101	0.441						
EF	80	0.235								
EJ	75	0.236	96	0.289	120	0.358	148	0.499	171	0.547
EP										
EU	73	0.423	94	0.454	118	0.503	146	0.587	169	0.554
FA										
FB										

	Date hatched	Death	Age at Death	Survivors G	Age	Weight	Age (d)	Weight (g)	Age (d)	Weight (g)
				%/day	days	grams	9/28/1996	9/28/1996	10/27/1996	10/27/1996
Live *Gammarus*	Yellow Treatment	n=24								
A	8/14/1996	9/3/1996	20		1	0.160				
C	8/14/1996	12/16/1996	124		1	0.181	45	0.238	74	0.251
D	8/14/1996	MIA			1	0.165				
H	8/17/1996	7/8/1997	325	0.172	1	0.183	42	0.249	71	0.249
AA	8/22/1996	9/28/1996	37		1	0.214	37	0.157	66	
AC	8/22/1996	3/24/1997	214	0.372	1	0.212	37	0.313	66	0.344
AD	8/22/1996	12/18/1996	118		1	0.206	37	0.249	66	0.279
AW	8/30/1996	10/28/1996	59		1	0.179	29	0.244	58	0.214
AY	8/30/1996	10/3/1996	34		1	0.203	29	0.197	58	
BF	9/1/1996	11/1/1996	61		1	0.161	27	0.200	56	0.189
BL	9/4/1996	11/25/1996	82		1	0.214	24	0.231	53	0.215
BU	9/4/1996	11/29/1996	86		1	0.227	24	0.248	53	0.268
BV	9/4/1996	12/2/1996	89		1	0.214	24	0.279	53	0.317
BX	9/4/1996	12/24/1996	111		1	0.209	24	0.246	53	0.251
CC	9/4/1996	11/27/1996	84		1	0.234	24	0.261	53	0.265
CO	9/4/1996	11/24/1996	81		1	0.205	24	0.260	53	0.271
CY	9/5/1996	11/8/1996	64		1	0.224	23	0.235	52	0.183
DF	9/5/1996	12/9/1996	95		1	0.229	23	0.246	52	0.280
DN	9/5/1996	11/18/1996	74		1	0.252	23	0.300	52	0.293
DT	9/5/1996	10/23/1996	48		1	0.230	23	0.211	52	
DZ	9/5/1996	12/28/1996	114		1	0.239	23	0.263	52	0.295
EG	9/6/1996	5/26/1997	262	0.307	1	0.212	22	0.200	51	0.263
EO	9/11/1996	12/15/1996	95		1	0.178	17	0.182	46	0.173
ER	9/11/1996	11/1/1996	51		1	0.193	17	0.214	46	0.195

	Age (d)	Weight (g)	Age (d)	Weight (g)	Age (d)	Weight (g)	Age (d)	Weight (g)	Age (d)	Weight (g)
	11/24/1996	11/24/1996	12/15/1996	12/15/1996	1/8/1997	1/8/1997	2/5/1997	2/5/1997	2/28/1997	2/28/1997
Live *Gammarus*										
A										
C	102	0.218	123	0.231						
D										
H	99	0.279	120	0.267	144	0.290	172	0.256	195	0.279
AA										
AC	94	0.405	115	0.446	139	0.450	167	0.430	190	0.486
AD	94	0.308	115	0.243						
AW										
AY										
BF										
BL	81	0.165								
BU	81	0.241								
BV	81	0.28								
BX	81	0.254	102	0.259						
CC	81	0.218								
CO										
CY										
DF	80	0.255								
DN										
DT										
DZ	80	0.353	101	0.265						
EG	79	0.281	100	0.288	124	0.329	152	0.363	175	0.410
EO	74	0.152	95	0.128						
ER										

	Date hatched	Death	Age at Death	Survivors G	Age	Weight	Age (d)	Weight (g)	Age (d)	Weight (g)
				%/day	days	grams	9/28/1996	9/28/1996	10/27/1996	10/27/1996
F. *Gammarus*	Green Treatment	n=34								
G	8/17/1996	12/6/1996	111		1	0.193	42	0.210	71	0.202
M	8/22/1996	12/2/1996	102		1	0.203	37	0.202	66	0.185
O	8/22/1996	11/22/1996	92		1	0.202	37	0.217	66	0.214
Z	8/22/1996	1/13/1997	144		1	0.179	37	0.215	66	0.226
AI	8/23/1996	9/3/1996	11		1	0.186				
AV	8/30/1996	12/28/1996	120		1	0.187	29	0.221	58	0.217
AX	8/30/1996	9/23/1996	24		1	0.192				
BA	8/30/1996	10/6/1996	37		1	0.202	29	0.212		
BI	9/4/1996	10/16/1996	42		1	0.213	24	0.200		
BM	9/4/1996	10/23/1996	49		1	0.185	24	0.188		
BP	9/4/1996	10/14/1996	40		1	0.188	24	0.199		
BQ	9/4/1996	10/2/1996	28		1	0.208	24	0.164		
CA	9/4/1996	10/2/1996	28		1	0.184	24	0.188		
CB	9/4/1996	10/23/1996	49		1	0.123	24	0.124		
CD	9/4/1996	11/8/1996	65		1	0.215	24	0.212	53	0.200
CL	9/4/1996	12/2/1996	89		1	0.214	24	0.228	53	0.217
CM	9/4/1996	12/2/1996	89		1	0.205	24	0.227	53	0.235
CP	9/4/1996	10/27/1996	53		1	0.220	24	0.208	53	0.164
CS	9/4/1996	10/30/1996	56		1	0.219	24	0.229	53	0.165
CX	9/5/1996	11/11/1996	67		1	0.223	23	0.201	52	0.195
DC	9/5/1996	12/6/1996	92		1	0.228	23	0.226	52	0.230
DE	9/5/1996	10/8/1996	33		1	0.181	23	0.169		
DH	9/5/1996	9/27/1996	22		1	0.170				
DI	9/5/1996	12/2/1996	88		1	0.191	23	0.189	52	0.172
DJ	9/5/1996	10/23/1996	48		1	0.183	23	0.210		
DS	9/5/1996	10/6/1996	31		1	0.215	23	0.216		
DV	9/5/1996	10/6/1996	31		1	0.152	23	0.180		
DX	9/5/1996	12/2/1996	88		1	0.240	23	0.224	52	0.221
ED	9/5/1996	12/15/1996	101		1	0.228	23	0.209	52	0.220

F. ***Gammarus***	**Date hatched**	**Death**	**Age at Death**	**Survivors G**	**Age**	**Weight**	**Age (d)**	**Weight (g)**	**Age (d)**	**Weight (g)**
				%/day	**days**	**grams**	**9/28/1996**	**9/28/1996**	**10/27/1996**	**10/27/1996**
EI	9/10/1996	10/27/1996	47		1	0.204	18	0.202		
EK	9/10/1996	11/24/1996	75		1	0.175	18	0.173	47	0.151
EL	9/10/1996	10/21/1996	41		1	0.157	18	0.160		
EM	9/10/1996	9/25/1996	15		1	0.133				
ES	9/11/1996	10/23/1996	42		1	0.179	17	0.186		

	Age (d)	Weight (g)	Age (d)	Weight (g)	Age (d)	Weight (g)
	11/24/1996	11/24/1996	12/15/1996	12/15/1996	1/8/1997	1/8/1997
F. *Gammarus*						
G	99	0.200				
M	94	0.139				
O						
Z	94	0.226	115	0.211	139	0.177
AI						
AV	86	0.212	107	0.165		
AX						
BA						
BI						
BM						
BP						
BQ						
CA						
CB						
CD						
CL	81	0.181				
CM	81	0.248				
CP						
CS						
CX						
DC	80	0.208				
DE						
DH						
DI	80	0.157				
DJ						
DS						
DV						
DX	80	0.177				
ED	80	0.181				

F. *Gammarus*	**Age (d)**	**Weight (g)**	**Age (d)**	**Weight (g)**	**Age (d)**	**Weight (g)**
	11/24/1996	**11/24/1996**	**12/15/1996**	**12/15/1996**	**1/8/1997**	**1/8/1997**
EI						
EK	75	0.112				
EL						
EM						
ES						

	Date hatched	Death	Age at Death	Survivors G	Age	Weight	Age (d)	Weight (g)	Age (d)	Weight (g)
				%/day	days	grams	9/28/1996	9/28/1996	10/27/1996	10/27/1996
F. Haddock	Red	n=29								
R	8/22/1996	9/23/1996	32		1	0.204				
U	8/22/1996	10/14/1996	53		1	0.213	37	0.210		
X	8/22/1996	10/2/1996	41		1	0.200	37	0.184		
AH	8/22/1996	9/30/1996	39		1	0.176	37	0.168		
AL	8/28/1996	9/30/1996	33		1	0.197	31	0.184		
AM	8/28/1996	9/30/1996	33		1	0.191	31	0.143		
AP	8/29/1996	10/28/1996	60		1	0.210	30	0.209	59	0.186
AR	8/29/1996	10/23/1996	55		1	0.173	30	0.174		
BJ	9/4/1996	9/27/1996	23		1	0.223				
BS	9/4/1996	10/2/1996	28		1	0.218	24	0.197		
BW	9/4/1996	10/6/1996	32		1	0.213	24	0.190		
BY	9/4/1996	9/18/1996	14		1	0.158				
CF	9/4/1996	10/28/1996	54		1	0.204	24	0.206	53	0.150
CG	9/4/1996	10/25/1996	51		1	0.184	24	0.193		
CQ	9/4/1996	10/2/1996	28		1	0.211	24	0.207		
CT	9/4/1996	10/23/1996	49		1	0.191	24	0.188		
CU	9/4/1996	10/12/1996	38		1	0.241	24	0.194		
CW	9/5/1996	10/23/1996	48		1	0.218	23	0.178		
CZ	9/5/1996	10/12/1996	37		1	0.238	23	0.192		
DL	9/5/1996	11/11/1996	67		1	0.185	23	0.190	52	0.183
DM	9/5/1996	10/10/1996	35		1	0.242	23	0.179		
DO	9/5/1996	11/4/1996	60		1	0.218	23	0.211	52	0.174
DP	9/5/1996	11/13/1996	69		1	0.246	23	0.212	52	0.211
DR	9/5/1996	11/1/1996	57		1	0.216	23	0.189	52	0.180
EB	9/5/1996	11/13/1996	69		1	0.232	23	0.232	52	0.178
EC	9/5/1996	11/18/1996	74		1	0.197	23	0.184	52	0.171
EH	9/10/1996	11/25/1996	76		1	0.169	18	0.171	47	0.185
EN	9/11/1996	10/6/1996	25		1	0.169	17	0.155		
EW	9/12/1996	10/6/1996	24		1	0.189	16	0.173		

Batch	Date hatched	Death	Age at Death days	Survivors G %/day	Age days	Weight grams	Age (d) 9/28/1996	Weight (g) 9/28/1996	Age (d) 10/27/1996	Weight (g) 10/27/1996
Worms	Pink									
ET	9/11/1996	10/28/1996	47		1	0.164	17	0.196	46	0.174
EV	9/12/1996	4/1/1997	201		1	0.187	16	0.212	45	0.190
EY	9/12/1996	35391	71		1	0.225	16	0.237	45	0.195
Starvation	Blank									
nf1	10/1-2/96	12/2/1996	61-62		1	0.168		Not born		
nf2	10/1-2/96	10/25/1996	23-24		1	0.145		Not born		
nf3	10/3/1996	11/29/1996	57		1	0.190		Not born	24	0.211
nf4	10/3/1996	12/2/1996	60		1	0.188		Not born	24	0.179
nf5	10/3/1996	12/2/1996	60		1	0.215		Not born	24	0.227
nf6	10/5-6/96	MIA 11/24/96	MIA		1	0.194		Not born	21-22	0.146
nf7	10/5-6/96	11/27/1996	52-53		1	0.136		Not born	21-22	0.140
nf8	10/8/1996	12/13/1996	66		1	0.196		Not born	19	0.158
nf9	10/8/1996	12/15/1996	68		1	0.187		Not born	19	0.178
nf10	10/8/1996	12/13/1996	66		1	0.172		Not born	19	0.228
nf11	10/8/1996	12/4/1996	57		1	0.157		Not born	19	0.149
nf12	10/9/1996	10/14/1996	5		1	0.134		Not born		
nf13	10/9/1996	12/2/1996	54		1	0.158		Not born	18	0.142
nf14	10/9/1996	10/25/1996	16		1	0.233		Not born		
nf15	10/9/1996	11/29/1996	51		1	0.148		Not born	18	0.153

Batch	Age (d) 11/24/1996	Weight (g) 11/24/1996	Age (d) 12/15/1996	Weight (g) 12/15/1996	Age (d) 1/8/1997	Weight (g) 1/8/1997
Worms						
ET						
EV	73	0.221	94	0.217	118	0.218
EY						
Starvation						
nf1						
nf2						
nf3	52	0.186				
nf4	52	0.153				
nf5	52	0.184				
nf6						
nf7	49-50	0.115				
nf8	47	0.116				
nf9	47	0.157	68	0.130		
nf10	47	0.165				
nf11	47	0.135				
nf12						
nf13	46	0.123				
nf14						
nf15	46	0.108				

		Birth Weight (g)	09/28/96 Weight (g)	10/27/96 Weight (g)	11/24/96 Weight (g)	12/15/96 Weight (g)	01/08/97 Weight (g)	02/05/97 Weight (g)	02/28/97 Weight (g)
Brittle stars	Avg	0.199	0.188	0.170					
	Std	0.023	0.026	0.031					
	n	29	27	13					
Corophium	Avg	0.196	0.258	0.294	0.360	0.396	0.473	0.606	0.718
	Std	0.027	0.040	0.052	0.072	0.095	0.134	0.181	0.203
	n	39	38	31	26	22	16	13	11
Live *Gammarus*	Avg	0.205	0.237	0.252	0.262	0.266	0.356	0.350	0.392
	Std	0.025	0.037	0.047	0.069	0.088	0.083	0.088	0.105
	n	24	22	19	13	8	3	3	3
Frozen *Gammarus*	Avg	0.193	0.200	0.201	0.186	0.188	0.177		
	Std	0.026	0.024	0.026	0.039	0.033	NA		
	n	34	30	16	11	2	1		
Fish	Avg	0.204	0.189	0.180					
	Std	0.024	0.019	0.016					
	n	29	26	9					
Worms	Avg	0.192	0.215	0.186	0.221	0.217	0.218	skipped	0.220
	Std	0.031	0.021	0.011	NA	NA	NA	NA	NA
	n	3	3	3	1	1	1		1
Starvation	Avg	0.175	NA	0.174	0.1442	0.130			
	Std	0.029	NA	0.034	0.029				
	n	15	NA	11	10	1			
All	Avg	0.197							
	Std	0.026							
	n	173							

Appendix 5

Bathypolypus arcticus growth at three different temperatures

This appendix contains the growth data at three different temperatures from Chapter 3. Time is reported in days from the beginning of the experiment and weight is reported in grams. Growth rates (G) have been calculated for octopuses in the first three treatments. Hatchling weight was measured for 15 randomly chosen octopuses before the experiment started.

On the final pages of this appendix, summary data are presented: “Avg” is the average weight in a given treatment at a given time, “Std” is the standard deviation, and “n” refers to the sample size.

Brood 2	Octo #	Birth Date	B. Weight	Day 1	Day 70	G	Day 120	G	Day 170	G
			(g)	1/17/1996	3/26/1996	(%/d)	5/15/1996	(%/d)	7/4/1996	(%/d)
2 Celcius										
2C	56	11/27/1995	0.276	0.319	0.344	0.108	0.357	0.074	0.328	-0.169
2C	63	12/7/1996	0.314	0.379	0.316	-0.260	0.314	-0.013		
2C	65	12/7/1996	0.275	0.322	0.255	-0.333	0.290	0.257	0.246	-0.329
2C	69	12/15/1996	0.204	0.412	0.410	-0.007	0.462	0.239	0.430	-0.144
2C	70	12/15/1996		0.402	0.393	-0.032	0.308	-0.487		
2C	77	12/24/1996		0.367	0.298	-0.298	0.334	0.228		
2C	78	12/24/1996	0.282	0.359	0.353	-0.024	0.390	0.199	0.635	0.975
2C	79	12/26/1996		0.372	0.343	-0.116	0.312	-0.189	0.435	0.665
2C	81	12/26/1996	0.256	0.376	0.356	-0.078	0.399	0.228		
2C	83	12/26/1996		0.372						
6 Celcius										
6C	54	8/11/1995		0.329	0.344	0.064	0.345	0.006		
6C	62	12/7/1995	0.287	0.395	0.378	-0.063	0.339	-0.218		
6C	64	12/7/1995		0.350	0.429	0.291	0.432	0.014		
6C	74	12/21/1995		0.418	0.489	0.224	0.405	-0.377		
6C	76	12/23/1995	0.283	0.347	0.398	0.196	0.429	0.150		
6C	80	12/26/1995	0.300	0.390	0.480	0.297	0.526	0.183	0.613	0.306
6C	85	12/26/1995		0.353	0.386	0.128	0.538	0.664	0.483	-0.216
6C	88	12/30/1995		0.352	0.427	0.276	0.583	0.623	0.505	-0.287
10 Celcius										
10C	55	11/15/1995		0.294	0.295	0.005				
10C	59	12/5/1995		0.446	0.542	0.278				
10C	60	12/7/1995	0.295	0.394	0.581	0.555				
10C	61	12/7/1995		0.302	0.489	0.688	0.547	0.224		
10C	67	12/14/1995	0.238	0.436	0.818	0.899	0.942	0.282		
10C	68	12/15/1995	0.294	0.461	0.776	0.744	1.562	1.399		
10C	72	12/17/1995		0.424	0.700	0.716	1.036	0.784	1.137	0.186
10C	73	12/19/1995	0.272	0.398	0.700	0.807				
10C	75	12/22/1995	0.280	0.331	0.660	0.986	1.008	0.847	1.794	1.153
10C	82	12/26/1995		0.414	0.700	0.750	1.059	0.828	1.512	0.712

Brood 2	Octo #	Birth Date	B. Weight	Day 1	Day 70	G	Day 120	G	Day 170	G
			(g)	1/17/1996	3/26/1996	(%/d)	5/15/1996	(%/d)	7/4/1996	(%/d)
Natural										
6C Natural	57	12/2/1996	0.319	0.463	0.396		0.456		0.430	
6C Natural	58	12/3/1996	0.275	0.433	0.521		0.466		0.478	
6C Natural	66	12/9/1995		0.433	0.565		0.525		0.528	
6C Natural	71	12/17/1995		0.425	0.367		0.547		0.874	
6C Natural	84	12/26/1995		0.305	0.386		0.778		0.435	
6C Natural	86	12/30/1995		0.371	0.411		0.477		0.423	
6C Natural	87	12/30/1995		0.323	?		0.389			
6C Natural	89	12/30/1995		0.356						
Summary Data			wt at birth	wt Day 1						
		Avg	0.278	0.378						
		Std	0.028	0.046						
		n	15	36						
2C Avg				0.368	0.341	-0.116	0.352	0.060	0.415	0.200
Std				0.030	0.047	0.150	0.056	0.254	0.146	0.581
n				10	9		9		5	
6C Avg				0.367	0.416	0.176	0.450	0.131	0.534	-0.066
Std				0.030	0.050	0.127	0.091	0.366	0.070	0.324
n				8	8		8		3	
10C Avg				0.390	0.626	0.643	1.026	0.727	1.481	0.684
Std				0.060	0.154	0.296	0.324	0.431	0.330	0.484
n				10	10		6		3	
6C Natural Avg				0.389	0.441		0.520		0.528	
Std				0.058	0.081		0.125		0.174	
n				8	7		7		6	

Appendix 6

Calculations used in Chapter 5

The following appendix documents all calculations used in Chapter 5 and is presented here so that others can see exactly where the numbers came from. L refers to laboratory data, F refers to data from the field. Most of the calculations are simply averages as reported in Chapter 5.

As mentioned in Chapter 5, there is almost certainly differences between populations of the same species of cephalopods. Furthermore, laboratory studies (See technical note 2) have found that there is also much variation, even between siblings. These numbers are meant to be as accurate as currently possible given the current basic life cycle data of cephalopods. Using averages for species that vary over five orders of magnitude in size at maturity greatly alleviates the effect of within species variation and allows interspecific comparisons.

Subclass Nautiloidea

Order Nautilida

Nautilus belauensis

Hatchling Size (LF) - See methods (Chapter 5)
Size at Maturity (F) - Saunders and Spinosa 1978 (pg 351)
Age at Maturity (LF)- Landman and Cochran1987 (pg 420)
Temperature (F) - Saunders and Ward 1987 (pg 147)

(9 + 17)/2 = 13

Subclass Coleoidea

Order Octopoda

Bathypolypus arcticus

Hatchling Size (L):

An average of all published values was used.
(0.208 + 0.283 + 0.150)/3 = 0.214 g
Values A and B are from Wood et al. 1998 (abstract) While C is from O'Dor and Macalaster 1983 (pg 402)

Size at Maturity (F) - O'Dor and Macalaster 1983 (pg 402)
Age at Maturity (L) - See methods (Chapter 5)
Temperature Estimate (F) - O'Dor and Macalaster 1983 (pg 403)

Octopus bimaculoides

Hatchling Size (L) - (pg 371) Forsythe and Hanlon 1988
Size at Maturity (L) - Forsythe and Hanlon 1988 (pg 372)

Octopuses weighed 517.39 g at day 324 and 597.53 g at day 370
Forsythe and Hanlon 1988 (pg 372)

(597.53g-517.39g)/(370d-324d) = 1.74g/d average weight gained per day during this period.

At day 341:
517.39g + 1.74g/d*(341d-324d) = 545g

Age at Maturity (L) - Forsythe and Hanlon 1988 (pg 376)

Broods laid on days 315, 316, 365 and 369

Average = 341 days.

Temperature Estimate (L) - Forsythe and Hanlon 1988 (22.98°C, pg 371).
I used the 23° treatment because the 18° treatment died before all animals were mature as a result of a power failure.

Octopus dofleini

Hatchling Size (F,?): (? Is likely field data)

(0.029 + 0.02165)/2 = 0.02525 → 0.025
From Cosgrove 1993 (abstract) and Hartwick 1983 (pg 280) respectively.

Size at Maturity (F, ?):

[(14+16)/2+(10+15)/2+(18+25)/2]/3 = 16.3 kg
A (15kg) from Cosgrove (pg 40) and B and C (12.5, 21.5) From Hartwick 1983 (pg 284).

Age at Maturity (F, ?):

(31 months)(year/12 months)(365d/year) = 943d
Cosgrove 1976 (pg 65)

(3 years)(356d/year) = 1095
Hartwick 1983 (pg 284)

(943 + 1095)/2 = 1019d

Temperature Estimate (F, ?):

[10.4 + (7 + 15)/2]/2 = 10.7°C
Cosgrove 1993 (pg 38) and Hartwick (pg 287)

Octopus joubini

Hatchling Size (L):

(0.040 + 0.035 + 0.045)/3 = 0.040
From Hanlon 1983 (pg 296), Forsythe 1984 (pg 397) and Forsythe and Hanlon 1981 (last page) respectively.

Size at Maturity (L) - Hanlon 1983 (pg 296)
Age at Maturity (L) - Hanlon 1983 (pg 296)

Temperature (L) - Hanlon 1983 (pg 296), Forsythe 1984 (Abstract)

Octopus maya

Hatchling Size (Data likely from his 1976 thesis, L) - Van Heukelem 1983 (pg 312)
Size at Maturity (L): Van Heukelem 1983 (pg 315)

Age at Maturity (L):

(242 + 272)/2 = 257
Van Heukelem 1983 (pg 318)

Temperature Estimate (L) - Van Heukelem 1983 (pg 315)

Octopus vulgaris

Hatchling Size (L) - Villanueva 1995 (Abstract)
Size at Maturity (F) - Mangold 1983 (pg 353)

I used the three populations for which temperatures were recorded.

[(2500 + 3000)/2 + (2500 + 4000)/2 + (1000 + 1500)/2]/3 = 2417

Age at Maturity (F): Mangold 1983 (pg 353)

[(6 + 8)/2 + (18 + 24)/2 + (12 + 18)/2]/3 = 14.3 months

(14.3 months)(1 year/12 months)(365 d/year) = 435 d

Temperature Estimate (F): Mangold 1983 (pg 353)

[(17 + 28)/2 + (10 + 20)/2 + (10 + 25)/2]/3 = 18.3

Order Sepiida
Sepia officinalis

According to Forsythe et al 1994, later generations of lab reared cuttlefish were larger than normal and had reduced fecundity. I chose size at maturity, age at maturity and temp data from the "1st generation French population" as it was the earliest generation that was reared at a stable temperature.

Hatchling Size (L): Forsythe et al 1994 (pg 180)

(0.15 + 0.20)/2 = 0.175g

Size at Maturity (L) - Forsythe et al 1994 (pg 182)
Age at Maturity (L) - Forsythe et al 1994 (pg 179)

(11/12)365 = 335d

Temperature Estimate (L): Forsythe et al 1994 (pg 179)

Sepia pharaonis

Hatchling Size (L): 0.1 Nabhitabhata 1995 (pg 28)
Size at Maturity (L): 84.1 Nabhitabhata 1995 (pg 28)
Age at Maturity (L): 110 Nabhitabhata 1995 (pg 27)
Temperature Estimate (L): 30 Nabhitabhata 1995 (pg 26)

Sepiella inermis

Hatchling Size (L): 0.01 Nabhitabhata 1995 (pg 28)
Size at Maturity (L): 36.6 Nabhitabhata 1995 (pg 28)
Age at Maturity (L): 90 d Nabhitabhata 1995 (pg 27)
Temperature Estimate (L): 30 Nabhitabhata 1995 (pg 26)

Order Sepiolida
Idiosepius pygmaeus

Hatchling Size: See methods (Chapter 5)
Size at Maturity (LF): Lewis and Choat 1993 (pg 24)

(150+200)/2 = 175 mg

Age at Maturity (LF): Lewis and Choat 1993 (pg 24)

(40 + 80)/2 = 60

Temperature Estimate (L): Lewis and Choat 1993 (pg 21)

(27.5 + 23)/2 = 25.2

Euprymna scolopes

Hatchling Size (L): Hanlon 1997 (pg 370)

(4.2+5.8)/2 = 5.0 mg

Size at Maturity (L) - Hanlon 1997 (pg 370) as per equation on pg 370 with time of 80 d.

$Y=0.0032968*e^{(0.08373)(80)} = 2.67$

Age at Maturity (L) - Hanlon 1997 (pg 370)
Temperature (L) - Hanlon 1997 (pg 366, 367)

Order Teuthoidea

Loligo forbesi

Hatchling Size (L): Forsythe and Hanlon 1989 (abstract)

(5 + 9)/2 = 7mg

Size at Maturity (F) - Martins 1982

$w = aL^b$ (pg 804)
Length (L) for mature females = 33.5 cm (pg 804)
$a = 0.425$ (pg 803)
$b = 2.184$ (pg 803)

$w = aL^b = 910g$

Age at Maturity (M): Forsythe and Hanlon 1989 (pg 12)

Temperature Estimate: See methods (Chapter 5)

(13 + 15)/2 = 14

36°N 28°W 13°C
40°N 28°W 15°C

Loligo opalescens

Hatchling Size (L) - Yang et al. 1986 (pg 783)
Size at Maturity (L) - Yang et al. 1986 (pg 783)
Age at Maturity (L): Yang et al. 1986 (Abstract)

(172 + 196)/2 = 184

Temperature (L): Yang et al. 1986 (pg 777)

Loligo vulgaris

Hatchling Size (?): Packard 1969 (pg 876)
Size at Maturity (F): Coelho et al. 1994 (pg 99)
Age at Maturity (F): Coelho et al. 1994 (pg 100)
Temperature Estimate (F):
pg 602 of Arkhipkin 94 - 12, 14, 15, 16 (14 avg)
Similar temperatures reported in Fuglister 1960

Sepioteuthis lessoniana

Hatchling Size (L): 44 mg Hanlon 1990 (pg 54)
Size at Maturity (L): 122.7 Nabhitabhata 1995 (pg 28)
Age at Maturity (L): 90 Nabhitabhata 1995 (pg 27)
Temperature Estimate (L): 30 Nabhitabhata 1995 (pg 26)

Illex illecebrosus

Hatchling Size (L) - O'Dor et al. 1986 (pg 59, Table 2)
Size at Maturity (F) - O'Dor 1983 (pg 187)
Age at Maturity (F) - O'Dor and Dawe 1998 (pg 94)
Temperature Estimate (LF): O'Dor 1983 (pg 180, 185)

$(10 + 18 + 14.3 + 16.3 + 7.0 + 9.7 + 10.3 + 15.5)/8 = 12.6$

Thysanoteuthis rhombus

Hatchling Size - See methods (Chapter 5)
Size at Maturity (F) - 8966g Nigmatullin 1995 (equation on pg 78, t = 240)
Age at Maturity (F) - 240d Nigmatullin 1995 (pg 79)
Temperature (F): Nigmatullin 1995 (pg 73)

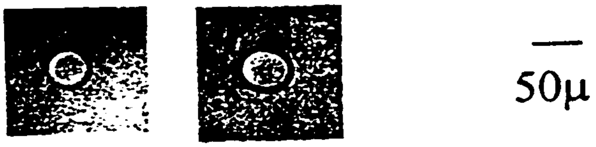
$(23 + 26)/2 = 24.5$
(pg 73)

Appendix 7

Does *Bathypolypus arcticus* contain antifreeze?

Many arctic, antarctic and temperate intertidal fish and invertebrates are resistant to freezing (Loomis 1995; Woehrmann 1997). One strategy used by some of these animals is the production of biological antifreeze. Antifreeze lowers the freezing point to below that of seawater (-1.9°C) without upsetting the animals osmotic pressure (Woehrmann 1997). Due to the cold arctic water that *B. arcticus* lives and is named after, I thought it would be interesting to investigate whether they contain antifreeze.

Tissue samples of *B. arcticus* were donated to Dr. Vanya Ewart of the NRC Institute for Marine Biosciences in Halifax NS Canada in order to determine if *B. arcticus* use antifreeze. Ice crystal photomicroscopy was used to analyze the sample. Round crystals were observed which indicate an absence of antifreeze activity.



Round crystals as seen above indicate an absence of antifreeze activity.

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