Vulnerability of Logfin Inshore Squid (Loligo Pealeii) to Predation: The Influence of Relative Prey Size and Behavior

VULNERABILITY OF LONGFIN INSHORE SQUID (*LOLIGO PEALEII*) TO PREDATION: THE INFLUENCE OF RELATIVE PREY SIZE AND BEHAVIOR

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DEDICATION

In memory of my grandmother, Martha Mary Cross Landau.

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ABSTRACT

VULNERABILITY OF LONGFIN INSHORE SQUID (*LOLIGO PEALEII*) TO PREDATION: THE INFLUENCE OF RELATIVE PREY SIZE AND BEHAVIOR

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Cephalopods provide forage to a wide range of predators in marine food-webs. Despite their ecological importance, a basic understanding of the mechanisms controlling predation risk and demand is lacking. This is true of one of the most common species of squid found in the northwest Atlantic, the longfin inshore squid (*Loligo pealeii*). In this dissertation, I address this shortcoming by investigating the role that size and behavior play in influencing squid's vulnerability to predation.

I used long-term food habits, population survey, and commercial landings data, to quantify size-based patterns of predation respective to 25 species of predators. Additionally, I estimated the amount of overlap between predatory consumption and the fishery catch for squid by size. I found that finfish and elasmobranchs generally consumed juvenile and sub-adult squid, while marine mammals primarily targeted adults. Consequently, marine mammals had the highest overlap with the fishing industry for squid size resources. Although large squid were not common in predator diets, predators

did not appear to be gape-limited when feeding on squid. This suggested that other factors, including behavior, were important in shaping size-based patterns of predation.

I used a laboratory-based approach to quantify attack and capture behaviors towards squid by two predators representing contrasting foraging tactics. Bluefish (*Pomatomus saltatrix*) and summer flounder (*Paralichthys dentatus*) were chosen as cruising and ambush predators, respectively. Patterns in attack rates suggested that size-selection on squid was constrained by passive processes rather than active choice in both predators. Size-dependent profitability functions were calculated by combining capture success rates, handling times, and relative prey mass, and determined that bluefish was the more efficient predator of squid. Lastly, I evaluated the occurrence and effectiveness of anti-predator responses used by squid in the presence of bluefish and flounder. Squid behavior depended on the type of predator present, and the survival value of primary and secondary defense behaviors differed during interactions with each predator.

The results of this project are intended to improve the quality of management of squid and their predators by providing a better understanding of predator-prey interactions in the northwest Atlantic.

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PREFACE

In the northwest Atlantic, decades of overfishing have led to the decline of many important commercial finfish populations. As the abundance of traditional stocks has progressively declined, the response by the fishing industry has been to target species at lower levels of the food chain (Pauly et al. 2002). Invertebrates such as squid are just one example of this emerging trend. Previous to the 1960's, longfin inshore (*Loligo pealeii*) squid were considered bait and had little commercial value in U.S. Atlantic coastal waters (Brodziak 1998). In contrast, today squid are one of the most valued fisheries in the region with commercial harvests increasing by several orders of magnitude from just a few decades ago (NMFS 2009). This reliance on smaller, faster growing species is representative of a global trend and has prompted concern as to whether such practices are sustainable (Pauly et al. 1998).

Improving our understanding of trophic interactions is essential to predict population responses to ecosystem changes that are precipitated by exploitation (Link 2002a, 2002b). Since predation can outweigh all other components of mortality (Buckel et al. 1999a; Overholtz et al. 2000), the comprehension and quantification of predator-prey interactions improves our ability to predict population abundance and behavior (Bax 1998). Furthermore, the recent progression towards developing more holistic, ecosystem-based management plans requires increased knowledge of predator-prey relationships to model population dynamics (Cury et al. 2005; Duplisea 2005; Moustahfid et al. 2009).

The consequences of overexploiting squid have potential ramifications that radiate throughout the food-chain. In the northwest Atlantic, squid have been identified

in food habits studies as a principal resource to many commercially and ecologically important predators. In a comprehensive survey conducted by Bowman et al. (2000), the food habits of 170 predators in the northwest Atlantic were reviewed. Of these, squid occurred in one-third of all species evaluated, the primary species being *Loligo* spp. In 17% of the predators evaluated, squid contributed 10% or greater by weight to their total diets. Dogfish, hake, billfish, flounder, and bluefish are just a few of the predators listed as relying heavily (> 10% by mass) on squid as a food resource (Bowman et al. 2000). Many of the predators listed above have been overexploited and their populations are either in an overfished or rebuilding status (NOAA 2009). Management efforts seeking to recover and sustain predator stocks may be less effective if the total squid biomass available as forage declines as a result of increased fishing (Pauly et al. 2002).

Despite the apparent importance of squid as a prey resource, a basic understanding of the mechanisms controlling predation risk and demand on their populations is lacking. While much attention has been given to size-influenced predation by piscivores (Juanes and Conover 1995; Mittelbach and Persson 1998; Manderson et al. 1999; Scharf et al. 2002; Dorner and Wagner 2003; Scharf et al. 2003), little to no information exists on the size-dependent relationships between squid and their predators. Previous studies that evaluated the importance of squid as prey have focused primarily on the weight contribution to predator diets. Mass-based descriptions of predator feeding habits determine the relative contributions of prey to predator diets and provide general estimates of predatory demand; however, to determine the total impact of a predator on a prey population, information on the sizes of prey consumed is also necessary. Size-based descriptions of predator diets help determine 1) the sizes of prey most important to

supporting predator growth, 2) the impacts of predation on different prey life stages, and 3) whether predators are competing for similar portions of the prey resource (Livingston 1993).

Relative prey size has a direct impact on capture success, retention, and handling time. These factors are fundamental to modeling foraging behavior and predicting predator diets (Juanes et al. 2002; Mittelbach 2002; Scharf et al. 2003). Morphological constraints (i.e. gape width), detection limits (vision), and swimming abilities are just a few factors which may limit the range of sizes a predator can successfully capture (Juanes and Conover 1995; Nilsson and Bronmark 2000; Sih and Christensen 2001). Given that squid have such a diverse number of defense and escape strategies as well as morphological differences (Hanlon and Messenger 1996), it is unknown if squid are more or less difficult to subdue in comparison to fish. We also do not know if predators attack relatively larger squid than they would fish. In addition to size-based relationships, the behaviors of predators and prey may be equally important in affecting encounter rates, selection, capture success, and ultimately prey vulnerability (Juanes et al. 2002; Scharf et al. 2003). Therefore, both quantitative and qualitative measures of prey responses will be informative in understanding predator foraging habits.

Squid are a unique organism for evaluating the ethology of predation. Although cephalopods are invertebrates, they have been likened to fish and other vertebrates in possessing advanced sensory systems, brain function, and behaviors (Hanlon and Messenger 1996). Packard (1972) describes cephalopods functionally as fish due to their similar habitats, range of body sizes, schooling behavior, and ontogenetic morphology. Thus, squid's vulnerability to predation may also be comparable to fish species which

they resemble ecologically (e.g., clupeids). Squid are soft bodied and have relatively compressed body depths. These characteristics may act to decrease handling time and make them more frequently targeted by predators. Conversely, squid utilize a wide variety of defense mechanisms to avoid predation, the majority of which common prey fish lack. For example, squid possess beaks, arms, and suckers that may be used to retaliate against a predator (Hanlon and Messenger 1996). Crypsis (camouflage, body pattern changes) is utilized to avoid detection and may have inconsistent success contingent on the predator's visual capabilities. Ejecting ink is another response unique to cephalopods which is employed to confuse a predator. Additionally, jet propulsion gives squid the ability to move both forwards and backwards and may allow squid to react in more directions than prey fish. All of these traits will influence squid's vulnerability to predation although their effectiveness will vary depending on the abilities of different predators.

This dissertation provides a comprehensive evaluation of how body length and behavior influence longfin inshore squid's vulnerability to predation. Chapter 1 provides an overview of size-based patterns in predation on longfin inshore squid in the northwest Atlantic ecosystem. A size-based perspective is also used to evaluate whether natural predators and the commercial fishing industry are competing for squid resources and the implications of these practices are discussed. Chapters 2 – 4 are based on experimental work completed at the Marine Biological Laboratory in Woods Hole, Massachusetts. These three chapters examine predator-prey interactions between longfin inshore squid and two model predators, bluefish (*Pomatomus saltatrix*) and summer flounder (*Paralichthys dentatus*). Chapters 2 and 3 evaluate interactions between squid and fish

from a predator perspective while Chapter 4 shifts primarily to the point of view of the prey. In comparison to bluefish, much less is known about the feeding tactics of summer flounder; consequently, Chapter 2 presents a detailed look at how summer flounder adapt their behavior in response to different types of prey, including squid and prey fish. Chapter 3 examines how selection, survival, capture success, handling time, and prey-profitability vary as a function of relative prey (squid) size in bluefish and summer flounder. The fourth and final chapter investigates whether squid use different anti-predator responses in the presence of bluefish and summer flounder and assesses the effectiveness of key defense behaviors.

CHAPTER 1

A SIZE-BASED APPROACH TO QUANTIFYING PREDATION ON LONGFIN INSHORE SQUID (*LOLIGO PEALEII*) IN THE NORTHWEST ATLANTIC

Abstract

Cephalopods are primary prey to a wide range of predators in global marine ecosystems. Despite their apparent ecological importance, little information exists on size-based predation for this taxon. Using long-term food habits, population survey and commercial landings data, I quantified size-based patterns of predation for 11 species of finfish, elasmobranchs, and marine mammals over ontogenetic time scales. General trends of size-selective and seasonal foraging behavior are also presented for 25 species of predators from the northwest Atlantic Ocean. The functional role of squid was evaluated by contrasting patterns in size-based predation between squid and fish prey types. Measurements of predator gape morphology and prey body depths ascertained if predators were physically limited when feeding on squid. Additionally, the amount of overlap between natural predators and the commercial fishing industry for squid size resources was estimated. Predation by finfish and elasmobranchs was generally focused on juvenile and sub-adult squid, while marine mammals primarily targeted adults. Consequently, marine mammals had the highest overlap with the commercial fishing industry for squid size resources. All predators exhibited size-selective feeding behavior and trends persisted across seasons. Predators fed on a wider range of fish than squid prey sizes and did not appear to be gape-limited when feeding on squid; however large squid were not common in predator diets. Results suggest squid behavior, availability in

the environment, and encounter rates are paramount in shaping size-based patterns of predation.

Introduction

Predators are opportunistic, switching between prey species based on their absolute and relative availabilities in the environment; however, to some extent all predators are selective (Bax 1998). In marine piscivores, the relationship between predator and prey body size directly influences foraging success and is one of the best indicators of the physical constraints on an individual (Peters 1983; Claessen et al. 2002). Other morphological features that change in proportion to a predator's body size, such as mouth gape, are informative and define the upper size limits of prey consumed both intra- and inter-specifically (Juanes 1994; Nilsson and Bronmark 2000; Juanes et al. 2002). As predators grow, the maximum size of prey consumed generally increases, but diets are often concentrated on, or continue to include, small prey (Juanes and Conover 1995; Scharf et al. 2000). Few marine predators feed exclusively on the largest prey they possibly can because (1) it is energetically costly to pursue large prey (Scharf et al. 2003), and (2) smaller individuals are exponentially more abundant in marine food-webs in comparison to larger ones (Brooks and Dodson 1965; Rice and Gislason 1996). Accordingly, the total range of prey sizes consumed by a predator depends largely on what it can physically manipulate, what is available in its immediate environment, and how energetically profitable it is to pursue increasingly larger prey.

The range of absolute prey sizes consumed by many marine predators will increase by orders of magnitude as their diets shift from planktivory during early life

stages to piscivory as adults. For this reason, an individual's trophic position within its community is more accurately described by body size rather than species (Jennings and Reynolds 2007). Alternatively, the range of relative prey sizes consumed ontogenetically by a predator, known as its size or ratio-based trophic niche breadth, often remains constant with predator ontogeny (Pearre 1986; Scharf et al. 2000). Size-based trophic niche breadths are useful for identifying physical limitations on a predator's feeding patterns, provide equivalent measures of resource use among species, and are appropriate for assessing competition for prey size resources (Bethea et al. 2003; Beauchamp et al. 2007).

While much attention has been given to size-based predation by piscivores (Juanes 1994; Mittelbach and Persson 1998; Manderson et al. 1999; Dorner and Wagner 2003), little to no information exists on the size-dependent relationships between cephalopods and their predators. Many top predators that are primarily piscivorous also include cephalopods in their diets over different seasonal, spatial, and ontogenetic scales (Smale 1996; Dawe and Brodziak 1998; Chase 2002; Staudinger 2006). For example, while cephalopods are virtually absent from predator diets in estuarine environments, there is a transition towards cephalopods in shelf, slope and open ocean habitats (Smale 1996). Previous studies have focused primarily on the weight contribution of cephalopods to predator diets and neglected to detail size-based patterns in feeding and behavioral interactions. In food habits studies where squid body sizes have been reported, large squid are often prevalent in predator diets (Kohler 1987; Smale 1996; Gannon et al. 1997; Chase 2002; Staudinger 2006). Small squid are rarely reported in diet analyses; consequently, natural mortality rates for paralarval squid are thought to be

relatively low in comparison to fish (Pierce and Guerra 1994). If predation pressure is concentrated during the later stages of life, this would suggest that predation may primarily act as a control on population structure and individual life history rather than recruitment success as is common in many species of fish (Claessen et al. 2002; Dorner and Wagner 2003).

Squid have been described as functionally similar to fish in many aspects of their ecology; they have analogous habitat distributions, schooling behaviors, body sizes and shapes as many fishes (Packard 1972; Hanlon and Messenger 1996; Pauly 1998; Pauly et al. 1998). For these reasons, size-based predation on squid may be comparable to fish that occupy analogous trophic roles (e.g., cluepids) (Packard 1972). Conversely, squid possess traits that could make them more susceptible to predation than prey fish. Squid lack hard defensive structures such as spines and bony plates. Squid also have soft, cylindrical body forms that may make larger individuals easier to engulf by predators. Optimal diet theory states that predators should select prey that provides the greatest energetic return for the least amount of effort to retain (Stephens and Krebs 1986; Sih and Christensen 2001). Additionally, when a higher quality food source becomes more abundant it should become more important in a predator's diet. The high nutritional value of cephalopods offers predators an added incentive of approximately 20% more digestible protein per unit body mass in comparison to fish (Lee 1994). The reward of a higher quality meal may motivate predators to pursue larger sized squid than fish. Currently, we do not know enough about size-dependent relationships between squid and their predators to predict how size, morphology, quality, and availability, interact to influence predator selection for squid in comparison to prey fish resources.

Overfishing has altered the trophic structure of marine food webs by systematically removing the largest individuals and depleting predator populations to fractions of their former abundance levels (Baum et al. 2003; Myers and Worm 2003). To replace yields lost by the collapse of more traditional fish stocks, commercial fisheries have increasingly targeted squid and other forage fish (Pauly et al. 2002; FAO 2007). Despite the overfished status of many teuthophagous species, predatory demand on squid populations has been estimated to exceed commercial landings by orders of magnitude and be equal to or greater than maximum sustainable yield (Buckel et al. 1999a; Overholtz et al. 2000). It has been suggested that the short life-cycles and high growth rates inherent to cephalopod populations have allowed them to rapidly increase productivity in response to reduced predation pressure (Caddy and Rodhouse 1998; Dawe and Brodziak 1998); however, it is uncertain if squid populations can endure the demands imposed by a community of predators as well as a growing fishing industry. To manage both cephalopods and their predators sustainably, a holistic approach that considers multispecies trophic-interactions is crucial. Natural and anthropogenic sources of mortality may inflict opposing or cumulative forces of size-selection on squid populations therefore, it is also important to evaluate how predation is concentrated relative to fishing pressure (Livingston 1993; Duplisea 2005).

The overall objective of this paper is to provide baseline information on size-dependent relationships between one of the most ecologically and commercially valuable species of cephalopod in the northwest Atlantic ecosystem, longfin inshore squid (*Loligo pealeii*), and its predators. Using long-term food habits data, population survey data, and commercial landings information, I (1) quantify how size-based patterns of predation on

squid vary among predator species, over ontogenetic scales, and during seasonal time-periods, (2) contrast the functional role of squid in comparison to other forage fish, (3) evaluate morphological characteristics in squid and teuthophagous predators that constrain size-dependent relationships, and (4) estimate the amount of overlap between natural predators and the commercial fishing industry for squid size resources.

Methods

Diet data

Predator and prey body size data were obtained from several sources. The largest dataset was collected as part of the long-term fishery-independent population survey conducted by the Northeast Fisheries Science Center (NEFSC). Surveys were conducted during the winter, spring, and fall seasons and spanned the region from Cape Lookout, North Carolina northward to waters off Nova Scotia, Canada. Survey details can be found in (Azarovitz 1981; NEFC [Northeast Fisheries Center] 1988). Datasets collected by several independent authors on finfish and marine mammal diets were also included (Gannon et al. 1997; Staudinger 2006; Ampela, *unpublished data*). Predator names, sample sizes, dates of food habits collection, and geographic ranges are listed in Table 1.1.

The majority of squid mantle lengths were measured directly using intact specimens found in predator stomachs. If prey remains were highly digested, the chitinous gladius (or pen) was used as an equivalent for mantle length. In several datasets, squid beaks were recovered during diet analyses and original body size was reconstructed using predictive equations relating the lower rostral length of the lower

beak to mantle length (Clarke 1986a; Staudinger et al. 2009). Grey seal (*Halichoerus grypus*) diets were collected from haul out sites on Muskeget and Monomoy Islands. The majority of seals at these sites were sub-adults and adults of mixed sex and were estimated to be in the range of 90 – 275 cm total length (Ampela, *personal communication*). Because squid remains were collected from scat, body lengths could not be confirmed for individual seals; therefore only prey length data were used for this predator species.

Size-based patterns of predation

To identify the sizes of squid most recurrent in predator diets and to determine at which stage of each squid's life-cycle size specific predation was most prevalent, absolute body size relationships between squid and their predators were evaluated using least squares and quantile regression techniques. Individual predator species were evaluated by graphing predator-prey length data as scatter-plots. Quantile regression was used to estimate the rate of change in the lower and upper bounds of predator-prey body size distributions respective to each predator species and over a wide range of predator body sizes (Scharf et al. 1998a; Scharf et al. 2000). Estimated lower and upper bounds were represented either by $5^{th}/95^{th}$, $10^{th}/90^{th}$, or $25^{th}/75^{th}$ quantiles depending on sample size restrictions as suggested in (Scharf et al. 1998a). Lastly, mean predator-prey body size relationships were estimated using ordinary least-squares regression.

Relative predator-prey body size relationships were used to quantify size-based trophic niche breadths of individual predator species and evaluate interspecific competition for squid size resources. Relative body sizes were calculated by dividing the

total length of each squid (length of squid mantle and arms, tentacles excluded (Staudinger et al. 2009)) by its corresponding predator length. Resulting predator-prey size ratios were examined as relative and cumulative frequency distributions to determine the percentages of relatively small (< 20% relative body size), medium or intermediate (20% ≥ and < 50% relative body size), and large (≥ 50% relative body size) squid in each predator's overall diet.

Size-based trophic niche breadths were determined by graphing relative size ratios as the dependent variable against predator size (independent variable) and displayed as scatter-plots (Scharf et al. 2000; Juanes 2003). Quantile regression was then used to estimate the lower and upper bounds of these scatter-diagrams. The 10th and 90th quantiles were chosen to evaluate all predator species because they adequately described the shapes of relative body-size distributions while remaining conservative even when sample size restrictions (Scharf et al. 1998a) were not strictly adhered to. This methodology ensured that estimates of size-based trophic niche breadths were standardized and comparable across predator species. An *F*-test was used to detect differences between the lower and upper bound slopes and determine if size-based trophic niche breadths were parallel, converging, or diverging (Scharf et al. 2000; Juanes 2003). No difference between lower and upper bound slopes indicated parallel size-based trophic niche breadths and hence a constant range of relative squid sizes consumed with predator ontogeny. Significant differences between slopes indicated either diverging and expanding, or converging and contracting size-based trophic niche breadths.

The mean size-based trophic niche breadth (TNB) respective to each predator was calculated using the equation:

$$TNB = \frac{\sum_{i=1}^{i=n} \left[(m_{90})(PL_i) + (b_{90})\right] - \left[(m_{10})(PL_i) + (b_{10})\right]}{n} \qquad \text{Eqn. 1.1}$$

Where i = an observation of predator length (PL), n = the total number of observed species-specific predator-prey lengths combinations; m = the slope, and b = the intercept calculated for the 10th and 90th quantiles of relative predator-prey size ratios regressed on predator size in each predator dataset. Size-based trophic niche breadths were then plotted as box and whisker plots and ordered from smallest to largest.

To determine if predators were feeding opportunistically or exhibiting size-selection on squid, size distributions of longfin inshore squid populations available in the environment were compared to squid lengths recovered from predator diets. If a predator was feeding opportunistically, the distribution of squid lengths in its diet was similar to the distribution of lengths in the environment. Negative size-selection occurred if predators had greater frequencies of relatively smaller squid lengths in their diets compared to lengths available in the environment. Conversely, positive size-selection was supported if a greater proportion of a predator's diet was comprised of relatively larger squid lengths than were most abundant in the environment.

Squid population data were collected as part of the NEFSC bottom-trawl survey and subset to correspond to the same time period (1991 - 2004) as when the majority of diet data were collected. Squid lengths were grouped into one centimeter mantle length increments to generate frequency distributions. All population and diet length

distributions were positively skewed and in violation of normality therefore, the Kolmogorov-Smirnov test was chosen to contrast differences (Zar 1984; Sokal and Rohlf 1995) and performed using the NPAR1WAY command in SAS (SAS 2003). When significant differences between predator diets and squid population data were detected, visual inspections of length frequency distributions were conducted to ascertain if negative or positive size-selection was occurring. Seasonal trends in size-selection were also evaluated for winter, spring, and fall; population data were not available for summer.

Functional roles of squid and forage fish

Predator diets in the northwest Atlantic contain a greater diversity of prey fish species than cephalopod species; however this does not necessarily mean that a greater range of fish prey sizes will be consumed. Size-based trophic niche breadths respective to squid and forage fish were compared to evaluate if predators were exploiting the two prey types similarly. Body size data on prey fish were collected as part of the food-web dynamics program (NEFSC) and correspond to the same predators and time periods that were used for calculations of squid size-based trophic niche breadths.

To determine how squid ranked on the spectrum of potential body shapes available to predators, measurements of squid body depth and width were compared with several common prey fish found throughout the northwest Atlantic. Longfin squid were collected from coastal waters off of Massachusetts by otter-trawl on the *RV* Gemma between May and August in 2007. Measurements of squid body depth and width were made at the maximum points on the mantle with digital calipers to the nearest 0.01 millimeter. The resulting relationship between squid body depth and total length was

compared with butterfish (*Peprilus triacanthus)*, sand lance (*Ammodytes americanus*), and Atlantic herring (*Clupea harengus*) using previously published morphometric equations (Scharf et al. 1998b). Sand lance and butterfish were representative of the minimum and maximum body depths, respectively, of prey fish available to predators in the northwest Atlantic (Ménard et al. 2006). Atlantic herring was assumed to most closely resemble squid in overall body form (Packard 1972).

Morphological constraints on size-dependent relationships

If predators are gape limited, prey body depth may be influential in constraining foraging behavior (Nilsson and Bronmark 2000). Relationships among squid body depth and width, predator length, and predator gape size were examined to determine if predators were feeding near their physical limits over ontogeny. Gape sizes were assessed from specimens of Atlantic mackerel (*Scomber scombrus*), fourspot flounder (*Paralichthys oblongus*), smooth dogfish (*Mustelus canis*), spotted hake (*Urophycis regia*), and summer flounder (*Paralichthys dentatus*) collected on the NEFSC 2008 spring bottom-trawl survey. All other predator gape relationships were adapted from (Scharf et al. 2000). Gape height was measured as the maximum linear distance from the upper and lower jaws with the mouth stretched open. Gape width was measured as the linear distance from the corners of the stretched open mouth. Squid length data were converted to body depths and widths using equations developed from the data collected above. Relationships between the limiting squid body metric and the limiting predator gape dimension were plotted as dependent variables against corresponding predator lengths (independent variables).

Overlap between predators and the commercial fishing industry

To evaluate whether predators were exploiting similar squid size resources as the fishing industry, squid lengths from predator diets were compared to those landed commercially. Predator diet data (Table 1.1) were pooled and grouped into one centimeter length increments. Commercial data were obtained from the most recent stock assessment (Northeast Fisheries Science Center 2002) and subset to match the primary period when predator diet information was collected (1991 – 2004). In addition to testing differences of location and distribution using a Kolmogorov-Smirnov test, the degree of overlap between predators and the fishing industry was estimated by calculating the overlapping area under the two distribution curves.

Results

Size-based patterns of predation

Juvenile and sub-adult squid (2 - 10 cm ML) were most important to finfish and elasmobranch predators while adults (≥ 15 cm ML) dominated (> 90%) the diets of marine mammals (Figure 1.1). The mean size of all predators sampled was 128 cm and the vast majority were ≤ 100 cm.

Of the 25 predators listed in Table 1.1, ten species had sample sizes large enough to meet minimum requirements suggested for conducting quantile regression analysis (n ≥ 40). Estimations of the lower bound (-0.01 to 0.35), mean (-0.03 to 0.51), and upper bound (-0.09 to 0.67) regression slopes for absolute body size relationships between squid and their predators spanned several orders of magnitude and ranged from negative

to positive. With the exception of Atlantic mackerel, all predators exhibited lower bound slopes that were moderate in comparison to mean and upper bound slopes (Table 1.2).

Small predators (< 40 cm PL) such as Atlantic mackerel, fourspot flounder, silver and spotted hakes exhibited rapid and simultaneous increases in the minimum and maximum sizes of squid in their diets (Figure 1.2). Consequently, a narrow range of squid lengths were consumed by small predators at any given size, but predation shifted across multiple squid life stages (e.g., juvenile to sub-adult) as predators grew. In contrast, large predators such as goosefish, pilot whales, smooth and spiny dogfish maintained relatively constant and broad ranges of squid sizes in their diets at all stages of growth (Figure 1.2). The two intermediate sized predators, bluefish and summer flounder, exhibited the greatest variation between the minimum and maximum size of squid in their diets. Both species expanded the overall distribution of squid sizes in their diets with ontogeny, although summer flounder's upper limit (25 cm ML) was much higher than bluefish (15 cm ML). Similar shapes in predator-prey body size distributions were observed among closely related predators (e.g., dogfish), yet the limits of size-based predation were highly species specific. For example, the onset of squid predation by spiny dogfish (25 cm PL) occurred at much smaller sizes in comparison to smooth dogfish (50 cm PL).

Most predators consumed squid that were < 30% relative to their own body sizes and diets were concentrated on squid within a 10 - 20% relative size range. The dominant size class in each predator's overall diet varied widely ranging from < 10% in pilot whales and 40% in fourspot flounder and spotted hake (Figure 1.3). Goosefish diets contained the greatest diversity and largest (up to 84%) relative squid sizes of all

predators examined. Some predators (e.g., summer flounder) displayed wide distributions of relative squid sizes in their diets, while others (e.g., Atlantic mackerel) exhibited definitive peaks, after which, the frequency of larger relative body sizes declined steeply (Figure 1.3).

Parallel size-based trophic niche breadths were most common among squid predators (Table 1.3; Figure 1.4). Spiny dogfish was the only predator evaluated to exhibit a converging size-based trophic niche breadth, and silver hake was the only predator exhibiting a diverging size-based trophic niche breadth. Visual inspection of several relative body size scatter plots suggest diverging (e.g., spotted hake and summer flounder) and converging (e.g., bluefish and goosefish) size-based trophic niche breadths, although differences between the lower and upper bound slopes were not statistically significant.

Comparisons between squid length distributions from population surveys and prey lengths recovered from predator diets determined that all predators were size-selective towards squid (D statistics were ≥ 0.89; p-values were < 0.0001). Atlantic mackerel was the only species to display negative size-selection. All other predators exhibited positive size-selection and was most pronounced in the two species of marine mammals (Figure 1.5). Trends of positive size-selection also persisted seasonally (D statistics were ≥ 0.36; p-values were ≤ 0.006). The largest differences were detected during spring, and secondarily during the winter.

Morphological constraints on size-dependent relationships

The relationship between squid body width (BW) and body length (BW = 0.154*ML + 0.680, r^2 = 0.94, n = 61 $p < 0.0001$)) increased at a slightly greater rate in comparison to the relationship between squid body depth (BD) and body length (BD = 0.148*ML + 0.581, r^2 = 0.92, $p < 0.0001$) but the difference between slopes was not significant.

Predator gape sizes measured in the present study (Table 1.4) and previously in Scharf et al. (2000) were much greater than squid body depths and widths. Consequently, most predators did not appear to be gape-limited when feeding on squid. Silver hake and spotted hake were the only predators found to target squid at or near their assumed physical limit and over ontogenetic scales; large silver hake even appeared to consume squid that were beyond their estimated gape (Figure 1.6). At small predator body sizes, spiny dogfish consumed squid that were comparable in depth to the span of their gape. No squid size data were reported for body sizes < 25 cm PL suggesting that spiny dogfish may be gape limited below this size. After approximately 50 cm PL, spiny dogfish gapes expanded more rapidly than the squid sizes they were feeding on and they no longer appeared to be gape limited.

Functional roles of squid and forage fish

Squid body shapes were intermediate to butterfish and sandlance and changed at a more moderate rate in comparison to Atlantic herring (Figure 1.7). This suggests that squid's window of vulnerability to predation would be protracted in comparison to the majority of forage fish common throughout the northwest Atlantic. Predators consumed

a smaller range of relative squid sizes in comparison to forage fish; however, mean trophic niche breadths for the two prey types differed only by about a 10% margin in the majority of predators (Figure 1.8). Goosefish, silver hake, and dogfish were the few predators that exploited considerably larger relative size ranges of fish prey sizes (20 - 50% greater) in comparison to squid.

Overlap between predators and the commercial fishing industry

The commercial fishing industry harvested significantly larger squid ($D = 0.431$, $p < 0.0001$) than were targeted by predators. The total estimated overlap between harvested and consumed lengths was 23% and peaked between 9 – 16 cm ML (Figure 1.9). Diet data were partitioned by the three major taxonomic predator groups; finfish, elasmobranchs, and marine mammals, and overlap with the fishing industry was calculated for each group. Marine mammals had the greatest amount of overlap (17%) with the commercial fishery for squid size resources (Figure 1.9). Finfish and elasmobranchs were nearly equal in their overlap with the fishery but progressively less than marine mammals (11% and 9% respectively).

Discussion

Although it is well known that predation is the dominant force structuring squid populations in the northwest Atlantic (Buckel et al. 1999a; Overholtz et al. 2000), information on size-dependent relationships between squid and their predators has been scarce. The results provided in this study shift from the broad perspective of community and down to individual species and to the best of our knowledge, represent the most

comprehensive evaluation of size-based predation on a cephalopod species conducted to date.

Size-based feeding patterns on longfin inshore squid varied widely among teuthophagous predators. Maximum squid sizes exhibited the greatest rates of change both within predator species and interspecifically. Teuthophagous predators consumed a narrower range of squid body sizes than were previously reported when all prey types (e.g., crustaceans, fish) were included in size-based diet analyses (Scharf et al. 2000; Ménard et al. 2006). Given the fact that our analyses were limited to a single prey species, this result is not completely unexpected. However, it is noteworthy that the contracted ranges of squid sizes found in predator diets were largely shaped by ontogenetic changes in the minimum size of squid consumed. Rates of change in minimum prey sizes were also greater than have been found in piscivorous predators (Scharf et al. 2000).

Small teuthophagous predators (e.g., mackerel, hakes, and flounders) increased both the minimum and maximum sizes of squid in their diets with growth and generally consumed narrow ranges of prey sizes. Consequently, size-based predation by small predators has the potential to fluctuate widely with variations in year class strength and overall demographic structure. Larger predators such as dogfish and pilot whales targeted a comparatively wide range of squid sizes and exhibited little to no change in the overall sizes of squid in their diets. Accordingly, size-based patterns in predation by these species would remain relatively consistent on all targeted squid life stages regardless of the size-composition of predator populations.

With the exception of silver hake and spiny dogfish, size-based trophic niche breadths of teuthophagous predators did not show significant trends of expansion or contraction. This is in contrast to Scharf et al. (2000) who found an increasing tendency towards narrowing trophic niche breadths in progressively larger piscivorous predators. Interspecific comparisons of mean size-based trophic niche breadths indicated high correspondence among predators for squid resources. Notable exceptions on both ends of the spectrum were goosefish and pilot whales. Species that co-occur will compete more directly, however the high amount of overlap observed for squid size resources was probably not limiting to predators since many populations are separated temporally and spatially on the shelf (Bethea et al. 2004). Relative frequency distributions and predator-prey body size relationships determined that the relative size class of squid favored in individual predator diets was highly species-specific and varied even among closely related species (e.g., fourspot and summer flounders). Differences among predators were likely shaped by morphological variations, foraging tactics (e.g., lie-and-wait, cruiser), habitat associations, and swimming abilities (Scharf et al. 2000).

Comparisons of squid and fish body shapes revealed that Atlantic herring increased body depth at greater rates than were observed for squid. This signifies that clupeids out-grow gape-limited predators faster and have a smaller window of predation than squid. The same would also be true of other important prey fish such as butterfish and scup (*Stenotomus chrysops*) which are deep bodied and co-occur seasonally with squid in shelf waters (Hendrickson 2005). Squid body shapes were considerably smaller than predator gape heights suggesting that predators were not gape-limited when foraging on squid (Nilsson and Bronmark 2000). Despite this, large squid were not common in

finfish and elasmobranch diets. Predators also possessed wider size-based trophic niche breadths for prey fish in comparison to squid indicating they were not only capable but actually did consume relatively larger prey. Since morphological restrictions were not limiting to predators, availability and behavioral components must be influential in shaping size-based feeding strategies on squid.

Size-based encounter rates between squid and their predators are largely dictated by overall species ranges, activity levels, ontogenetic habitat utilization, and migration patterns. Encounter rates will likely be elevated during the winter and spring seasons when squid and many of their predators aggregate in southern and offshore waters (Staudinger 2006). Unfortunately diet data were not extensive enough to evaluate seasonal trends that were also species-specific. Diel vertical migration is more pronounced in juvenile squid than adults (Brodziak and Hendrickson 1999); accordingly, vertically migrating predators will have the highest encounter rates with juvenile squid. Although juvenile and sub-adult squid were recurrent in finfish and elasmobranch diets, paralarval and small juveniles were rare. Previous studies have also noted this deficiency (Smale 1996; Dawe and Brodziak 1998). The focus of sampling efforts on demersal predators and environments explains to some degree why squid smaller than five centimeters were only occasionally observed in regional diets. Longfin inshore squid exhibit an ontogenetic descent from surface waters into demersal habitats at approximately five centimeters mantle length (Macy and Brodziak 2001). Not surprisingly then, Atlantic mackerel, one of the only pelagic species evaluated in this study, had a diet entirely composed of paralarval and juvenile squid. Atlantic mackerel was also the only predator observed to exhibit negative size-selection on squid.

Size distributions from population surveys suggested that squid sizes greater than 10 cm ML were relatively scarce in the environment. Despite this, large squid were favored in the diets of pilot whales and grey seals. Marine mammal diets often reflect considerably wider ranges of cephalopod sizes and species and may be more reliable indicators of cephalopod population distributions in comparison to conventional survey gears (Clarke 2006). Previous work conducted in the northeast Atlantic found that thirteen species of toothed whales and two species of tunas selected for larger prey than were locally abundant (MacLeod et al. 2006; Ménard et al. 2006). Mean squid lengths reported in the diets of harbor seals in the Gulf of Maine were also quite large (Williams 1999). These accounts provide mounting evidence that marine mammals and large pelagics target larger squid than are normally found in the diets of demersal shelf predators.

Sampling biases

Squid population and predator diet data were primarily obtained from bottom-trawl surveys conducted in continental shelf waters. This method of sampling likely under-represented large squid capable of out-swimming nets, prevalent at other depths in the water column, and that inhabit open ocean environments (Brodziak and Hendrickson 1999; Macy and Brodziak 2001; Hendrickson 2004). Sampling biases may also have influenced predator-prey size data. Food habits collected as part of the National Marine Fisheries Service (NMFS) bottom-trawl surveys identify squid from whole specimens and beaks when possible and length measurements were only made from intact specimens or pens. Species are not usually determined from pens, and the lower rostral

lengths of the lower beaks were not measured as part of NMFS survey protocols. These methods decrease the total number of species-specific squid lengths in diet analyses. Perhaps most importantly, small squid degrade faster in predator stomachs in comparison to large squid therefore small squid will more often be recovered in a highly digested state and based on the methods of collection noted above, lead to under-representation of small body sizes (Santos et al. 2001).

Because our study compiled diet data from several sources, different methods were employed to collect prey length data. Sampling of marine mammal diets was more opportunistic than for fish predators. Pilot whale diets were derived from individuals that had been killed incidentally or stranded. It is uncertain if diets from these individuals were representative of healthy animals and the greater population (Gannon and Waples 2004). Grey seal diets were determined from scat and could not be traced back to specific individuals. In both pilot whales and grey seals, lower rostral lengths of the lower beaks were used to reconstruct original body size using the equations found in Staudinger et al. (2009). Estimating original length from digested remains can be subject to back-calculation errors and beaks that are highly digested may be eroded by digestion. To compensate for digestion effects, beaks recovered from grey seal scat were adjusted using a digestion coefficient (Grellier and Hammond 2006).

Impacts of fishing pressure

Finfish and elasmobranch predation was focused primarily on pre-recruit squid suggesting that overlap with the fishing industry was low. Since commercial landings data may not include biomass removed as bycatch, it is possible that size-based removals

of pre-recruit squid may be higher than indicated in our analyses (Hall et al. 2000). The observed differences in size-based feeding patterns among predator taxa revealed that marine mammals had the highest overlap and therefore the greatest potential for conflict with the commercial fishing industry. Marine mammals have high energetic demands and are often specialized in their feeding ecology (MacLeod et al. 2006); however, foraging habits are also strongly associated with prey availability and seasonal changes (Andersen et al. 2007). Unfortunately our results were limited to general depictions of size-based reliance on squid populations; to fully assess competition for squid resources between predators and the fishery, higher seasonal and spatial resolution is needed.

Fishing has eroded the predation landscape by decreasing the prevalence of larger body sizes in exploited populations (Jennings and Reynolds 2007). All of the fish and elasmobranch species examined here are harvested commercially or caught incidentally by fishing gear. Maximum body sizes of the nine fish species evaluated using quantile regression analyses were 20-50 cm smaller than their reported maximum sizes (Table 1.5). Information on squid-predator body-size relationships became scarcer for all species with increasing predator body size. This is almost certainly due to the low frequency of large individuals in the ecosystem rather than from diminishing predation rates on squid. Predators that were found to rapidly increase the maximum size of squid in their diets and have the potential to attain substantially larger body sizes than were common over the past several decades (e.g., bluefish, summer flounder), will likely increase their predatory demand on adult squid if their population size-structure becomes less truncated. Increased abundance of large fish may also lead to heightened competition with fisheries for adult squid.

Commercial fisheries have increasingly targeted mid-trophic level species including squid to supplement or replace the yields lost from traditional fisheries such as groundfish and large pelagics (Pauly et al. 1998; Essington et al. 2006). Squid biomass is believed to be artificially inflated due to a release from predatory demand from depressed populations of higher level predators (Caddy and Rodhouse 1998; Duplisea and Castonguay 2006). If this is true, fisheries have been taking advantage of excess biomass specifically in the adult component of squid populations that may no longer be in surplus if predator population structure and abundance is recovered under current rebuilding efforts.

Species such as squid that serve as forage to higher level predators and form the "waists" of marine food-webs dictate the dynamic properties of the surrounding food web (Rice 1995). Key forage species have been found to be most sensitive to overfishing at high levels of predation (Collie and Gislason 2001). Because squid are known to be important mid-trophic level species, target harvest rates and long-term potential yields have been set using precautionary approaches (Brodziak 1998). However, fisheries management plans generally do not include estimates of natural mortality rates on pre-recruits in setting biological reference points (BRPs) and total mortality (Z) thresholds (Collie and Gislason 2001). Furthermore, single-species management plans fail to consider trophic interactions and do not account for changes in predator population structure (Buckel et al. 1999a; Pikitch et al. 2004). Because large predators have been functionally absent from the community for several decades, it is uncertain how stock rebuilding will influence predation on squid populations. For example, harvesting adult squid could result in increased survival of pre-recruit squid due to reductions in density

dependent mortality rates. Although intraspecific predation was not included in our analyses, cannibalism within longfin squid populations can be considerable (Macy 1982; Dawe 1988). A similar scenario has been suggested for walleye pollock (*Theragra chalcogramma*) and their predators in the eastern Bering Sea (Livingston 1993).

Conclusions

Over the past 50 years the northwest Atlantic has undergone profound shifts in species abundance and composition and is considered one of the most overfished marine ecosystems in the world (Link and Garrison 2002; Briggs 2008). Depletion of higher level predators and the prevalence of fishing down the food web are more common in the North Atlantic than in other world oceans (Essington et al. 2006). This will be increasingly important as the demographic structure in predator populations is rebuilt. Even if management efforts are not successful in recovering large predators, knowledge of size-based feeding strategies will be useful in predicting the responses of existing populations to exploitation as well as seasonal, annual, and decadal shifts in environmental conditions. Taken as a whole or in parts the information presented in this study can be used to expand single-species management plans into more holistic multispecies and ecosystem-based approaches.

TABLES

Table 1.1: List of predators of longfin inshore squid. N = indicates sample sizes of squid lengths, bolded values indicate predators included in quantile regression analyses. All data were collected between 1991-2004 except for grey seals (2004-2007) and pilot whales (1989-1991).

Predator common name	Predator scientific name	N	Region	References
Atlantic cod	*Gadus morhua*	5	1	
Atlantic halibut	*Hippoglossus hippoglossus*	3	1	
Atlantic mackerel	*Scomber scobrus*	**45**	1	
Black sea bass	*Centropristis striata*	7	1	
Bluefish	*Pomatomus saltatrix*	**267**	1, 4	6
Clearnose skate	*Raja eglanteria*	2	1	
Fourspot flounder	*Paralichthys oblongus*	**136**	1	
Goosefish	*Lophius americanus*	**96**	1, 4	6
Grey seal	*Halichoerus grypus*	**84**	2	7
Little skate	*Leucoraja erinacea*	9	1	
Long-finned pilot whale	*Globicephala melas*	**497**	3	5
Offshore hake	*Merluccius albidus*	2	1	
Pollock	*Pollachuius virens*	8	1	
Red hake	*Urophycis chuss*	11	1	
Sea raven	*Hemitripterus americanus*	19	1	
Silver hake	*Merluccius bilinearis*	**82**	1, 4	6
Smooth dogfish	*Mustelus canis*	**255**	1	
Spiny dogfish	*Squalus acanthias*	**615**	1	
Spotted hake	*Urophycis regia*	**58**	1	
Striped bass	*Morone saxatilis*	28	1	
Summer flounder	*Paralichthys dentatus*	**277**	1, 4	6
Weakfish	*Cynoscion regalis*	25	1	
White hake	*Urophycis tenuis*	3	1	
Windowpane	*Scophthalmus aquosus*	5	1	
Winter skate	*Leucoraja ocellata*	32	1	

[1]Cape Lookout, North Carolina - Nova Scotia, Canada; [2]Massachusetts; [3]North Carolina - New York; [4]New Jersey - Massachusetts; [5]Gannon et al. 1997; [6]Staudinger, 2006; [7]Ampela, K. Unpublished data.

Table 1.2: Equations of absolute body size relationships between longfin inshore squid and their predators. Quantile regression was used to estimate the lower and upper bound equations of scatter data; mean equations were estimated from ordinary least squares regression. M_{Lmin} and M_{Lmax} indicate the minimum and maximum sizes of squid lengths consumed by each predator respectively. ML = mantle length, PL = predator length, all body lengths are in centimeters. N = number of prey lengths. 25th and 75th quantiles were used for 40 < N < 100; 10th and 90th quantiles for 100 < N < 200; and 5th and 95th for 200 < N < 1000. *NS* indicates a non-significant p-value.

Predator species	N	Quantiles analyzed	ML_{min}	ML_{max}	Lower bound	Mean	Upper bound
Atlantic Mackerel	45	25th / 75th	1.0	5.6	ML = 0.14*PL - 1.71	ML = 0.12*PL -0.25	ML = 0.11*PL + 0.51^{NS}
Bluefish	267	5th / 95th	1.2	15.0	ML = 0.03*PL +0.90	ML = 0.06*PL + 2.88	ML = 0.18*PL + 1.93
Fourspot flounder	136	10th / 90th	0.5	18.5	ML = 0.24*PL - 3.40	ML = 0.36*PL - 4.40	ML = 0.46*PL - 4.86
Goosefish	96	25th / 75th	0.9	25.0	ML = -0.01*PL + 6.63^{NS}	ML = -0.03*PL + 12.08^{NS}	ML = -0.09*PL +18.18^{NS}
Pilot whale	497	5th / 95th	4.5	36.6	ML = -0.01*PL +12.50^{NS}	ML = 0.01*PL + 16.73^{NS}	ML = 0.02*PL + 24.01
Silver hake	82	25th / 75th	0.5	26.1	ML = 0.35*PL - 5.42	ML = 0.51*PL - 7.90	ML = 0.67*PL - 10.67
Smooth dogfish	255	5th / 95th	1.1	21.0	ML = 0.01*PL + 1.01^{NS}	ML = 0.01*PL + 4.59^{NS}	ML = 0.03*PL + 8.40^{NS}
Spiny dogfish	615	5th / 95th	1.3	25.0	ML = 0.00*PL + 2.50^{NS}	ML = 0.01*PL + 6.53^{NS}	ML = 0.07*PL + 10.27^{NS}
Spotted hake	58	25th / 75th	2.0	15.5	ML = 0.27*PL - 2.53^{NS}	ML = 0.43*PL - 5.54	ML = 0.53*PL - 6.48
Summer flounder	277	5th / 95th	0.9	25.0	ML = 0.10*PL - 2.00	ML = 0.23*PL - 2.22	ML = 0.39*PL - 3.58

Table 1.3: Equations for relative body size relationships between longfin inshore squid and their predators. The 10th and 90th quantiles were used estimate the lower and upper bounds, respectively, for all predator species. *F* = the F-test statistic for differences between lower and upper bound lines; *p*-value = significance of *F*-test. RBS = relative predator-prey body size, and PL = predator length.

Predator species	Lower bound	Upper bound	*F*	*p*-value	Trophic niche breadth
Atlantic Mackerel	RBS = -0.004*PL + 0.29	RBS = -0.01*PL + 0.58	0.84	0.36	Parallel (decreasing)
Bluefish	RBS = -0.002*PL + 0.24	RBS = -0.003*PL + 0.47	3.12	0.08	Parallel (decreasing)
Fourspot flounder	RBS = 0.002*PL + 0.22	RBS = 0.003*PL + 0.41	0.09	0.76	Parallel (constant)
Goosefish	RBS = -0.003*PL + 0.32	RBS = -0.01*PL + 0.98	2.41	0.12	Parallel (decreasing)
Long-finned pilot whale	RBS = -0.0002*PL + 0.12	RBS = -0.0002*PL + 0.20	0.001	0.98	Parallel (decreasing)
Silver hake	RBS = 0.001*PL + 0.24	RBS = 0.02PL + 0.10	6.33	0.01	Diverging
Smooth dogfish	RBS = -0.001PL + 0.14	RBS = -0.001PL + 0.30	0.98	0.32	Parallel (decreasing)
Spiny dogfish	RBS = -0.002PL + 0.22	RBS = -0.004PL + 0.57	48.53	0.0001	Converging
Spotted hake	RBS = -0.01PL + 0.50	RBS = 0.004PL + 0.45	3.66	0.06	Parallel (constant)
Summer flounder	RBS = -0.002PL + 0.25	RBS = 0.0004PL + 0.43	2.41	0.12	Parallel (constant)

Table 1.4: Linear regression equations for predator length (PL) to gape width (GW) and gape height (GH) for Atlantic mackerel, fourspot flounder, smooth dogfish, spotted hake and summer flounder. All lengths were measured in centimeters. All regression equations were highly significant ($p < 0.0001$).

Predator	N	Equation	r^2
Atlantic mackerel	32	GH = 0.117*PL + 0.334	0.86
		GW = 0.094*PL + 0.188	0.67
Fourspot flounder	24	GH = 0.156*PL - 0.795	0.93
		GW = 0.116*PL - 0.161	0.84
Smooth dogfish	35	GH_{Sq} = 0.060*PL - 0.753	0.93
		GW_{Sq} = 0.065*PL - 0.253	0.96
Spotted hake	40	GH = 0.092*PL - 0.014	0.88
		GW = 0.118*PL - 0.403	0.96
Summer flounder	37	GH_{Sq} = 0.130*PL - 0.195	0.95
		GW_{Sq} = 0.114*PL - 0.261	0.96

Table 1.5: Historical and sampled maximum fish lengths (L_{Max}). Historical lengths were reported in Collette & Klein-MacPhee (2002) and Fishbase, Froese and Pauly (Ed.) (2008).

Species	Historical L_{Max} (cm)	Sampled L_{Max} (cm)
Atlantic Mackerel	60	38
Bluefish	130	77
Fourspot flounder	45	43
Goosefish	120	101
Silver hake	76	41
Smooth dogfish	150	123
Spiny dogfish	160	110
Spotted hake	42	40
Summer flounder	94	74

FIGURES

Figure 1.1: Community spectrum of predation over the life span of longfin inshore squid. Arrows point to the mean size of squid consumed by each predator species. Dashed lines indicate predators with sample sizes ≤ 10. Predator scientific names are listed in Table 1.1. Squid life stages are approximated to mantle lengths reported in Jacobson (2005).

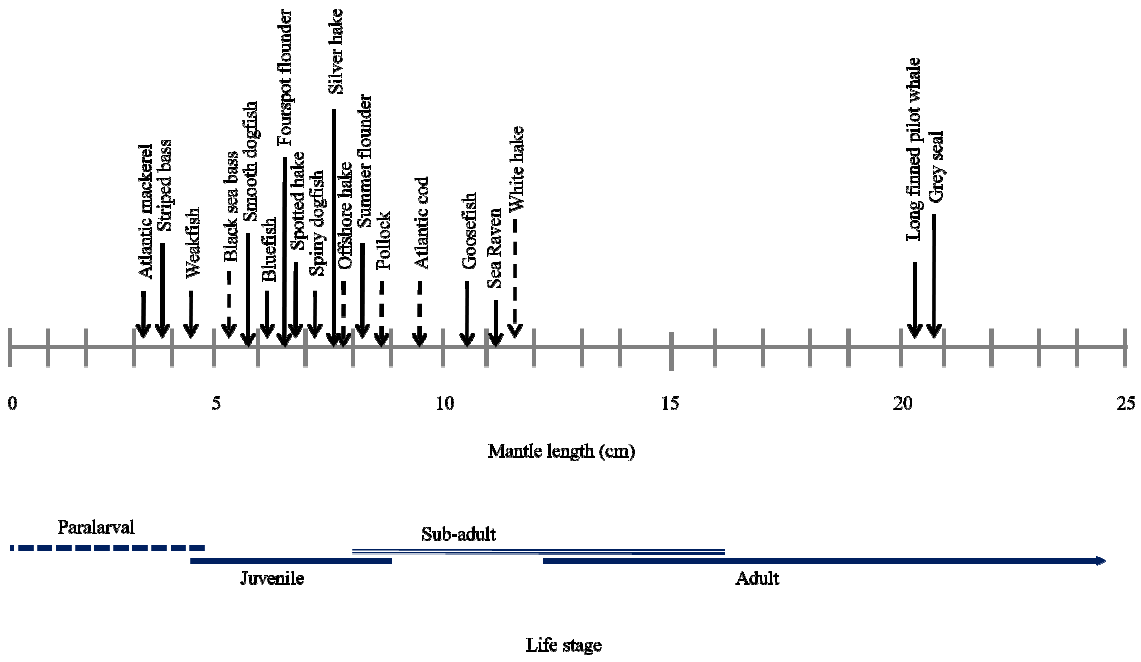


Figure 1.2: Scatter-plots of absolute predator-prey relationships for longfin inshore squid and A) Atlantic mackerel B) bluefish C) fourspot flounder D) goosefish E) long finned pilot whale F) silver hake G) smooth dogfish H) spiny dogfish I) spotted hake J) summer flounder. Each (o) represents a single prey consumed by a predator. Solid lines represent the lower and upper bound regression lines estimated from quantile regression analyses. Dashed lines indicate mean slopes estimated from ordinary least squares regression. All line equations are listed in Table 1.2.

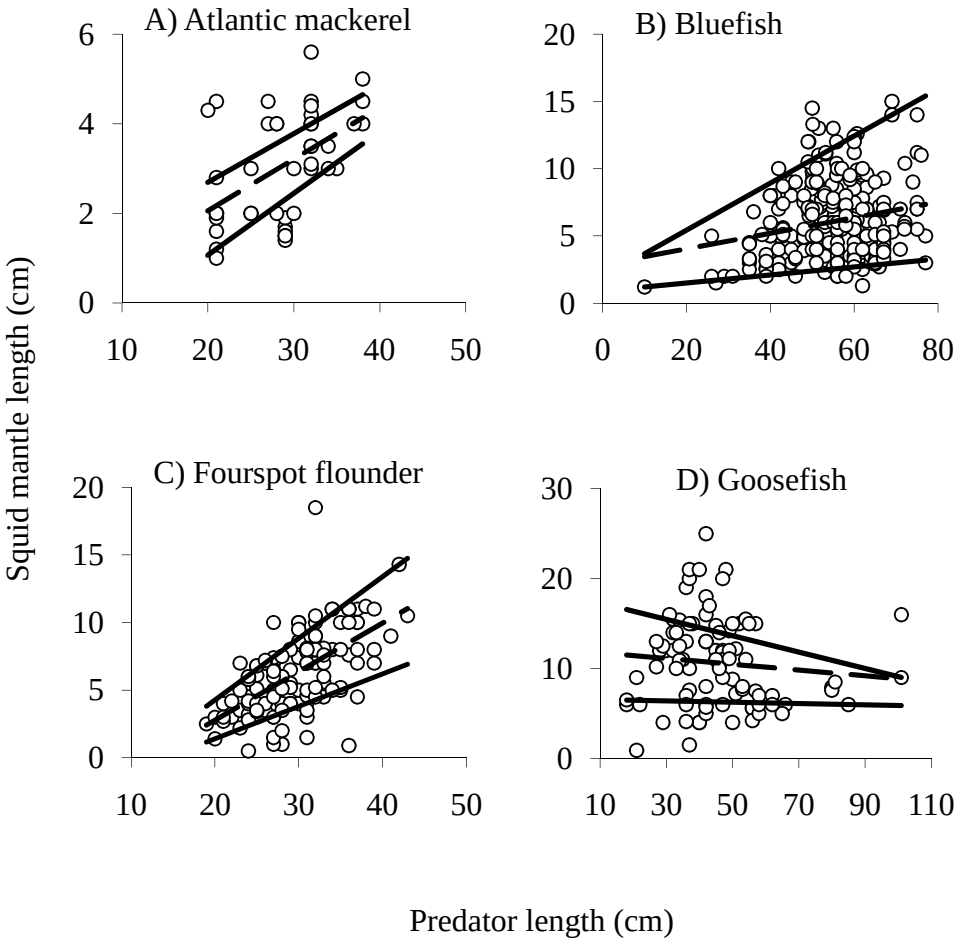


Figure 1.2 continued

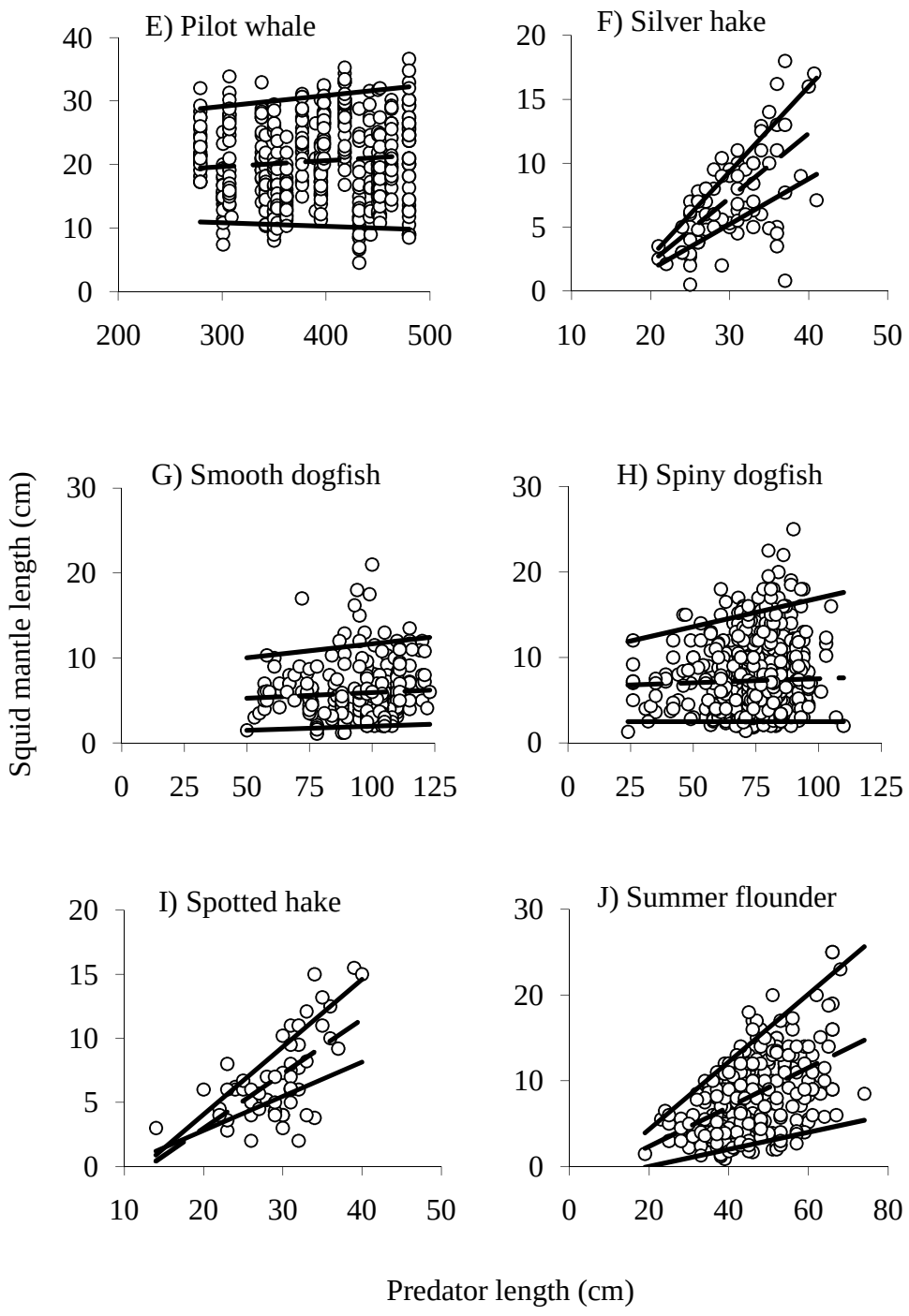
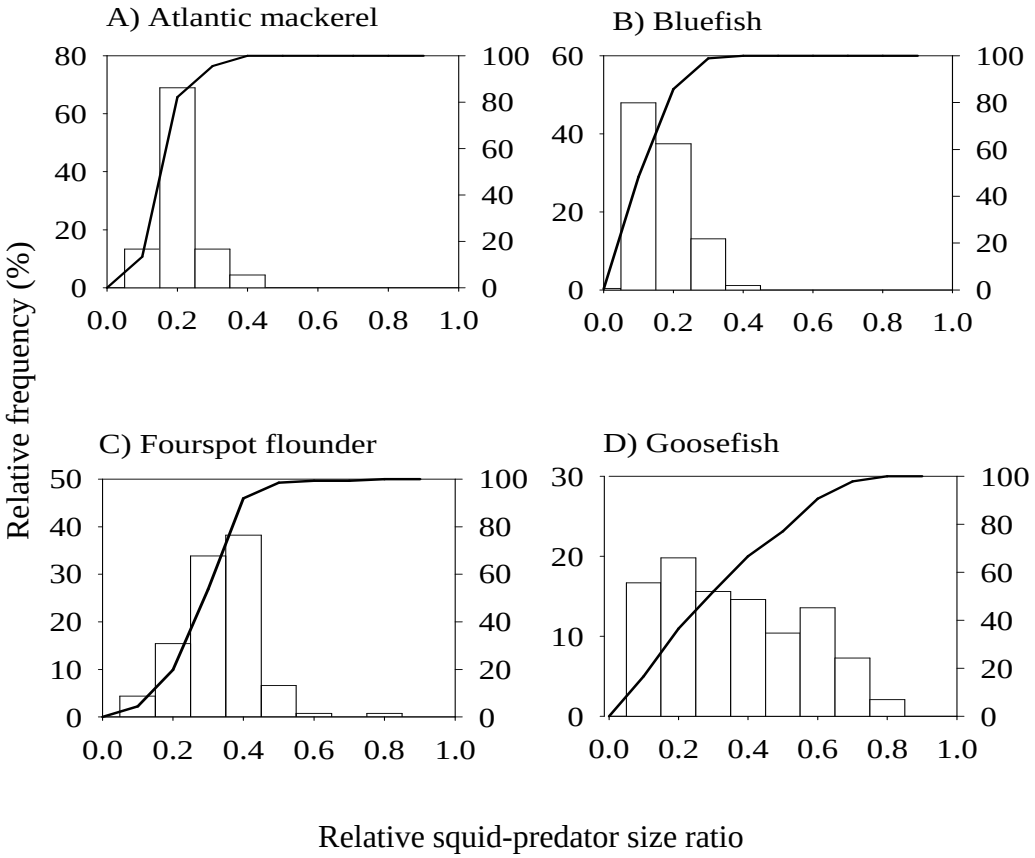


Figure 1.3: Relative and cumulative frequency distributions of relative predator-prey size-ratios for longfin inshore squid and A) Atlantic mackerel B) bluefish C) fourspot flounder D) goosefish E) long-finned pilot whales F) silver hake G) smooth dogfish H) spiny dogfish I) spotted hake and J) summer flounder. Bars indicate relative frequencies by percent. Solid lines indicate cumulative frequencies by percent.



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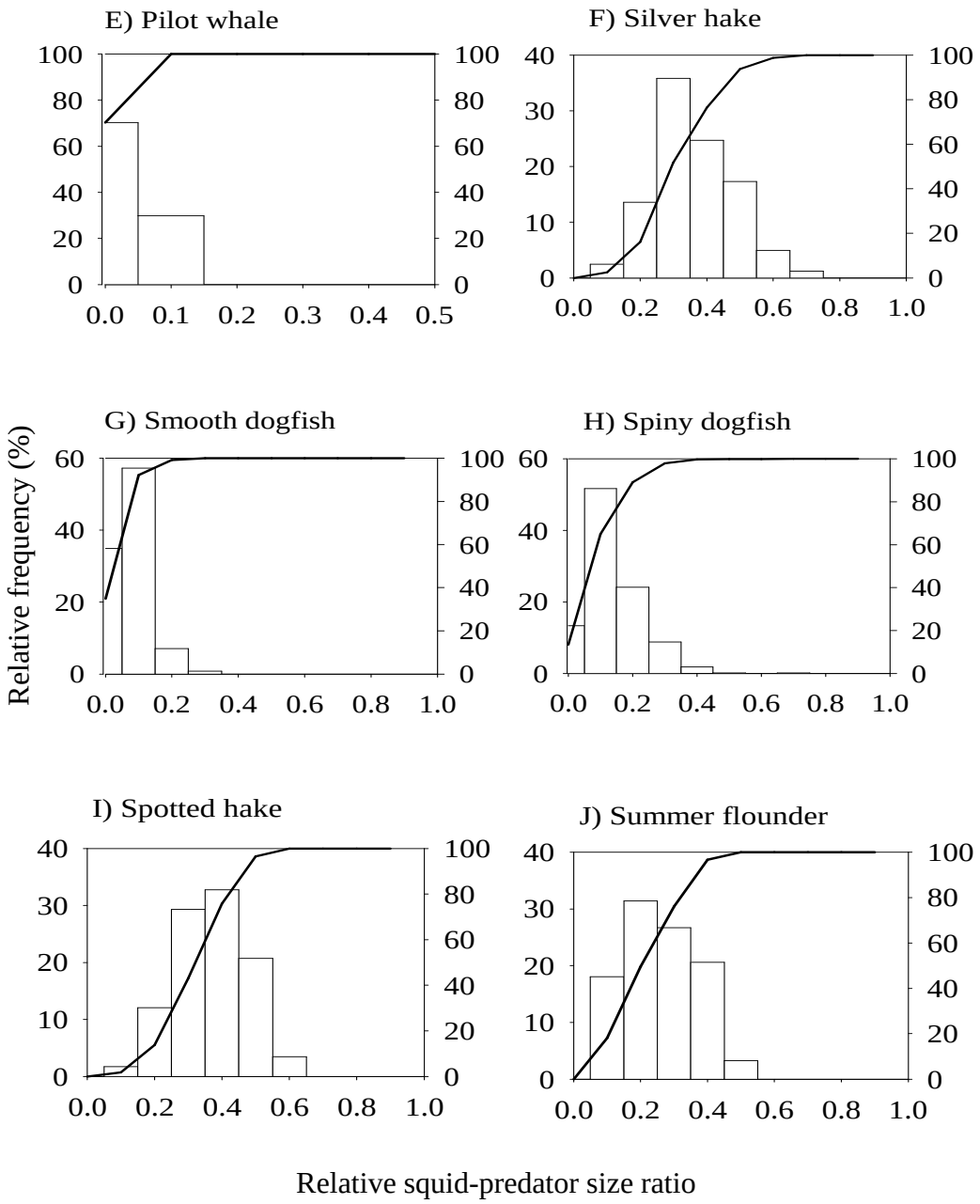
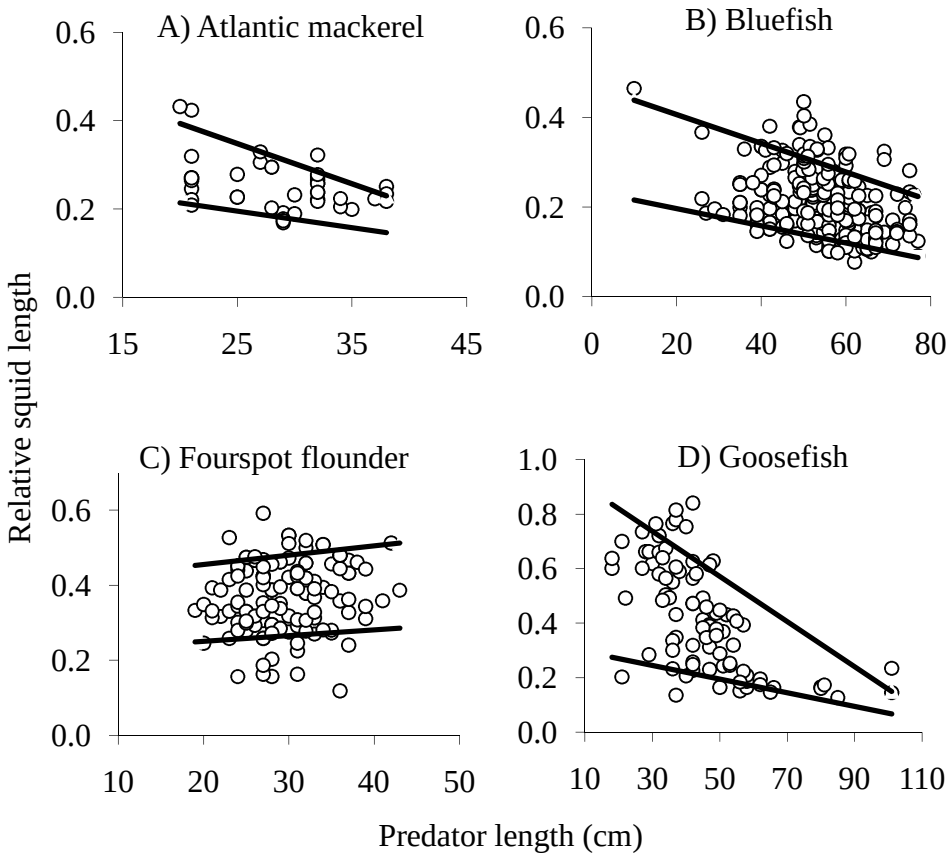


Figure 1.4: Scatter-plots of relative predator-prey size ratios for longfin inshore squid and A) Atlantic mackerel B) bluefish C) fourspot flounder D) goosefish E) long-finned pilot whales F) silver hake G) smooth dogfish H) spiny dogfish I) spotted hake and J) summer flounder. Each (o) represents a single prey consumed by a predator. Lower and upper bound regression lines represent the 10th and 90th quantiles respectively. All line equations are listed in Table 1.3.



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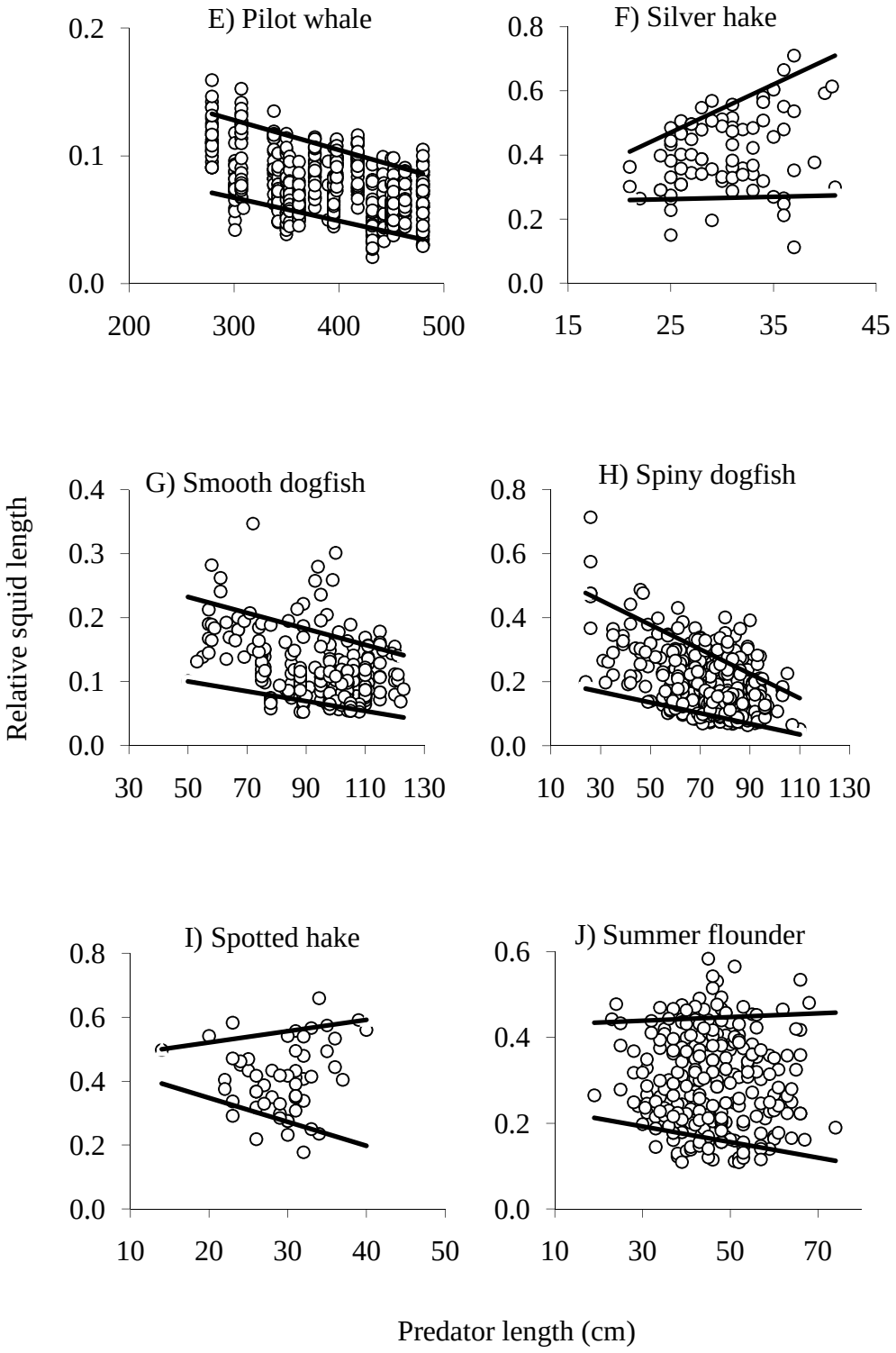
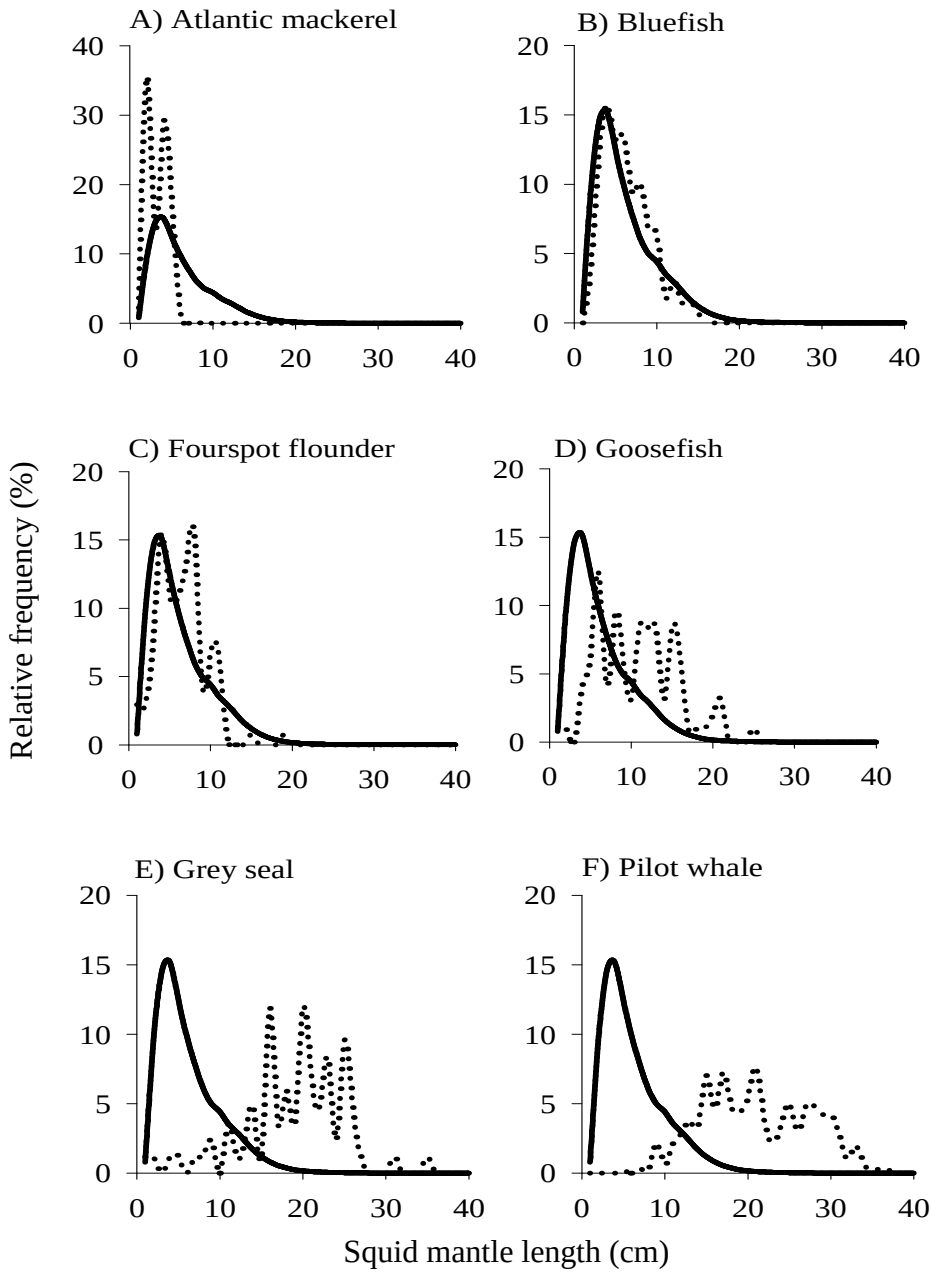


Figure 1.5: Relative frequency distributions of longfin inshore squid mantle lengths available in the environment (solid line) and in the diets (dotted line) of A) Atlantic mackerel B) bluefish C) fourspot flounder D) goosefish E) grey seals F) long-finned pilot whale G) silver hake H) smooth dogfish I) spiny dogfish J) spotted hake and K) summer flounder.



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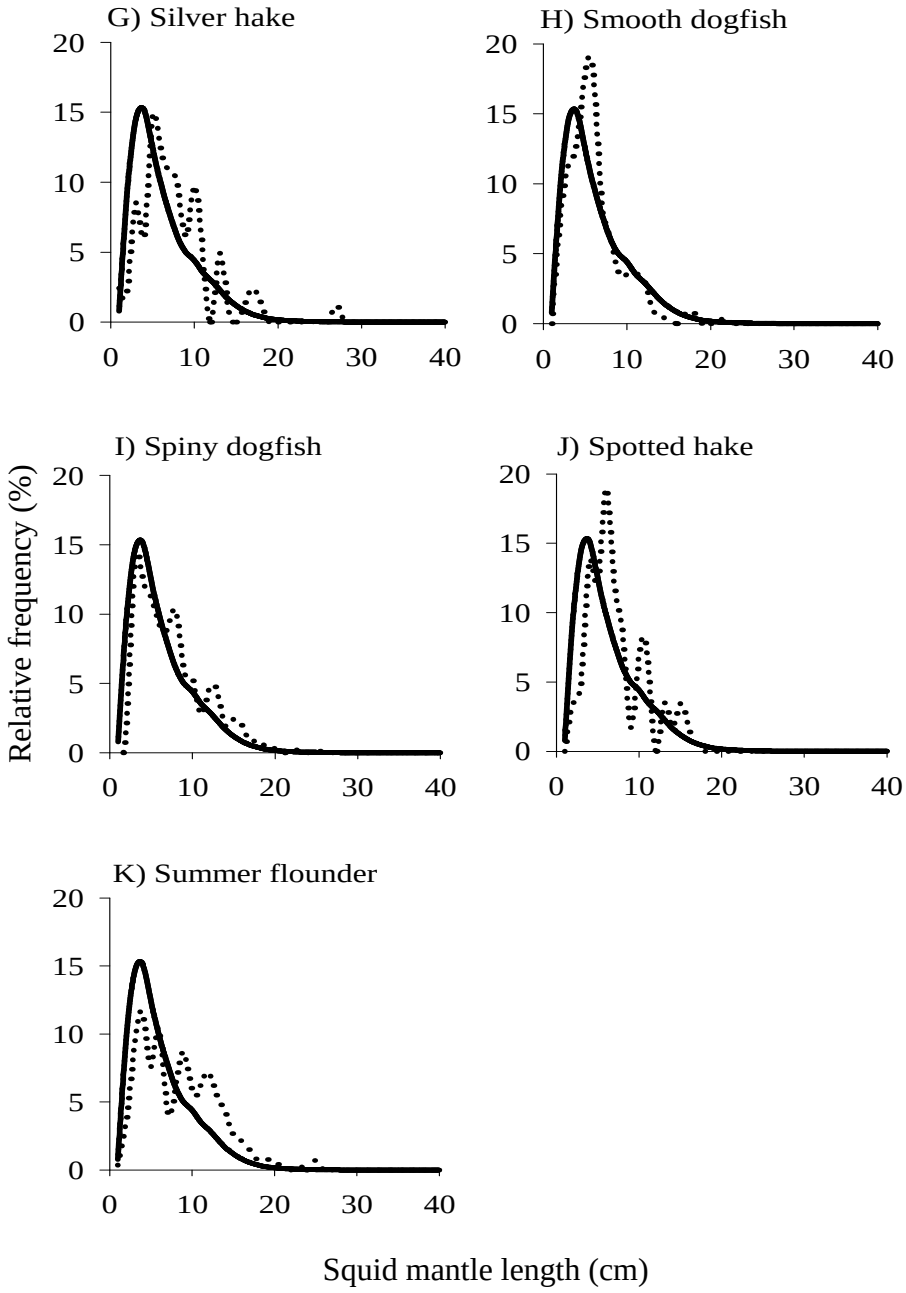
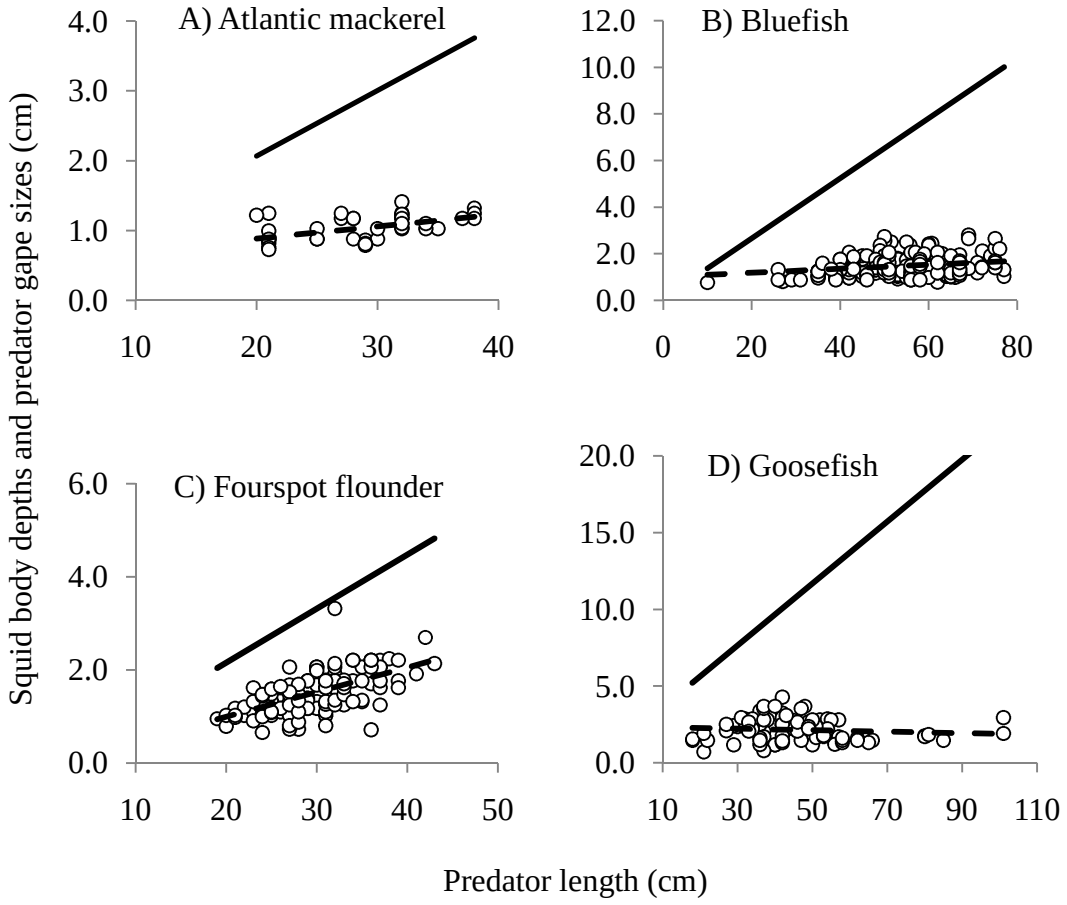


Figure 1.6: Scatter-plots of longfin inshore squid body depth to predator length for A) Atlantic mackerel B) bluefish C) fourspot flounder D) goosefish E) silver hake F) smooth dogfish G) spiny dogfish H) spotted hake I) summer flounder. Each (o) represents a single squid consumed by a predator. All relationships for predator gape width and height are reported in Table 1.4. Solid lines represent the relationship between predator length and predator gape width for all predators except spotted hake. Gape height was found to increase at a lesser rate than width in spotted hake and was therefore plotted as the limiting morphometric gape relationship. Dashed lines indicate linear regressions between predator length and squid body depth.



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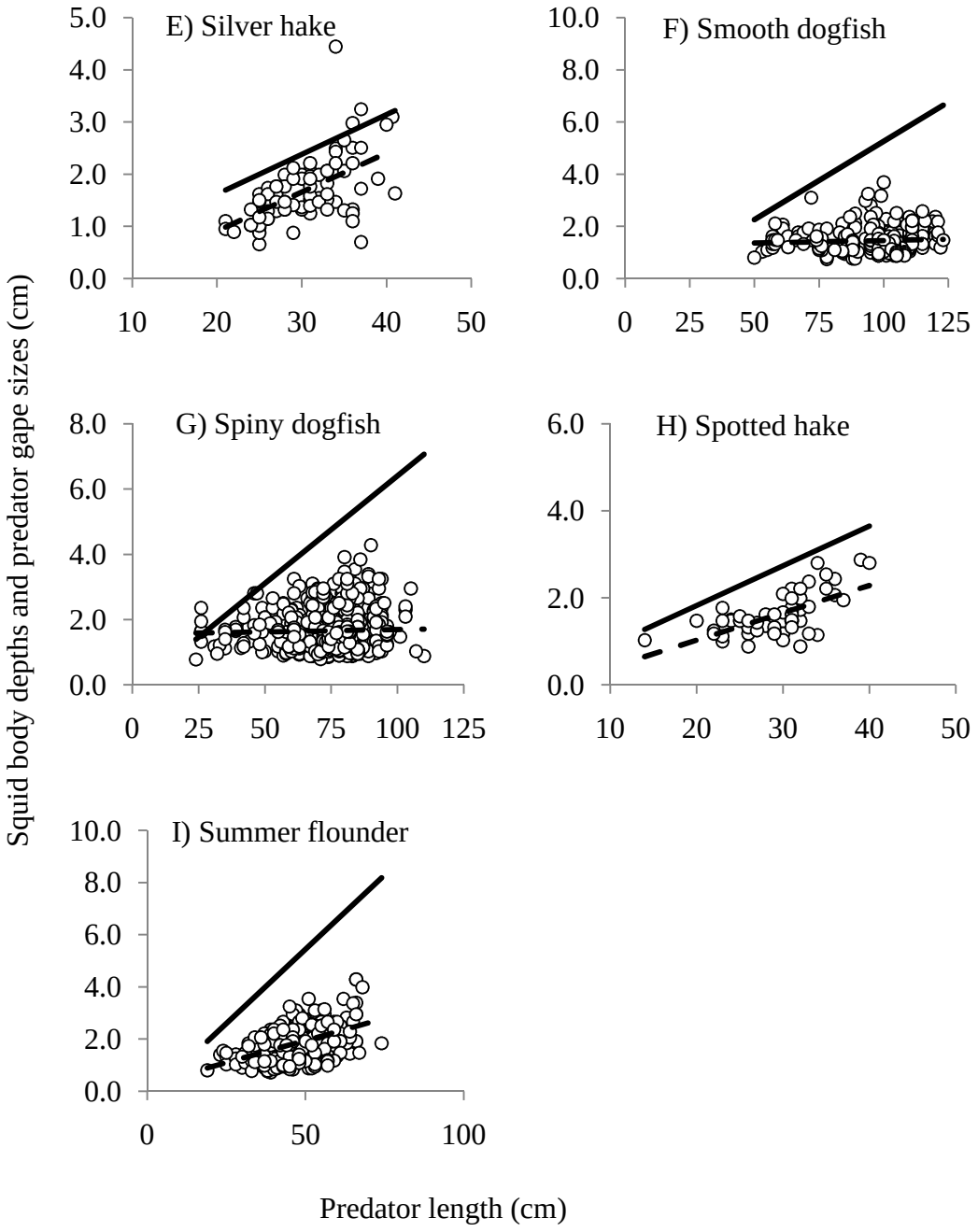


Figure 1.7: Regression lines of the relationship of prey total length and prey body depth for Atlantic herring, butterfish, longfin inshore squid, and sandlance. Squid lengths were measured directly; equations for Atlantic herring, butterfish, and sandlance were adapted from Scharf et al. (1998b).

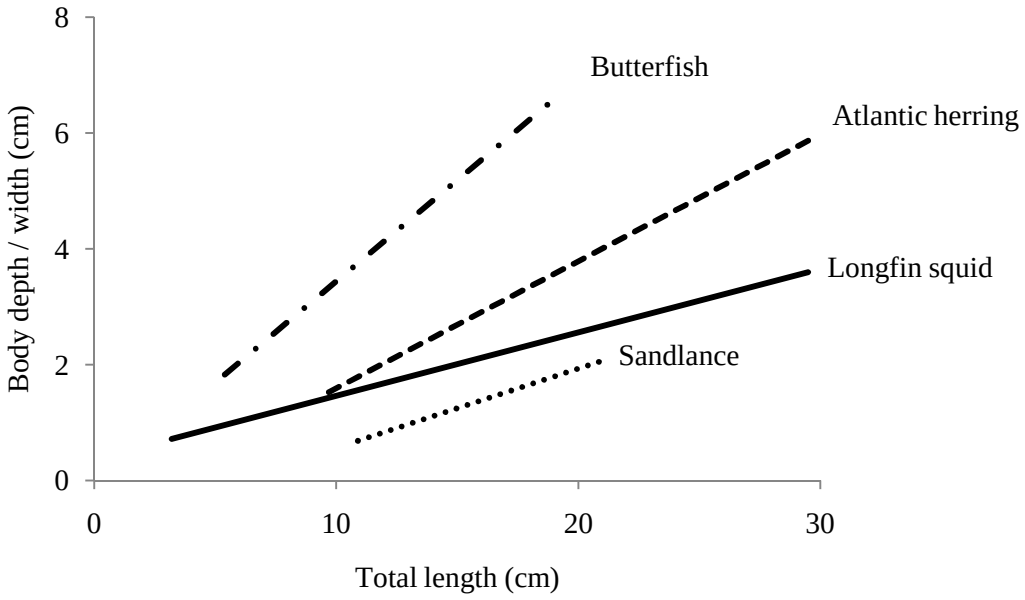


Figure 1.8: Measurements of size-based trophic niche breadths for A) longfin inshore squid and B) prey fish. Box boundaries represent 25th and 75th percentiles, lines within boxes mark the median. Error bars indicate the 90th and 10th percentiles. Circles show outliers in the 5th and 95 percentiles. Values indicate the mean trophic niche breadth of each predator.

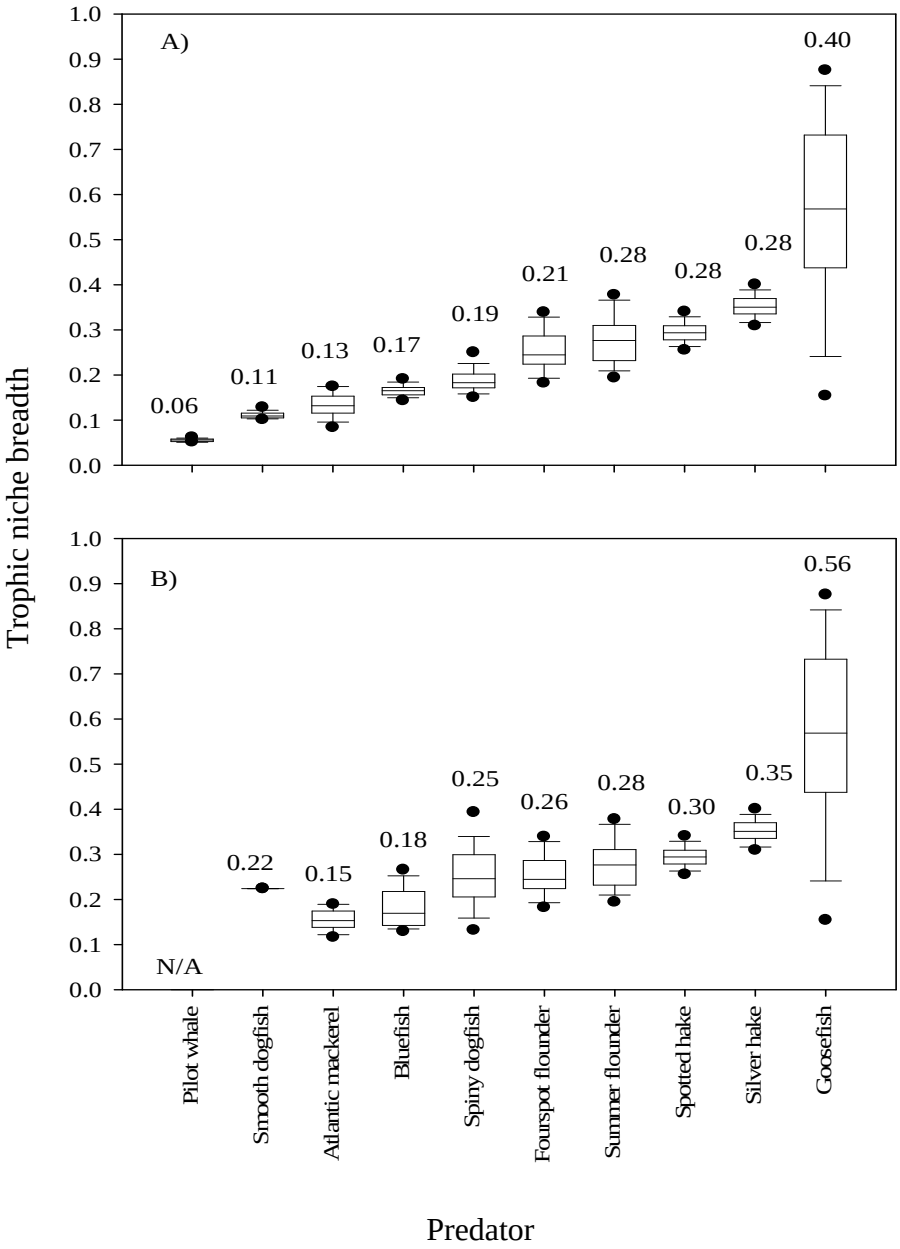
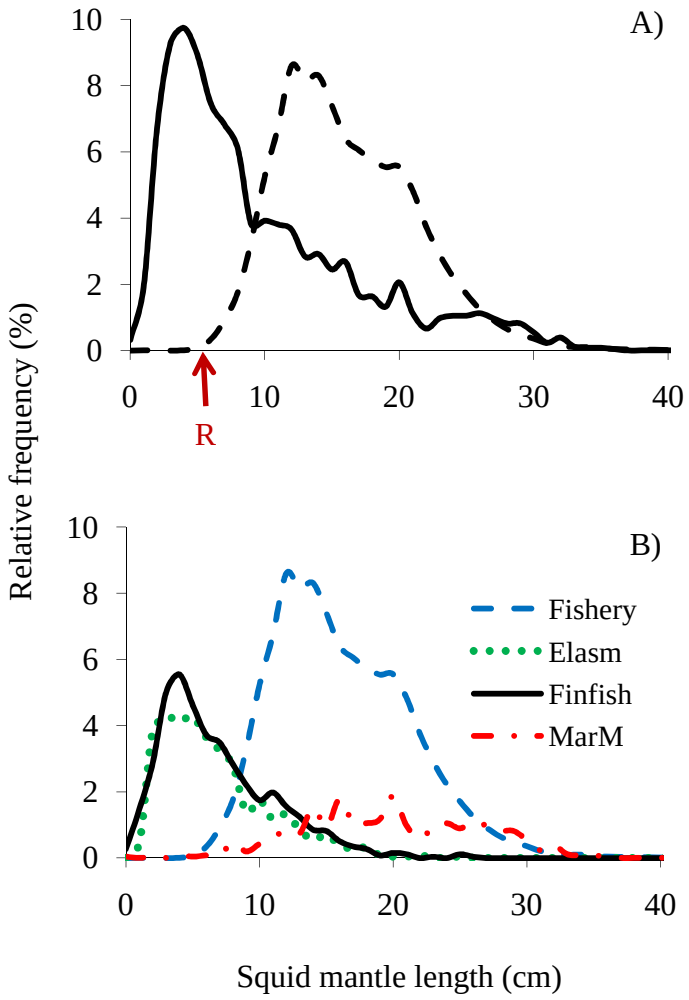


Figure 1.9: Relative frequency distributions of longfin inshore squid consumed by A) all predators (solid lines) and harvested by the commercial fishing industry (dashed lines) and B) by major predator assemblages. Groups analyzed included the commercial fishery (fishery), elasmobranchs (Elasm), finfish (Finfish) and marine mammals (MarM). Arrow and "R" indicate the size when squid (8 cm ML) recruit into the commercial fishery.



CHAPTER 2

FEEDING TACTICS OF A BEHAVIORALLY PLASTIC PREDATOR, SUMMER FLOUNDER (*PARALICHTHYS DENTATUS*)

Abstract

In a series of laboratory experiments, the feeding behaviors of summer flounder (*Paralichthys dentatus*) were examined in response to squid and fish prey. Attack and capture tactics were evaluated for their influence on capture success, handling time, and prey-type selectivity. The ambush tactic was the primary behavior (50.6%) used to attack squid. Secondary attack types included active pursuit (42.7%) and stalking (6.7%). Regardless of the prey species targeted or the type of attack employed, summer flounder were equally efficient in capturing prey; capture success rates ranged from 50%–83%. The majority of prey was swallowed in a headfirst orientation (55.3% of squid), however swallow alignment did not significantly affect handling time. Approach times during ambush attacks were greater overall in comparison to active attacks, and relative prey size significantly affected capture times. Despite additional costs in handling time, summer flounder actively selected for mummichogs (*Fundulus* spp.) (attack rate (attacks per minute)=0.11) over longfin squid (*Loligo pealeii*) (0.08) and Atlantic silversides (*Menidia menidia*) (0.02). Differential attack rates favoring mummichogs suggests a preference towards demersal prey. In the presence of relatively large, fast moving, and pelagic prey, summer flounder used a greater diversity of attack tactics than have been observed previously under controlled conditions. The behavioral plasticity exhibited

by summer flounder is likely mediated by prey behavior and local availability of prey resources in inshore and offshore environments.

Introduction

Factors that influence predatory behavior and hence prey selectivity occur on two scales. On a broad scale, encounter rates between predators and prey are controlled by prey abundance, habitat overlap, and foraging tactics (e.g., lie and wait, active pursuit). At finer scales, behavior is limited by relative body size, detection abilities, defense mechanisms, activity levels, attack and response behaviors (Mittelbach 2002). Although the relationship between predator and prey body size has been cited as the most important factor constraining predation (Cohen et al. 1993; Cowan et al. 1996; Lundvall et al. 1999; Juanes et al. 2002; Dorner and Wagner 2003; Floeter and Temming 2003; Scharf et al. 2003), prey behavior may be equally influential in mediating capture success, handling times, and attack rates (Juanes et al. 2002; Scharf et al. 2003). Consequently, a comprehensive understanding of prey behavior and predator responses to different prey types may be necessary to model foraging behavior and to predict predator diets (Juanes et al. 2002; Mittelbach 2002; Scharf et al. 2003).

Flatfish are important predators in benthic habitats. In coastal and offshore environments of the northwest Atlantic, summer flounder (*Paralichthys dentatus*) are regarded as an ecologically and commercially valuable species. Summer flounder have been described as active, day-time feeders (Olla et al.1972; Stickney et al. 1973; Manderson et al. 2000) and are known to exploit both demersal and pelagic prey types (Olla et al. 1972; Manderson et al. 2000; Link et al. 2002; Staudinger 2006; Latour et al.

2008). Encounter rates with different prey types as well as prey availability vary widely with summer flounder ontogeny and seasonal migration patterns. Estuarine and bay ecosystems are key habitats for juveniles year-round, whereas adults are only present in these areas during the summer and fall (Packer and Hoff 1999). As adults transition between inshore environments and waters of the continental shelf and slope, their food habits shift dramatically from small crustaceans such as shrimp (Powell and Schwartz 1979; Manderson et al. 2000; Latour et al. 2008) to fish and squid (Link et al. 2002; Staudinger 2006).

Previous work has shown that flatfish exhibit a complex array of feeding behaviors that are dependent on both visual and olfactory cues (DeGroot 1971; Holmes and Gibson 1983). However, prey offered to predators under controlled conditions have generally been prepared feeds, worms (Stickney et al. 1973; Gibb 1995; Bels and Davenport 1996), or mysiids and shrimps (Olla et al. 1972; Holmes and Gibson 1983; Bergstrom and Palmer 2007). Although there are exceptions (e.g., (Manderson et al. 2000)), few studies have reported flatfish predatory responses to fish and large invertebrates as prey. Squid in particular have been neglected from predator-prey behavioral studies despite their importance to the diets of flatfish and a variety of other piscivorous predators (Smale 1996; Dawe et al. 1998). Squid and other cephalopods possess an array of defense mechanisms and pose unique behavioral challenges to their predators (Hanlon and Messenger 1996). These behaviors may in turn require or elicit more complex responses by predators to capture and subdue squid in comparison to common fish and crustacean prey.

The purpose of this paper is to describe and quantify attack and capture behaviors used by summer flounder in the presence of large, highly mobile prey. Specifically, I will test summer flounder behavioral responses to longfin inshore squid (*Loligo pealeii*) as prey. In a series of feeding experiments, I evaluate the effects of attack strategy and swallow orientation on handling time and capture success. I also test whether summer flounder exhibit active selection towards squid and two species of prey fish, one demersal and one pelagic.

Methods

Laboratory experiments

Experiments were conducted at the Marine Resources Center of the Marine Biological Laboratory (MBL) (Woods Hole, Massachusetts) between May and September of 2006. Summer flounder ranging in size from 30 - 48 cm total length (TL) were collected by otter-trawl from Buzzards Bay (Massachusetts), transported to the MBL, and held in recirculating seawater tanks for approximately one month prior to use in behavioral trials. Flounder were maintained on a diet of live and frozen fish and squid. Longfin inshore squid to be used as prey in behavioral trials were collected daily by otter-trawl and transported to the MBL in a live-well tank aboard the *RV* Gemma. Atlantic silversides (*Menidia menidia*) and mummichogs (*Fundulus* spp.) were obtained from local bays and estuaries by seining. All prey were acclimated for a minimum of 6 hours prior to use in experiments.

Behavioral trials were conducted in a tank that was 3.1 meters in diameter, 0.8 meters in height, and contained approximately 28,000 liters of filtered and circulating

seawater. The bottom of the experimental tank was lined with a mixture of gravel and sand approximately 2 - 4 cm deep, allowing flounder to behave normally and bury beneath the substrate when resting. Water temperatures ranged from 16 to 20 degrees Celsius and during experiments two 500 watt lights were positioned above the tank to aid with filming clarity. All experiments were conducted during the day-time, generally between 09:00 to 13:00 h.

Prior to the start of each trial, three summer flounder of approximately equal size were introduced into the experimental tank and food was restricted for 24 hours to standardize hunger levels. An opaque polyvinyl chloride cylinder approximately 1.5 meters in diameter and one meter in height was lowered into the experimental tank and fifteen prey were added to the interior partitioned area. Prey were allowed to acclimate to the experimental tank for a minimum of 3 hours prior to the start of a trial. A trial commenced when the partition was raised out of the tank, exposing prey to predators. Trials lasted approximately 30 minutes from the time when the partition was initially raised. All predator-prey interactions were recorded by video cameras mounted at two lateral viewing windows and a third camera mounted above the experimental tank.

Squid-flounder behavioral experiments

In the first set of trials, summer flounder feeding behavior was evaluated exclusively in response to longfin squid as prey. Summer flounder were offered longfin squid ranging in size from 3 – 21 cm dorsal mantle length (DML). Observed feeding behaviors included attack type, swallow orientation, capture success, and handling time,

and were quantified using frame-by-frame analysis of video tape recorded during experimental trials.

Differences in the frequency of total attacks among attack types and swallow orientations were compared using a chi-squared test and executed using the PROC FREQ command in SAS (SAS 2003). When an attack was made by a summer flounder it was classified as either successful or unsuccessful. In a successful attack, a prey was captured and ingested by the attacking predator. In an unsuccessful attack, the prey was not consumed. Capture success was defined as the proportion of successful attacks divided by the total number of attacks made over the course of all trials. Proportions of capture successes among attack types were compared using a modified contingency table (Zar 1984).

Handling times consisted of the following three components: approach, capture, and transport. I defined approach as the time from when a flounder began a lunge sequence and made first contact with a prey. Capture was quantified as the difference in time from the first point of contact with a prey until the time when no part of the prey was visible exterior to the predator's mouth. Transport was measured as the difference in time between the end of the capture sequence and the completion of the last post-manipulation event. All handling time variables were found to be non-normally distributed and were not sufficiently corrected using $\log_{10}$ transformation. A Kruskal Wallis test, the equivalent of a non-parametric ANOVA, was used to contrast differences in handling times among attack types, and swallow orientations with the PROC NPAR1WAY command in SAS (2003).

The effects of relative prey size and swallow orientation were evaluated for their influences on handling time using a two-way ANOVA (SAS 2003). It was expected that capture and transport times would be most affected by these factors since prey manipulation occurs during these two periods; however, due to low samples sizes for transport, only capture times were included in the model. Relative prey size was calculated as the ratio of total squid size (the sum of the lengths of the mantle and arms) (Staudinger et al. 2009) divided by summer flounder total length and grouped into 10% increments (e.g., 0.10 - 0.19, 0.20 - 0.29). Capture times were distributed normally within relative size groups therefore a parametric test was appropriate for this analysis.

Prey-type selectivity

Selective feeding is defined as an observed difference in the distribution of prey types or sizes present in a predator's diet compared to what is in the surrounding environment (Juanes and Conover 1994). Assuming all prey types are equally available, the prey that a predator attacks most is considered "preferred" and the prey that is attacked least is "avoided".

In prey-type selection trials, summer flounder were given a choice of equal numbers of three prey species: longfin inshore squid, Atlantic silversides, and mummichogs. All squid and fish prey were size-matched as closely as possible. No prey used in a single trial exceeded a 0.15 relative size margin from each other and the mean relative size of all prey was 0.25. To determine if summer flounder were feeding randomly or exhibiting preference for certain prey-types, two competing hypotheses were tested. H_0: attack rates on different prey types do not vary; H_1: active choice is evidenced

through differential attack rates and summer flounder exhibit preference for one of the prey types. If the null hypothesis was accepted, selection for different prey-types in summer flounder was considered to be passive. Conversely, if the alternative hypothesis was accepted, selection was considered active (Juanes and Conover 1994; Juanes et al. 2002). In prey-type selection trials, attack rates were measured as the total number of attacks made on each prey species over the total time of all replicate trials, and compared using a chi-squared test. Differences in attack types, capture success rates, and handling times among prey types were assessed using frame-by-frame analyses of video tape recorded during trials and analyzed using a chi-squared test, a modified contingency table, and a Kruskal Wallis test, respectively, as described above.

Results

A total of 39 trials were conducted and 121 attacks were observed overall. Predator-prey interactions between summer flounder and longfin squid were assessed in 34 trials. Selectivity and feeding behaviors towards multiple prey-types were evaluated in an additional five replicate trials.

Behavior: attack types

Three attack types were exhibited by summer flounder and described as either ambush, active, or intermediate. Summer flounder also displayed a mock-attack behavior.

Summer flounder initiated ambush attacks from a stationary position either buried beneath or resting on top of the substrate. Prior to making an ambush attack, summer

flounder often appeared alert and displayed a raised pectoral fin, pointed vertically, or with its head lifted at an angle from its body above the substrate. When an ambush attack was started from a resting position on top of the sediment, summer flounder were observed to deeply arch their body and brace themselves against the substrate using their anal and dorsal fins. Fin contact with the bottom likely allowed flounder to push off and gain additional momentum when attacking an approaching prey.

Summer flounder were observed to make active attacks after energetically swimming in the water column. Active attacks were also observed after summer flounder glided either laterally, upwards or downwards through the water column. Often when a flounder made an active attack it paused just prior to making a dramatic thrust with its caudal fin and increased its speed as a strike was initiated.

Intermediate attacks were characterized by small movements that advanced a flounder towards a targeted prey while supporting themselves on their anal and dorsal fins. Summer flounder always remained in contact with the substrate prior to completing an intermediate attack. Movements along the bottom could be slow or rapid. Summer flounder were also observed to swivel and change directions during an intermediate approach. A summer flounder executing an intermediate attack might pause for several seconds before completing a strike sequence. The primary difference between an intermediate attack and an active attack was that flounder remained in contact with the substrate and did not swim upwards into the water column.

On multiple occasions, summer flounder demonstrated mock-attacks towards prey. This behavior did not qualify as an actual attack because summer flounder did not execute a definitive strike with an open mouth indicating a true attempt to bite and

consume prey. Flounder exhibiting mock-attacks recognized, oriented, and swam towards prey, but swimming speeds decreased rather than increased when flounder came in close proximity to a prey. Mock-attacks were displayed by flounder either actively swimming through the water column or in direct contact with the substrate, approaches similar to active and intermediate attacks, respectively. The majority of mock-attacks were observed by summer flounder approaching one or more prey at the water's surface. During these encounters, flounder swam upwards through the water column towards prey and then glided into the cluster of prey. When longfin squid were approached in this manner, they inked and jetted erratically in response, and prey fish darted in at least one random direction before actively swimming away from the approaching flounder. Mock-attacks were also made on longfin squid resting on the bottom. Summer flounder initially displayed stalking behaviors, approaching prey while remaining in contact with the substrate with their anal and dorsal fins, and then increased its speed of approach. If an approaching flounder was detected, prey fled upwards into the water column and in the opposite direction of the oncoming predator. Flounder then glided towards where the prey had been and either came to rest on the substrate or continued to swim actively along the bottom or in the water column.

Behavior: handling times

Approaches on prey were made either from the substrate or while swimming in the water column depending on the attack type employed. The capture portion of the ingestion cycle included biting or chewing of prey or suction of prey into the buccal cavity. All prey were swallowed whole. Relatively large prey were manipulated intact,

but forced down the esophagus using repeated buccal and opercular pumping. During transport, prey passed from the buccal cavity through the pharyngeal cavity, the esophagus, and eventually the stomach. Post-manipulation behaviors were characterized by physical movements made by a flounder including jaw protrusions, buccal and opercular pumping, and forced expulsion of debris (e.g., ink) through the opercular cavity. This final portion of the ingestion cycle varied radically in duration with relative prey size and prey alignment as it was swallowed. Summer flounder were observed to clear their pharyngeal and opercular cavities following the successful capture of a prey for up to 4 minutes after the capture cycle was completed. When the frequency of post-manipulation behaviors exceeded one minute between events, the primary period of the transport cycle was considered to have ended.

Behavior: swallow orientation

Summer flounder oriented towards and swallowed prey in three positions; anterior, posterior, and perpendicular. Prey swallowed in the anterior position were ingested headfirst, and tailfirst when swallowed in the posterior position. Capture of prey in the perpendicular orientation was discerned by an initial bite that was oriented somewhere along the length of the body of the prey. The perpendicular orientation was only observed for large prey (> 0.30 relative body size) and usually required the flounder to reposition the prey into either an anterior (33%) or posterior (44%) orientation before completion of the capture cycle. Reorientation of a prey was often preceded by violent thrashing of the prey and even striking the prey against the substrate, perhaps to stun the prey while the flounder manipulated it into a more manageable position for ingestion.

Attack behavior, capture success, and handling times

Out of the 89 attacks observed in behavioral trials using longfin squid as prey, 50.6% of all attacks were classified as ambush attacks (Table 2.1). The ambush or "lie-and-wait" tactic was therefore the primary tactic used by summer flounder when feeding on longfin squid (X^2 = 30.30, df = 2, $p < 0.0001$). Active attacks were the second most frequent attack type exhibited by summer flounder and were observed in 42.7% of all attacks. Intermediate attacks were observed least often, comprising only 6.7% of all attacks made.

Although summer flounder were found to alternate between ambush and active attacks, and to a lesser extent intermediate attacks, significant differences were not detected among capture success rates resulting from each attack type (X^2 = 0.15, df = 2, $p > 0.05$) (Table 2.1). Summer flounder were therefore equally effective in capturing longfin squid regardless of the tactic employed.

The three handling time components, approach, capture, and transport were contrasted among attack types (Figure 2.1). Intermediate attacks were excluded from this analysis due to small sample sizes ($n \leq 3$). The only handling time component found to differ between ambush and active attack types was approach (X^2 = 6.69, df = 2, p = 0.04). Ambush and active approach times ranged from 0.07 – 0.33 seconds (median = 0.13 seconds) and 0.03 – 0.30 seconds (median = 0.10 seconds), respectively. Capture (X^2 = 2.11, df = 2, $p > 0.05$), and transport (X^2 = 2.70, df = 2, $p > 0.05$) handling times were not significantly influenced by attack type.

Summer flounder predominantly attacked and swallowed squid in the anterior position (X^2 = 8.54, df = 2, p = 0.014) (Table 2.2). Of the 38 attacks where swallow

position was discernible, 55.3% of squid were swallowed headfirst. Longfin squid swallowed in the posterior and perpendicular orientations were observed less often and in nearly equal frequencies, 23.7% and 21.1% respectively. None of the handling time components were found to vary significantly among swallow orientations (Figure 2.2).

Relative prey-size predator-size ratios of longfin squid and summer flounder ranged from 0.20 – 0.50. The effects of swallow orientation and relative prey size were analyzed for 29 attacks. Capture times were significantly impacted by relative prey size but not by swallow orientation (Table 2.3); the interaction between dependent variables was also not significant ($p = 0.57$). Overall, capture times increased with increasing relative squid size ($R^2 = 0.52$, $p < 0.0001$) (Figure 2.3). The smallest capture time (0.10 seconds) was observed for a squid 0.26 relative size during an anterior swallow orientation. The longest capture period (42.43 seconds) was observed for a 0.51 relative sized squid during a posterior swallow alignment.

Prey-type selection

A total of 31 attacks were observed during the 5 replicate prey-type selection trials. Attack rates among prey-types varied significantly ($X^2 = 8.57$, df = 2, $p = 0.01$) indicating summer flounder used active selection when choosing among longfin squid, mummichogs, and Atlantic silversides as prey. Summer flounder exhibited preference for mummichogs, the demersal prey fish, and avoidance towards Atlantic silversides, the pelagic prey fish (Table 2.4). Attack rates on longfin squid (0.08) were higher than attack rates on Atlantic silversides (0.02) but slightly lower than were observed for mummichogs (0.11) (Figure 2.4A). Although differences in attack rates among prey

types were detected, capture success rates were found to be comparable across all prey-types (X^2 = 0.02, df = 2, p > 0.05). Capture success rates ranged from 83% when attacking longfin squid to 67% when foraging on Atlantic silversides (Figure 2.4B).

In prey-type selection trials, summer flounder used all three attack types interchangeably when attacking longfin squid (X^2 = 0.0018, df = 2, p = 1.0) and mummichogs (X^2 = 3.69, df = 2, p = 0.16) (Figure 2.5). Only three attacks were observed on Atlantic silversides, an ambush attack which was not successful, an intermediate attack which did result in ingestion, and a third attack where the approach was out of view and could not be classified. Approach and capture times were equivalent among all prey-types (all $p \geq 0.05$). Alternatively, transport times were an order of magnitude higher for mummichogs (median = 14.9 seconds) in comparison to longfin squid (median = 0.83 seconds) (p = 0.037); no post-manipulation behaviors were observed when Atlantic silversides were consumed (Figure 2.6).

Discussion

Attack behavior

Summer flounder exhibited several different types of attacks ranging from lie-and-wait to active pursuit when feeding on longfin squid, mummichogs, and Atlantic silversides. Ambush attacks were the primary tactic used by summer flounder to capture longfin squid and fish prey. Similar to other species of flatfish, summer flounder spend the majority of their lives on or near the bottom and possess morphological characteristics that are well suited for a lie-and-wait predator. Therefore, it should not be surprising that ambush attacks were displayed most frequently; however, lie-and-wait

attacks have rarely been observed in summer flounder in the laboratory. Previous studies reported intermediate attacks (e.g., crawling and stalking) as the principal tactic employed to capture demersal prey such as winter flounder (*Pseudopleuronectes americanus*), Atlantic silversides, sand shrimp (*Crangon septemspinosa*), and grass shrimp (*Palaemonetus vulgaris*) (Olla et al. 1972; Manderson et al. 2000). This is in contrast to the present study where intermediate attacks were only observed in 6.7% of attacks made on longfin squid and 25% of attacks made on prey fish. Manderson et al. (2000) hypothesized that the lie-and-wait tactic would be more effective for capturing pelagic prey in certain habitats. I found that capture success rates did not differ among the three attack types suggesting that although summer flounder modify attack behavior in response to prey type and behavior, the tactic chosen does not influence efficiency.

Anti-predator displays exhibited by prey in response to mock attacks indicated summer flounder demonstrated some form of predatory intent and that a threat was apparent. I suggest that mock-attacks were used by summer flounder to investigate potential prey (similar to predator inspection by prey (see (Krause et al. 2002))), or to scatter a group of prey. Another possibility is that if prey detected and reacted to summer flounder at distances too great for a flounder to execute an effective attack sequence, a directed attack was abandoned.

Handling times

In behavioral trials using longfin squid as prey, approach was the only handling time component found to differ significantly among attack types. When a summer flounder used ambush attacks, the distance covered during the approach sequence was

entirely contingent on the height in the water column at which a prey was swimming. Conversely, when summer flounder utilized intermediate and active attacks, the distance at which an attack was initiated was largely controlled by the pursuing flounder. Active attacks made in the water column appeared to enable summer flounder to get closer to longfin squid in comparison to ambush attacks, and likely resulted in shorter approach times.

Stephens and Krebs (1986) define handling time as the pursuit, capture, and consumption of a prey item. Depending on the question addressed, how handling time is quantified may vary substantially among studies and is largely subjective depending on how long an individual fish is observed and the range of species specific post-ingestion behaviors displayed. Of the three components measured in the present study, the capture period was the most reliably measured and the most translatable among studies and species.

Bels and Davenport (1996) suggested extending the handling time cycle to include post buccal cavity manipulation behaviors in plaice (*Pleuronectes platessa*) and dab (*Limanda limanda*) since food not completely cleared from the opercular and orobranchial cavities could potentially impede other activities such as respiration. Because visual confirmation of what was occurring internally was not possible, transport was the handling time component measured with the greatest amount of uncertainty. Although transport was likely underestimated, this component was considered an important aspect of the handling time process since no summer flounder were observed to initiate a new search or attack sequence or transition into a resting position (e.g., burying beneath the substrate) while exhibiting post-manipulation behaviors.

Swallow orientation

The orientation of prey capture has been found to vary widely among predator-prey species combinations (Reimchen 1991; Juanes and Conover 1994; L'Abee-Lund et al. 1996; Ellis and Gibson 1997; Vehanen et al. 1998). Headfirst swallow orientations have been associated with predators that utilize ambush attack strategies while tailfirst alignments are more characteristic of predators that actively chase their prey (Juanes and Conover 1994). When making both ambush and active attacks, summer flounder displayed an Ω-shaped body position prior to initiating an approach sequence and rarely made repetitive attempts to capture missed prey. This style of predation is characteristic of a lunger as opposed to a pursuer (Hunter 1984). Although squid regularly swim backwards (leading tailfirst), ambush attacks yielded capture in the anterior swallow orientation more frequently in comparison to other alignments. During active attacks made on squid swimming at the surface, squid faced towards an attacking flounder and displayed anti-predator arm postures (e.g., v-curl) and body patterns (e.g., deep red coloration) (Hanlon and Messenger 1996). When these anti-predator displays failed to deter an approaching flounder, squid were more often captured by summer flounder in the headfirst orientation. If a fleeing squid was pursued, swimming backwards would orient the squid's anterior end toward its attacker and make it more susceptible to headfirst capture.

Swallowing prey fish in the headfirst orientation is thought to minimize handling times and abrasion caused by morphological traits such as spines, opercula, or fin rays (Reimchen 1991; LAbeeLund et al. 1996). In gape limited predators, swallowing large prey in orientations other than headfirst alignment has the potential to impact normal

respiration, or even choke a predator (Bels and Davenport 1996). Squid possess physical characteristics such as beaks, arms, and suckers which pose an unusual challenge to their attackers (Hanlon and Messenger 1996). When captured in the posterior or perpendicular orientations, occasionally longfin squid would wrap their arms around the operculum and head (blind and eyed sides) of summer flounder. During these attacks, in an attempt to release a retaliating squid's grasp, summer flounder were observed to thrash and even strike squid against the substrate. I had expected that perpendicular and posterior attacks would result in longer handling times, however the small sample sizes ($n < 10$) were likely responsible for the inability to detect significant effects on handling times among the three swallow orientations.

In studies examining feeding behavior in cutthroat trout (*Oncorhynchus clarki*) and brown trout (*Salmo trutta*), headfirst swallow orientation was beneficial in minimizing escape, handling time, and swallowing success (Reimchen 1991; LAbeeLund et al. 1996). These trends were more prominent when prey size increased and approached maximum predator gape sizes. Gape sizes in summer flounder are large relative to squid body depth and width; consequently, summer flounder are not thought to be gape limited when feeding on squid (Chapter 1). Alternatively, squid's behavioral defenses may become limiting to summer flounder regardless of swallow orientation at larger relative sizes (>0.40) as evidenced by sharply increasing handling times. Longfin squid captured in the perpendicular orientation were generally larger (> 0.30 relative prey size) than squid captured in anterior and posterior alignments. This difference indicates that larger squid may be more difficult to pursue and orient towards when attacked due to higher swimming speeds and escape velocities (Ellis and Gibson 1997). There were only

two occurrences (4% of all successful attacks) of squid escaping summer flounder once they were captured; once from a perpendicular alignment and once from a posterior alignment. This post capture escape loss is comparatively lower than has been reported (9% escapement) for other piscivorous predators (Reimchen 1991; Scharf et al. 2003).

Prey-type selection

In the field, encounter rates with demersal prey are likely to be higher in comparison to pelagic prey, and may explain summer flounder preference for demersal prey types in the present study (mummichogs) and previously (Manderson et al. 2000). Regardless of the prey type chosen, summer flounder were consistently efficient and highly successful (all capture success rates > 65%) in obtaining all prey types. Relative prey sizes of squid and fish used in behavioral experiments correspond to the most common sizes found in summer flounder diets in the northwest Atlantic ecosystem (Chapter 1). When feeding on fish and squid, all three attack types were used interchangeably indicating summer flounder could adapt their behavior opportunistically. The only limitation identified when feeding on the different prey types was in the transport component of handling times. However, no post-manipulation behaviors were observed when summer flounder fed on Atlantic silversides. Conversely, transport was an order of magnitude higher for mummichogs in comparison to longfin squid. Summer flounder actively chose mummichogs over other prey types despite the additional cost in handling time. This result suggests that some aspect of prey behavior must have been influential in shaping summer flounder feeding preferences. For example, differential prey reaction distances and activity levels explained a large fraction of the variation in

susceptibility to predation of various forage fishes when attacked by bluefish (*Pomatomus saltatrix*) and striped bass (*Morone saxatilis*) (Scharf et al. 2003).

Summer flounder foraging ecology

Throughout their range in waters along the eastern coast of North America, summer flounder are known to exhibit major shifts in diet with ontogeny (Witting et al. 2004), over regional scales (Rountree and Able 1992; Latour et al. 2008), and between inshore and offshore habitats (Link et al. 2002; Staudinger 2006). For example, juvenile and adult summer flounder in the Chesapeake Bay primarily consume mysiid shrimp (*Neomysis* spp.) and bay anchovy (*Anchoa mitchilli*) (Latour et al. 2008), while in the Navesink River in New Jersey, sand shrimp and winter flounder are the dominant prey (Manderson et al. 2000). Alternatively, adult summer flounder found in waters on the continental shelf and slope are primarily piscivorous and teuthopagous; major prey species include clupeiformes, sand lance (*Ammodytes americanus)*, butterfish (*Peprilus triacanthus*), and longfin squid (Link et al. 2002; Staudinger 2006).

The diversity of feeding behaviors observed in the present study suggests that in addition to being opportunistic in what they eat, summer flounder are also flexible in changing how they capture prey. Based on previous studies, summer flounder use intermediate attack behaviors such as crawling, stalking, and shambling when feeding on shrimps, mysiids, and other demersal prey (Olla et al. 1972; Manderson et al. 2000). As shown here, larger, fast-moving, and pelagic prey types may require more complex behaviors including surprise attacks.

Summer flounder appear to alternate between ambush, active, and intermediate attack tactics without compromising efficiency. However in the field, capture success rates will likely differ from observations made under ideal conditions in the laboratory. Factors such as light intensity, turbidity, and the structural complexity of the surrounding environment (e.g., vegetation) can impede visual location of prey (Lindholm et al. 1999; Manderson et al. 2000). As a consequence, the distance at which a summer flounder can detect an approaching prey will be reduced, pursuit times will be higher, and there will be a higher occurrence of failed attacks (Ellis and Gibson 1997).

Adult summer flounder may reserve intermediate attack behaviors for predating on demersal prey, and intermediate strategies may only be used during the times of year when their diets are focused on small crustaceans (e.g., in estuarine habitats). Intermediate attack types may be more characteristic of juvenile foraging behavior for two reasons; first, juveniles are confined to inland habitats where shrimps and mysiids comprise the majority of their diets year-round. Secondly, intermediate attacks allow summer flounder to remain camouflaged against the substrate and capture prey using subtle movements, thereby reducing their risk of being detected by predators. Larger, adult flounder are less vulnerable to predation which may enable them to take greater risks and pursue prey in the water column. Longer handling times and higher frequencies of post-manipulation behaviors associated with larger prey may also influence the types and sizes of prey preferred by summer flounder.

Conclusions

Flatfish exhibit a wide range of tactics to hunt and capture prey, and feeding behavior is adapted to the behavior of their prey (Holmes and Gibson 1983). It is likely that because the food habits of summer flounder encompass such a diversity of prey types, the breadth of their behavioral capabilities is also quite varied. Summer flounder have the flexibility to alternate among approaches that are fast and agile to pursue mobile prey; patient and surprising, to ambush a passing pelagic prey, or stealth, to creep up on slow-moving or benthic prey. Although the lie-and-wait tactic was the most commonly employed, summer flounder also exhibited active and intermediate attacks to capture longfin squid and fish without compromising efficiency. Since few studies have presented flatfish with large, highly-mobile prey, and to the best of our knowledge this is the first evaluation of predator-prey behavioral interactions using squid as prey, it is uncertain if the behavioral plasticity observed by summer flounder in response to these prey types are characteristic of other piscivorous flatfishes (e.g., Bothiae, Pleuronectidae, and Psettodidae).

TABLES

Table 2.1: Statistical results from behavioral trials using longfin squid as prey. $N_{Successful}$, $N_{Unsuccessful}$, and $Total_{Attacks}$ are the frequencies of successful, unsuccessful, and total number of attacks made by summer flounder. Capture success is the percentage of successful attacks made using each of the three attack types. Frequencies of each attack type behavior were compared using a chi-squared test. Differences in the proportions of capture successes among attack types were compared using a modified contingency table.

Attack Type	$N_{Successful}$	$N_{Unsuccessful}$	$Total_{Attacks}$	X^2	*df*	*p*-value
Ambush	31	14	45	30.30	2	< 0.0001
Active	20	18	38			
Intermediate	3	3	6			
Total	54	35	89			

Attack Type	Capture success (%)	X^2	df	*p*-value
Ambush	68.9	0.15	2	> 0.05
Active	52.6			
Intermediate	50.0			

Table 2.2: Frequencies of the positions in which longfin squid were attacked and swallowed by summer flounder during behavioral trials. Differences were tested using a chi-squared test.

Swallow orientation	$N_{Observed}$	% Observed	X^2	*df*	*p*-value
Anterior	21	55.3	8.54	2	0.014
Posterior	9	23.7			
Perpendicular	8	21.1			
Total	38				

Table 2.3: Results of a two-way ANOVA testing the effects of the dependent variables, swallow orientation and relative prey size, on capture time. Relative prey size was calculated as the total length of each longfin squid consumed, divided by the total length of the attacking summer flounder. Capture time was measured in seconds. *df* = degrees of freedom, *SS* = Sum of Squares, *MS* = Mean Square.

Dependent variable	*df*	*SS*	*MS*	*F-value*	*p-value*
Swallow orientation	2	9.6	4.8	0.05	0.95
Relative prey size	3	2069.0	689.7	6.75	0.003
Swallow orientation*relative prey size (interaction)	3	209.2	69.7	0.68	0.57

Table 2.4: Attack results of prey-type selection trials. $N_{Successful}$, $N_{Unsuccessful}$, and $Total_{Attacks}$ are the frequencies of successful, unsuccessful, and total number of attacks made by summer flounder, respectively. Differences in attack frequencies over the course of all trials were tested using a chi-squared test.

Prey type	$N_{Successful}$	$N_{Unsuccessful}$	$Total_{Attacks}$	X^2	*df*	*p*-value
Mummichogs	12	4	16	8.57	2	0.01
Longfin squid	10	2	12			
Atlantic silversides	2	1	3			
Total	24	7	31			

FIGURES

Figure 2.1: Handling times of ambush and active attacks made by summer flounder on longfin squid. Intermediate attack types were excluded from analyses due to low sample sizes. Measurements of A) approach B) capture and C) transport are displayed in seconds. Box boundaries represent 25th and 75th percentiles, lines within boxes mark the median. Error bars indicate the 90th and 10th percentiles. Circles show outliers in the 5th and 95 percentiles.

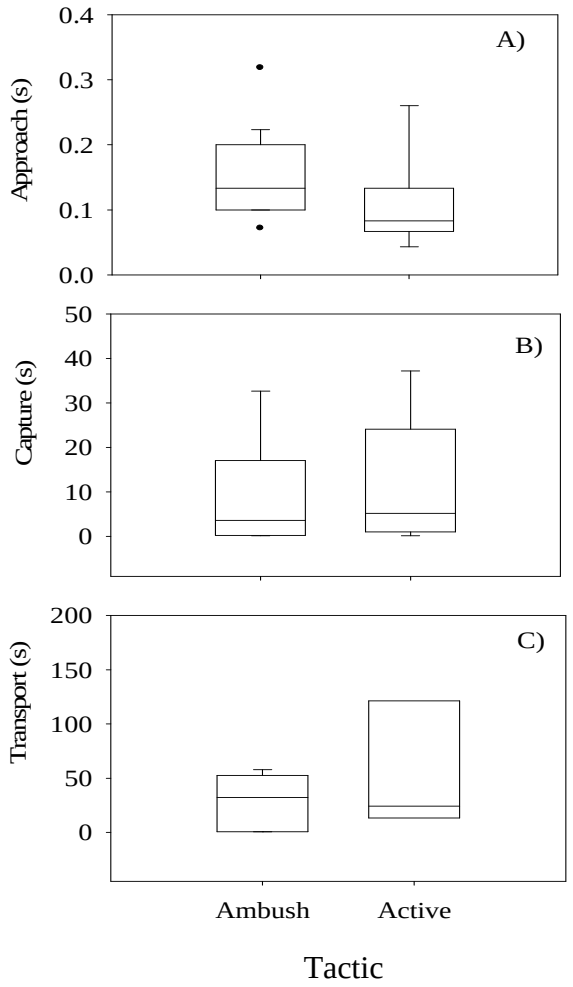


Figure 2.2: Handling times of longfin squid swallowed by summer flounder in the anterior, posterior, and perpendicular orientations. Measurements of A) approach B) capture and C) transport are displayed in seconds. Box boundaries represent 25th and 75th percentiles, lines within boxes mark the median. Error bars indicate the 90th and 10th percentiles. Circles show outliers in the 5th and 95 percentiles.

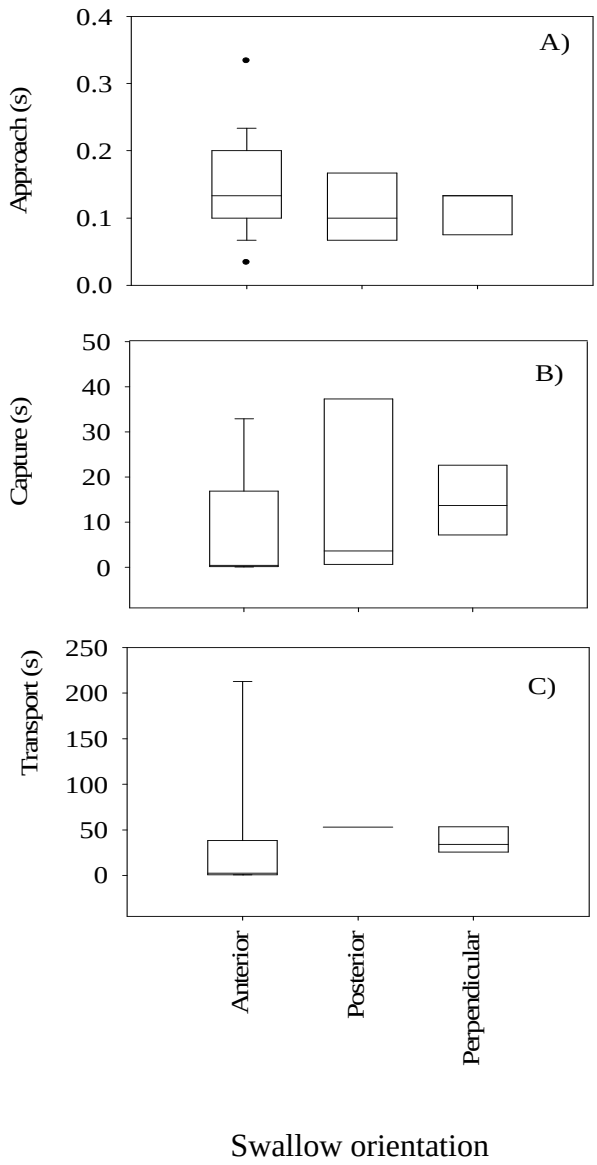


Figure 2.3: Scatterplot of capture times (seconds) of relative longfin squid sizes swallowed in anterior (open circles), posterior (closed circles), and perpendicular orientations (triangles) by summer flounder.

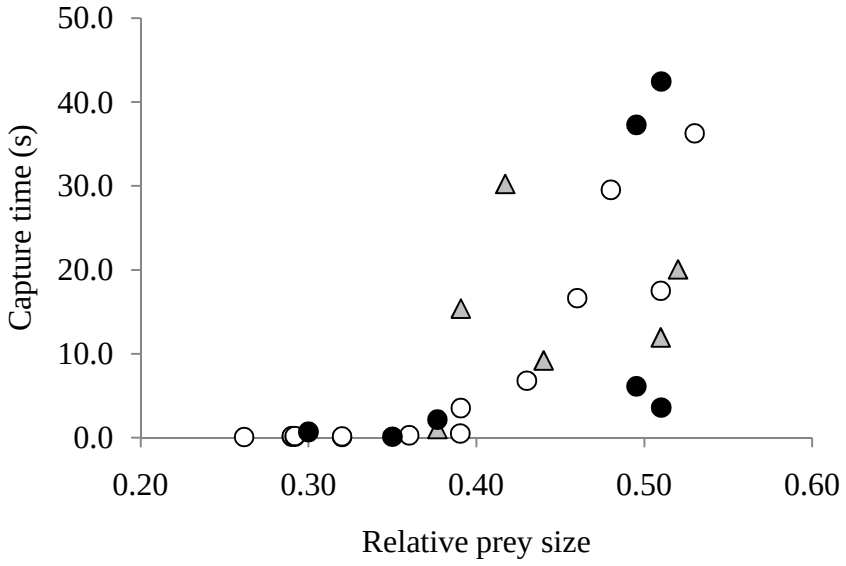


Figure 2.4: Prey-type selection trial results. A) Attack rates and B) percent capture successes made by summer flounder on mummichogs, longfin inshore squid, and Atlantic silversides. Vertical bars represent one standard error.

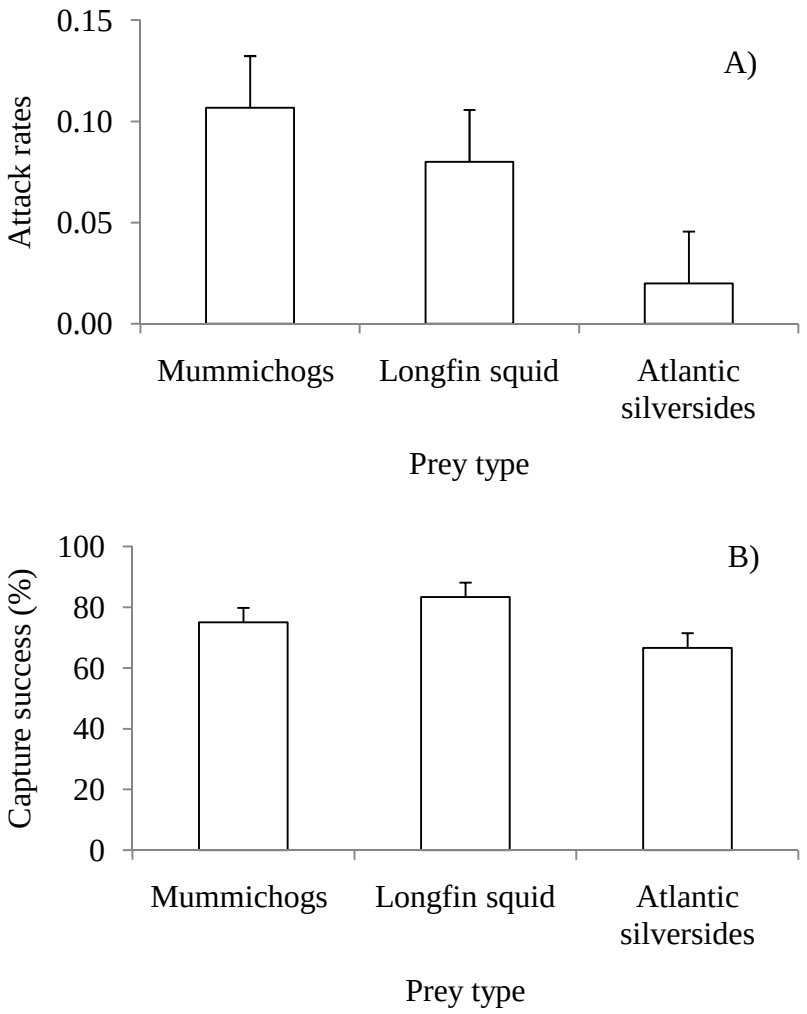


Figure 2.5: Frequencies (displayed as percentages) of ambush, active, and intermediate attacks made by summer flounder when attacking mummichogs, longfin inshore squid, and Atlantic silversides during prey-type selection trials.

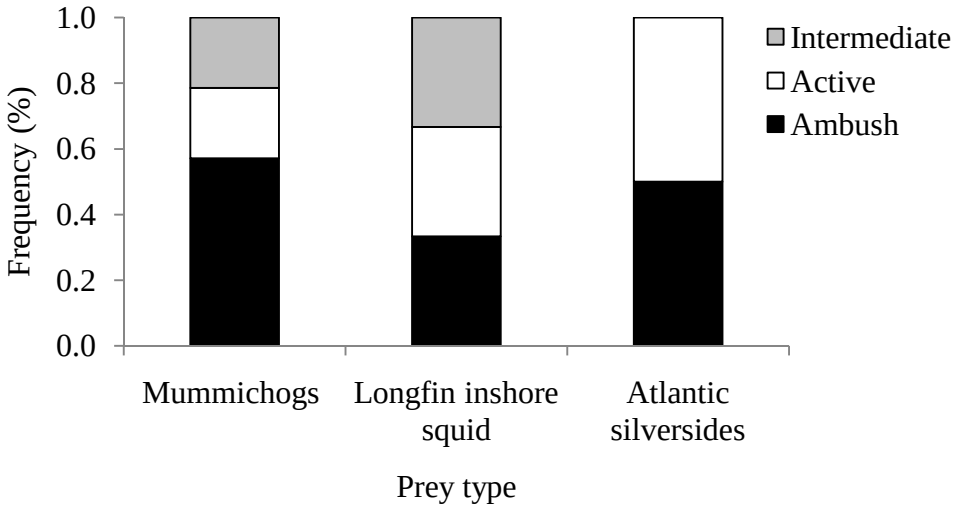
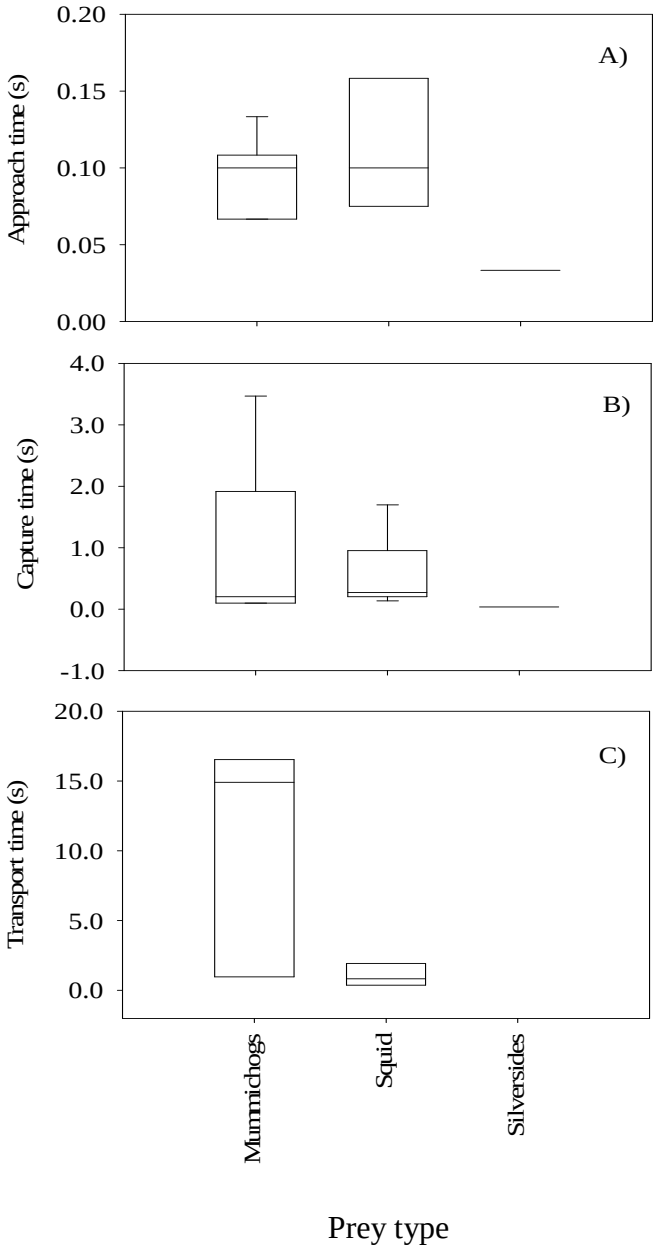


Figure 2.6: A) Approach B) capture and C) transport handling times of flounder attacks on mummichogs, squid, and Atlantic silversides during prey-type selectivity trials. Transport times were not observed during attacks on silversides. All measurements are displayed in seconds. Box boundaries represent 25th and 75th percentiles, lines within boxes mark the median. Error bars indicate the 90th and 10th percentiles.



CHAPTER 3

SIZE-DEPENDENT VULNERABILITY OF SQUID TO ATTACK AND CAPTURE BY TWO PREDATORS

Abstract

Cephalopods are primary prey to a wide range of predators in marine food-webs, yet a basic understanding of the mechanisms controlling predation risk and demand on their populations is lacking. Feeding experiments were conducted to evaluate how relative prey size and behavior mediate squid's vulnerability to predation. Attack and capture behaviors towards longfin inshore squid (*Loligo pealeii*) were quantified using two contrasting predators: bluefish (*Pomatomus saltatrix*) a pelagic, cruising predator, and summer flounder (*Paralichthys dentatus*) a demersal, ambush forager. Size-dependent estimates of predator selectivity, prey susceptibility, and prey profitability were quantified from predator-prey interactions during behavioral trials. Patterns in attack rates suggested that size-selection on squid was constrained by passive processes rather than active choice in both predators. Squid's susceptibility to predation by bluefish was strongly dependent on relative prey size but flounder were equally efficient at capturing all sizes of squid offered. Profitability was dome-shaped in both predators. Profitability values peaked at larger relative predator-prey sizes and were of greater magnitude for bluefish indicating that they were the more efficient predator of squid. Flounder foraging efficiencies were largely constrained by handling times which increased exponentially with relative prey size. Squid's vulnerability to bluefish and flounder was also assessed by measuring time-dependent mortality rates and comparing

survivorship with two species of forage fish. Squid were found to be more susceptible to predation and succumbed to greater mortality rates than Atlantic silversides (*Menidia menidia*) and mummichogs (*Fundulus sp*.) suggesting that when equal amounts of squid and fish are available in the environment, squid will be selectively ingested by bluefish and flounder. It was concluded that interspecific differences in prey behavior and predator foraging tactics influenced predator success and squid vulnerability. Results should be useful to predict how size-based patterns in predation by bluefish and flounder shift with changes in squid population dynamics in the northwest Atlantic ecosystem.

Introduction

Age and size structure of prey populations is strongly impacted by the selective feeding behaviors of their predators (Brooks and Dodson 1965; Rice et al. 1993; Christensen 1996; Claessen et al. 2002). Selection is evidenced when the distribution of prey sizes or types found in a predator's diet differs from what is available in the nearby environment (Ivlev 1961; Juanes and Conover 1994). When selectivity is observed, differences between diet and the environment may reflect active choice by the predator, or selection may be passive due to morphological and behavioral limitations or disparate capabilities of predators and prey (Werner 1974; Christensen 1996; Ellis and Gibson 1997). Attack rates differentiate between active and passive modes of selection, but due to the difficulties of obtaining data on attack rates in the field, measurements are generally confined to the laboratory. Active choice implies that a predator is responding to inherent differences in prey and chooses to pursue some more frequently than others. When attack rates are found to differ among prey, active choice is demonstrated, and the

prey that is attacked most is deemed “preferred”. Alternatively, if all prey encountered by a predator are attacked equally but the diet is skewed towards a range of prey sizes or types, then selection must be passive (Juanes et al. 2002) (Figure 3.1).

Selective foraging behaviors have been well explored in piscivorous fishes and are strongly dependent on the relative sizes of predators and their prey (Rice et al. 1993; Juanes and Conover 1995; Mittelbach and Persson 1998; Scharf et al. 1998c; Dorner and Wagner 2003). Behavioral components of predator-prey interactions such as handling time and capture success vary as a function of relative prey size and provide quantitative measures of the costs associated with acquiring progressively larger prey (Werner 1977; Rice et al. 1993; Scharf et al. 2003). Predators should select prey that minimize costs of capture and maximize net energetic rewards and overall profitability (Schoener 1971; Greene 1986; Stephens and Krebs 1986; Sih and Christensen 2001). Interspecific differences in predator foraging tactics and prey response behaviors will also play a significant role in shaping predator selectivity and prey vulnerability (Sih and Moore 1990; Manderson et al. 2000; Juanes et al. 2002; Scharf et al. 2002).

A prey’s vulnerability to a given predator is contingent on the probabilities of being encountered, attacked, and ingested (Greene 1986; Bailey and Hood, 1989; Scharf et al. 2003; Taylor 2003). Although attack and capture probabilities have been quantified for many important forage fish including Atlantic silverside (*Menidia menidia*), bay anchovy (*Anchoa mitchilli*), Atlantic menhaden (*Brevoortia tyrannus*), winter flounder (*Pseudopleuronectes americanus)* and shiners (*Notropis spp*.) (Manderson et al. 1999; Hartman 2000; Manderson et al. 2000; Scharf et al. 2003; Taylor 2003), comparable studies do not exist for cephalopods. Cephalopods are consumed by a broad assortment

of predators and are important constituents of marine food webs (Smale 1996; Bax 1998; Bowman et al. 2000). Many predators that consume fish also eat squid, and the two prey types alternate in predator diets over seasonal and ontogenetic time scales (Smale 1996; Staudinger 2006).

Squid have been described as functionally similar to fish in terms of habitat distributions, range of body sizes, and schooling behavior (Packard 1972); accordingly, squid should experience similar encounter rates with predators as the forage fish which they resemble ecologically. Squid are soft bodied, have relatively compressed body depths, but unlike fish, squid are deficient of bones and spines. These physical characteristics may increase squid's vulnerability to predators by decreasing handing times and increasing capture probabilities (Werner 1977; Rice et al. 1993). Alternatively, squid utilize a variety of anti-predator defenses including jet propulsion, ink, and camouflage (Hanlon and Messenger 1996). These characteristics, in addition to highly advanced visual capabilities, may help squid detect and avoid predators better than common forage fish (Bailey and Hood, 1989).

Because quantitative evaluations of attack probabilities, handling times, and selection towards squid are scarce (Chapter 2), we do not know if vulnerability to predation is primarily dependent on squid's availability in the environment, or if upon encounter squid are more susceptible to attack and capture than fish. This information is fundamental to understanding predator-prey interactions and predicting how populations respond to fluctuations in community structure and abundance caused by environmental and anthropogenic pressures (Rice et al. 1993; Mittelbach and Persson 1998; Scharf et al. 2003).

The purpose of this study was to determine the role that size and behavior play in mediating squid's vulnerability to predation. To accomplish these objectives, a series of laboratory experiments were conducted to quantify attack and capture behaviors towards longfin inshore squid (*Loligo pealeii*) by two predators representing contrasting foraging tactics. Bluefish (*Pomatomus saltatrix*) was selected as a pelagic, cruising predator and summer flounder (*Paralichthys dentatus*) was chosen as a benthic, lie-and-wait predator. Attack rates established whether predators used active or passive selection when foraging on squid and if preference was exhibited towards a specific size range. Size-dependent capture success rates and handling times quantified the costs associated with obtaining progressively larger squid and were combined with information on relative prey mass to estimate size-dependent foraging efficiencies (profitability functions) respective to each predator. Lastly, time-dependent survival rates were used to evaluate if risk to predation by bluefish and flounder varied as a function of relative squid size and if squid were more susceptible to predation in comparison to two species of forage fish.

Methods

Collection and maintenance of experimental animals

Summer flounder ranging in size from 36 – 47 cm Total Length (TL) were collected from Buzzards Bay, MA as part of the Massachusetts Division of Marine Fisheries spring 2006 trawl survey. Bluefish ranging in size from 31 – 63 cm (TL) were caught by hook and line from local bays and estuaries surrounding Woods Hole, MA during summer 2007. After capture, fish were immediately transported to the Marine Resources Center at the Marine Biological Laboratory (MBL) in Woods Hole, MA. All

fish were maintained in tanks with recirculating, biofiltered seawater and fed a diet of live and frozen fish and squid. Water temperatures (16° - 20° Celsius) and photoperiods mimicked late spring-early summer conditions. In addition to natural light from adjacent windows, fluorescent lighting was maintained on a 10:14 hour (h), light:dark schedule. Flounder holding tanks were lined with a mixed gravel and sand substrate approximately 2 - 4 cm deep which allowed flounder to bury. Bluefish and flounder were acclimated to captivity for approximately 1 month and were considered ready for use in feeding trials when live food was accepted on a daily basis.

Longfin inshore squid ranging in size from 2 – 21 cm Mantle Length (ML) were collected daily from Vineyard and Nantucket Sounds using a modified trawl net. Squid were transported back to the MBL facility in a live-well tank and upon arrival, immediately transferred into the experimental tank. If it was necessary to hold squid overnight prior to use in trials, they were housed in a 1 m diameter (d) tank and fed live fish and small squid. No squid used in trials were held for more than 48 h. Squid were handled as little as possible and careful attention was given to identifying individuals that were vigorous and had suffered little to no physical damage during collection. Squid that had visible abrasions to their epithelium or showed signs of lethargy were not used in trials. Atlantic silversides and mummichogs (*Fundulus sp*.) ranging in size from 6 – 11 cm (TL) were collected by beach seine approximately 24 to 48 h prior to use in feeding trials and maintained on pellet feed. Fish were held in partitioned screen boxes in the same holding tanks as squid.

Experimental set-up

Feeding trials using summer flounder as predators were conducted between June and August of 2006, and bluefish trials were conducted between June and August of 2007. All trials took place in a 28 x 10^3 Liter (L), 3.1 x 0.8 m (d, height (h)) tank filled with seawater from the same lines as specimen holding tanks. A mixed gravel and sand substrate (similar to holding tanks) approximately 2 - 4 cm deep lined the bottom of the experimental tank allowing squid to camouflage. The area surrounding the experimental tank was lined with black plastic sheeting to prevent disturbance to acclimating animals and during filming. Additionally, two 500 watt lights were positioned above the tank to aid with filming clarity.

Three predators of approximately equal size were chosen for each trial and allowed to acclimate to the experimental tank for at least 24 h. When predators were used on successive days, a minimum of 24 h elapsed between trials. Predator hunger levels were standardized by withholding food for 24 h prior to the start of each trial. Approximately 3 h prior to the start of each trial, an opaque PVC cylinder 1.5 x 1 m (d, h) was lowered into the center of the experimental tank using a pulley system, and 15 prey were placed on this inner portioned area and allowed to acclimate. The partition allowed predators and prey to acclimate simultaneously; although visual contact was restricted, water movement between the compartments likely resulted in some exchange of olfactory information. A trial commenced when the partition was raised above the tank and predators and prey were allowed to interact. Trials were recorded using Panasonic miniDV PV GS500 video cameras which were manually operated at two lateral viewing windows located on opposite sides of the experimental tank, and from a third camera

mounted above the tank. Predator-prey behaviors were assessed using frame-by-frame analysis (30 frames/s) of video recorded during each trial.

Experiment 1: Size-selection

In size-selection trials, patterns in attack rates among relative squid size classes were assessed. Three predators of approximately equal size were simultaneously presented with 5 squid from 3 different relative size-classes, for a total of 15 prey. The range of relative prey size-classes offered to predators corresponded to the sizes of squid found regularly in bluefish and summer flounder diets (Chapter 1). Squid size-classes were grouped in 10% increments of relative predator-prey size ratios (total prey length / total predator length; e.g., 0.10 – 0.19, 0.20 – 0.29) and relative sizes ratios ranged from 0.10 – 0.79. Each trial lasted 30 minutes. After each trial was completed, all remaining squid were removed from the experimental tank and measured to determine which individuals from each size-class had been consumed.

Experiment 2: Time-dependent survival

During time-dependent survival trials, survival rates among relative squid size-classes were evaluated. Three size-matched predators were offered 15 squid that were all within the same relative size-class (e.g., 0.20 – 0.29). Predator-prey interactions were recorded for the first 30 minutes of each trial and after 30 m, visual counts were made of the number of squid surviving at 1, 2, and 3 h from the start of the trial.

Experiment 3: Prey-type vulnerability

In the last series of trials, squid's vulnerability to predation was contrasted with that of two prey fish by examining differences in survival rates over a 24 h period. The two species of prey fish, Atlantic silversides and mummichogs, are present in the diets of bluefish and summer flounder during late spring and summer. Three size-matched predators were simultaneously offered 5 squid, 5 Atlantic silversides, and 5 mummichogs ranging from 0.15 to 0.35 relative prey size. Attacks on all prey were recorded for the first 30 m, and visual counts of the number of individuals remaining in each prey group were made at 1, 2, 3, 6, 9, 12, and 24 h.

Repeated Measures

To determine whether repeated use of individual predators affected feeding behaviors over the course of all trials, I assessed 1) whether predators reached feeding saturation with successive use in trials and 2) if predator feeding efficiency increased with repeated use over time. Feeding saturation was measured by comparing the total number of attacks (successful + unsuccessful) made by each group of three predators over successive days of use. Predator efficiency examined whether capture success rates varied for individuals groups of fish (identified by TL) over all trials. Capture success was determined by dividing the number of successful attacks by the total number of attacks made during each trial. Unsuccessful attacks did not result in ingestion but included attempts where a prey was obtained by the predator and escaped or was rejected during the manipulation period.

Distributions of total attacks and capture success rates were found to be highly skewed; therefore, a Friedman's nonparameteric two-way ANOVA was used to test treatment differences using a randomized complete block design (Zar 1984). Fish groups were treated as subject variables and the number of days used in trials was assigned as the treatment variable. All tests yielded nonsignificant *p*-values (all *p*-values > 0.18), suggesting that repeated use of bluefish and flounder did not influence feeding behaviors.

Size selection, capture success, and handling times

Attack rates from size-selection trials were used to determine if predators actively chose squid from a particular relative size-class or if selection was passive (random). Attack rates were calculated by:

$$Attack\ rate = \frac{Total\ attacks}{(Trials \times Time)} \qquad \text{Eqn. 3.1}$$

Total attacks was the total number of attacks made on each relative prey size group (e.g., 0.20 – 0.29) during all trials, Trials was total number of trials conducted for each relative size group, and Time was the length in minutes of each trial (30 m). A Kruskal Wallis test was used to compare attack rates among relative squid size classes. Based on previous studies using piscivores, attack rates were was expected to decrease with increasing prey size (Juanes and Conover 1994).

$$Capture\ success = \frac{Successful\ attacks}{Total\ attacks} \qquad \text{Eqn. 3.2}$$

Capture success was determined by dividing the number of Successful attacks by the total number of attacks made (Total attacks) on each relative squid size-class. Capture success rates from size-selection and time-dependent survival trials were pooled when significant differences were not detected between the two trials types using an

ANOVA (all p-values > 0.49). Least squares regression analysis was then used to assess the strength of the relationship between relative prey size (independent variable) and capture success.

Handling times were quantified as the time in seconds from when a predator made first contact with a prey during an attack until the time when the prey was no longer visible outside the predator's mouth and rapid opercular movement and gulping ceased (Christensen 1996; Scharf et al. 2003). Because some attacks were obscured, handling time information was not available on every attack made. Handling times from size-selection and time-dependent survival trials were combined and analyzed as a function of relative prey size (independent variable) using least squares regression. Handling time data appeared to be nonlinear therefore, both linear and exponential functions ($y = ae^{bx}$) were fit to the data and the model with the strongest coefficient of determination (R^2) was selected to explain trends for each predator.

Profitability

Prey profitability was estimated using the equation:

$$Profitability = \frac{Relative\ prey\ mass}{Handling\ time} \times Capture\ success \qquad \text{Eqn. 3.3}$$

and was expected to be a dome shaped function of relative squid size (Scharf et al. 1998c; Hartman 2000; Scharf et al. 2003). Handling times and capture success represented the energetic costs of obtaining squid and were estimated using the equations and mean values derived from size-selection and time-dependent survival trials. Relative prey mass (prey mass / predator mass) was assumed to be roughly equivalent to energetic intake and represented the benefit received by each predator for ingesting a squid

(Steimle and Terranova, 1985; Scharf et al. 2003). Lengths of each prey and predator were measured directly and converted to mass using length-weight relationships provided in Lange and Johnson (1981) and Wigley et al. (2003). Profitability curves were generated by inserting relative prey mass, handling time, and capture success values respective to each 0.01 relative predator-prey length increment for all possible combinations between 0.05 and 1.0, and fit using the quadratic polynomial function ($y = y_0 + ax + bx^2$). Because capture success can play a significant role in shaping foraging efficiencies of some (e.g., piscivores) but not all predators (e.g., planktivores) (Stephens and Krebs 1986; Scharf et al. 1998c; Sih and Christensen 2001), profitability curves were estimated with and without the variable to determine the overall influence on the model.

Survival analysis

Survival rates from time-dependent survival and prey-type vulnerability trials were evaluated using survival or time-to event analysis (Cox and Oates 1984; Allison 1995). To test whether survival rates differed among relative squid size-groups in time-dependent survival trials and prey species in prey-type vulnerability trials, Cox's proportional hazards regression was executed using the PROC PHREG command in SAS (SAS 2003). The Hazard (or risk) ratio estimated the percent increase per unit time in predation risk experienced by each relative size group and prey type respective to each predator. Additionally, the hazard function determined if predation risk for each squid size group and prey type increased, decreased, or remained constant over the course of trials. The Cox regression technique was chosen because it does not require knowledge of the underlying probability distribution of the hazard function and therefore is less

restrictive than other models. Because time is ranked, the model is not sensitive to multiple time intervals. The model also allows for stratification of variables and tied events and was the most appropriate model for the datasets analyzed in this study (Castro-Santos and Haro 2003).

Results

Twenty-four bluefish were used over the course of 40 trials, and 61 attacks were observed over 60 hours of observation; 24 summer flounder were used in 34 feeding trials and 118 attacks were observed over 57 hours of observation (Table 3.1). The average number of squid consumed by summer flounder in each trial was approximately twice as high as by bluefish and was likely due to the greater number of attacks made by summer flounder. A wider range of relative squid size classes was tested using summer flounder (0.10 – 0.70) in comparison to bluefish (0.10 – 0.50) (Table 3.2). During both years of trials, the smallest (≤ 0.19) and largest relative squid size-classes (≥ 0.50) had the fewest number of replicates due to limited availability in local waters, or in the case of small squid, because of difficulties in keeping individuals alive in captivity for extended periods of time.

Size-selection, capture success, handling times

Attack rates did not differ ($p > 0.05$) among squid size-groups and size-selection was inferred to be passive in both bluefish ($X^2 = 6.69$, $df = 4$, $p = 0.15$) and summer flounder ($X^2 = 5.49$, $df = 6$, $p = 0.50$) (Figure 3.2). Capture success rates decreased linearly with increasing relative squid size in bluefish ($R^2 = 0.844$, $F = 16.25$, $p = 0.03$),

but a significant trend was not detected in flounder (R^2 = 0.002, F = 0.01, p = 0.94) (Figure 3.3). Capture success rates were greatest for the smallest (0.10) and largest squid size classes (0.60) tested using summer flounder; however, few attacks were made on these two size-classes (n ≤ 2), and overall trends were not affected when they were excluded from analyses. Handling times increased exponentially with increasing relative squid size in bluefish ($y = 1.58e^{3.33x}$, R^2 = 0.46, n = 27, p = 0.0005) and flounder ($y = 0.23e^{10.94x}$, R^2 = 0.68, n = 27, p < 0.0001) (Figure 3.4). Mean handling times were greater (16.37 seconds) and corresponded to greater relative size ratios in flounder (0.40 – 0.50) (t-value = 2.40, df = 56, p = 0.02) in comparison to bluefish (3.64 seconds; 0.20 – 0.40 relative size ratios).

Profitability

Profitability was a dome shaped function of relative prey size for both bluefish and flounder. Peak profitability values were greater for bluefish (0.004) in comparison to flounder (0.002) and corresponded to relative squid sizes between 0.40 - 0.50 in bluefish and 0.20 - 0.30 in flounder (Figure 3.5). Removal of capture success from the bluefish model increased peak values by an order of magnitude and shifted the curve so that maximum values corresponded to relative squid sizes near 0.70. The influence on flounder profitability values were less pronounced; peak profitability shifted to relative squid sizes of approximately 0.60 and extended to larger relative sizes. Overall, flounder profitability values did not vary considerably between models that included and excluded capture success.

Time-dependent survival

In trials using bluefish as predators, significant differences in survival rates were observed among relative squid size groups (X^2 = 6.90, *df* = 2, *p* = 0.009). The 0.10 – 0.19 relative squid size group was determined to be the most vulnerable to bluefish predators, and risk of predation (Hazard ratio = 0.96) decreased by 4.3% (per unit time (m)) in progressively larger size groups (Figure 3.6). Significant differences in survival rates among relative squid size groups were not detected in the presence of summer flounder (X^2 = 2.02, *df* = 3, *p* = 0.16) (Figure 3.6); however, when data from all size groups were combined, squid survivorship declined exponentially (Log likelihood = -166.2, X^2 = 4.85, *p* = 0.03). Hazard functions increased with time for both bluefish and summer flounder suggesting that neither predator reached feeding saturation, and all squid sizes remained at risk during the entire course of trials.

Prey-type vulnerability

Risk of predation differed significantly among prey types in the presence of both bluefish (X^2 = 21.63, *df* = 2, *p* < 0.0001) and summer flounder (X^2 = 13.11, *df* = 2, *p* < 0.0003). Differences in predation risk among prey types was more pronounced in flounder (Hazard ratio = 0.50) compared to bluefish (Hazard ratio = 0.35) (Figure 3.7). Of the three prey types, squid experienced the highest mortality rates, silversides had the lowest mortality rates, and mummichogs were intermediate. As was seen in time-dependent survival trials, overall mortality rates were higher in the presence of bluefish for all prey types in comparison to flounder.

Discussion

Patterns in bluefish and summer flounder attack rates suggest that selection on longfin squid was the result of passive processes rather than active choice however the primary constraint on selection differed between the two predators. In bluefish, capture success was identified as the limiting factor. Bluefish were more effective at capturing smaller squid, and as a result, time-dependent mortality rates were greater in progressively smaller relative size groups. Flounder were equally efficient at capturing all squid sizes, and squid survival did not vary as a function of relative prey size. When capture success was excluded from profitability calculations, the estimated reward to bluefish increased nearly four-fold but had only a marginal effect on flounder profitability. In contrast, predation efficiency in flounder was primarily constrained by handling time. Handling times increased exponentially in both predators. The rate of increase was several orders of magnitude higher for flounder and reached maximum values up to five times greater than bluefish. The steep rise in handling times associated with larger squid sizes yielded considerably lower profitability estimates for flounder. Given these results, I conclude that the costs associated with pursuing large squid appeared to be more limiting to flounder in comparison to bluefish; although I note there were fewer attack data available for bluefish towards the largest sizes (≥ 0.50) of squid.

Size-selection

Regardless of the combination of relative squid size classes offered to bluefish in size-selection trials, the smallest squid available (relative sizes ranging from 0.12 – 0.25) were successfully attacked and captured most often. Squid appeared to school with the

smallest individuals on the periphery and squid < 5 cm ML (≤ 0.10 relative size) often separated themselves from the rest of the school in loose aggregations or swam near the surface as isolated individuals. These behaviors appeared to predispose the smallest squid to predation by bluefish. Step-wise attack patterns on small, medium, and large Atlantic silversides have been reported previously by Juanes and Conover (1994). Additionally, Scharf et al. (2002) found bluefish to target solitary bay anchovy (*Anchoa mitchilli*); however, it was the largest bay anchovy that separated from schools and were selectively consumed by bluefish. Results from the present study add to existing evidence that size-dependent vulnerabilities of different prey are highly species-specific and mediated in part due to the attack strategy used by the predator and the schooling behavior of prey.

Capture success

The high capture success rates achieved by bluefish and flounder suggest that once squid are encountered they are highly susceptible to predation. Squid were more susceptible to capture at similar relative sizes compared to bay anchovy, Atlantic silversides, Atlantic menhaden (*Brevoortia tyrannus*), and age-0 striped bass (*Morone saxatilis*) (Scharf et al. 2002; Scharf et al. 2003). Soft, narrow body depths and the absence of hard defensive structures such as spines likely contribute to squid's relatively high susceptibility. Defense behaviors such as arm postures and body pattern changes were used by squid to try and deter approaching predators (Hanlon and Messenger 1996). Arm postures were generally exhibited from a stationary position at the water's surface. Since many predators respond to movement (Keenleyside 1979; Scharf et al. 2002), staying still and trying to appear like something else is a good strategy for prey to remain

inconspicuous and conserve energy that would alternatively be used for flight (McFarland 1987; Hanlon and Messenger 1996). This strategy has been hypothesized to decrease a predator's ability to predict the direction in which a prey may escape (Hanlon and Messenger 1996; Juanes et al. 2002; Scharf et al. 2002). When and if anti-predator displays fail to deter an attack, swimming speeds become crucial to avoiding predators. In size-selection experiments where mixed size-assemblages of squid were present, large squid noticeably out-swam smaller squid and likely contributed to reduced capture success rates in the presence of pursuing bluefish. Flounder relied more on camouflage and the element of surprise to capture squid; consequently, swimming speeds may have been less influential in avoiding ambush attacks and would explain why flounder capture success rates were close to chance (50:50) across relative squid size classes.

Ontogenetic behavior was another factor that appeared to influence susceptibility to capture. Longfin squid are considered juveniles until sizes of approximately 8 – 12 cm ML at which point they become sexually mature (Jacobson 2005). In behavioral trials, squid < 5 cm ML (≤ 0.10 relative size) seemed naive to predators and particularly susceptible to capture. During several trials, small squid failed to react to predator attacks on squid schooling with them or in adjacent schools. They did not flee to another part of the tank or regroup after an attack (Staudinger, *personal observation*). This often left them prone to repeated attacks, especially by bluefish, within a minute or less of an initial attack. When larger subadult and adult squid ≥ 6 cm ML were attacked, they inked, jetted erratically, and then swam away from the location of the attack, regrouped in tight schools on the opposite side of the tank, and maximized the distance between themselves and the threatening predator (Staudinger, *personal observation*). Juvenile

squid < 5 cm ML may not yet have developed sophisticated anti-predator defense behaviors, which would explain why they were less responsive to predators (McFarland 1987).

It should be noted that the high capture success rates achieved by both predators may have been an artifact of conducting experiments in the confines of captivity. Squid may have been more vulnerable than in open waters where flight could be used when predators were first detected (Hanlon and Messenger 1996). Tanks sizes were large enough that squid were able to elicit a range of defensive behaviors and substrate was provided for crypsis. High light intensities may have increased detection of squid and produced artificially high capture success rates especially in flounder which are usually associated with demersal, low-light environments.

Handling times

Bluefish gape allometries are slightly larger than summer flounder (Chapter 1) and may have contributed to facilitated capture and manipulation of squid. Nevertheless, the sizes of squid offered to bluefish and flounder did not approach maximum gape thresholds and neither predator was considered gape limited (Chapter 1). Flounder took considerably longer than bluefish to reposition and swallow large squid and were often observed with the arms or mantle protruding from their mouths during the capture process. In contrast, bluefish generally swallowed squid in one sweeping gulp if the squid was attacked in the water column or in a few short and continuous bites when a squid was grabbed off the substrate (Staudinger, *personal observation*). Tooth structure and shearing dentition allow bluefish to sever their prey into small pieces and yield

reduced handling times in comparison to other piscivorous predators (Scharf et al. 1997; Hartman 2000; Scharf et al. 2009). During several attacks, squid were bitten into chunks and the discarded pieces were scavenged by other bluefish. Occasionally bluefish expelled or regurgitated large pieces of squid several minutes after an attack was completed. These behaviors were not observed in summer flounder which swallowed their prey whole. Due to the paucity of studies to quantify foraging behaviors in marine fish other than bluefish, striped bass were the only additional predator available to compare our findings with summer flounder. Striped bass and summer flounder swallow their prey whole and compared to bluefish, exhibited higher handling times and lower profitability estimates. Presumably this was due to the overall costs of the different modes of attacking and processing progressively larger prey (Scharf et al. 2009).

Differences between the fusiform and laterally compressed body forms characteristic to bluefish and flounder and their associated behaviors provide further explanation for why prominent differences in handling times were observed between the two predators. Bluefish are constant swimmers and as a result may be able to approach and overtake prey more easily than flounder which begin attacks more frequently from a stationary position. Bluefish are ram ventilators; therefore, water is constantly flowing through the opercular and buccal cavities (Freadman 1979), which may help bluefish pass food to the stomach faster than flounder. After consuming large prey, flounder frequently rested on the bottom with their heads raised at an angle above the substrate and exhibited jaw protrusions and opercular expansions (Bels and Davenport 1996). These behaviors demonstrated that completion of the transport process was highly strenuous to flounder especially when large prey sizes were consumed. Ultimately the

high energetic costs associated with long handling times lowered overall profitability values to flounder and decreased their overall foraging efficiency in comparison to bluefish.

Despite larger gape sizes and other morphological advantages, the bluefish diet contains a narrower range of relative squid sizes than summer flounder (Chapter 1). In northwest Atlantic waters, bluefish consume relative size ratios of longfin squid between 0.10 – 0.50, but almost 90% of the bluefish diet is composed of relative squid sizes ≤ 0.30, and 50% of all squid in bluefish diets are between 0.10 - 0.19 (Chapter 1). The distribution of relative squid sizes found in regional summer flounder diets closely match attack rate patterns derived experimentally in this study. Flounder diets include high frequencies of relatively large squid (≥ 0.20) and consume a wider range of relative squid sizes than bluefish (up to 0.60 relative body size) (Chapter 1).

Adult longfin squid are primarily demersal and sometimes rest on the bottom during the day to conserve energy (Macy 1982; Hanlon et al. 1983). When the threat of predation was not imminent, adult squid rested on the bottom of experimental tanks, this behavior has also been reported in other tank studies when predators were absent (Hanlon et al. 1983). Additionally, Stevenson (1934) observed longfin squid to drop to the substrate in a camouflage pattern when predatory fish swim overhead. Consequently, summer flounder and other bottom-dwelling predators may be more likely to encounter relatively large squid in comparison to pelagic predators such as bluefish. In contrast, juvenile squid spent the majority of time during experiments near the surface of the tank and under natural conditions are known to migrate through the water column to feed at the surface more frequently than adults (Brodziak and Hendrickson 1999). Increased

residence time in the water column would expose juveniles to pelagic predators and may explain the propensity of bluefish diets towards small squid. Juveniles also prefer nearshore and shallow water habitats that may elevate encounters with bluefish seasonally (Staudinger 2006).

Profitability

The present study reports the first estimates of prey profitability for a flatfish predator and the only known quantifications using squid as prey. Previous studies have assessed the profitability of Atlantic silversides, striped bass, Atlantic menhaden, bay anchovy, and sand shrimp (*Crangon septemspinosa*) to bluefish predators (Scharf et al. 1998c; Juanes et al. 2001; Scharf et al. 2002; Scharf et al. 2003; Scharf et al. 2009), and for striped bass foraging on Atlantic silversides, shiners (*Notropis atherinoides*), and bay anchovy (Hartman 2000; Scharf et al. 2003; Scharf et al. 2009). During interactions with bluefish, squid profitability peaked at greater relative sizes than were determined for Atlantic menhaden and striped bass as prey (Scharf et al. 1998c; Scharf et al. 2003), but were lower than bay anchovy (Scharf et al. 2002; Scharf et al. 2003; Scharf et al. 2009). Relative squid sizes that maximized profitability in bluefish were most comparable to Atlantic silversides (Scharf et al. 1998c; Scharf et al. 2001; Scharf et al. 2003; Scharf et al. 2009).

Squid profitability peaked in summer flounder at lower relative prey sizes than have been reported previously for all other predator-prey combinations except between striped bass and shiners (Hartman 2000). The exclusion of capture success rates in the summer flounder profitability model shifted peak values towards larger relative prey

sizes but did not produce a noticeable change in profitability values. Since there are no other estimates of capture success and profitability for flatfish or any other demersal predator, it is uncertain whether this is a species-specific constraint associated with obtaining squid, or a general disadvantage of the lie-and-wait predation strategy.

Prey- type vulnerability

Prey-type selection could not be quantified in bluefish because few attacks (n = 5) were made during the first 30 minutes of all prey-type vulnerability trials; however behavioral observations (n = 3) indicated squid were more susceptible to bluefish than prey fish. Additionally, in the presence of both bluefish and summer flounder, time-dependent mortality rates of longfin squid were greater than prey fish. This was an unexpected result since squid were assumed to have a more complex array of defense behaviors than Atlantic silversides or mummichogs.

Summer flounder have been shown to prefer demersal prey over pelagic prey (Manderson et al. 2000; Chapter 2); accordingly, mummichogs and squid succumbed to higher mortality rates in comparison to Atlantic silversides. In the presence of flounder, mortality rates were initially higher for all squid sizes and prey types. This trend likely reflects the more opportunistic foraging tactic of a lie-and-wait predator. Ambush predators such as flounder may be motivated to attack more often since they often wait for prey to come to them. Overall, time-dependent mortality rates were higher for all prey types in the presence of bluefish and suggest that bluefish have greater foraging abilities than flounder.

Food habits studies indicate that squid rarely dominate the total diets of summer flounder and bluefish, yet consumption of squid fluctuates considerably over seasonal and spatial scales (Bowman et al. 2000; Buckel et al. 1999b; Staudinger 2006). The relative abundance and diversity of prey fish available to predators in coastal waters of the northwest Atlantic exceeds the relative number of cephalopods. Therefore, I conclude that encounter rates primarily control the proportions of squid and fish prey in predator diets; however, based on lower survival probabilities and higher capture success rates determined in this study, when the relative proportions of both prey types are equivalent, squid will be selectively ingested.

Conclusions

Understanding how prey vulnerability fluctuates in response to different predator species and identifying the limiting factors on selective feeding patterns will be crucial to predict how populations respond to changes in community structure and predator abundance (Scharf et al. 2003). Bluefish and summer flounder are known to be voracious predators capable of impacting squid populations (Buckel et al. 1999a; Overholtz et al. 2000) and size-dependent foraging efficiencies determined in the present study will be useful to modeling predatory demands associated with shifts in predator size-structure and prey abundance.

Because of the species-specific nature of predator and prey behaviors, it is uncertain if the findings of this study can be generalized to other demersal and pelagic predators and species of squid. Our findings suggest that while relative size establishes the lower detection limits and upper physical boundaries on which prey are attacked and

captured, predator foraging tactics and prey behaviors play a decidedly important role in controlling squid vulnerabilities and predator success.

TABLES

Table 3.1: Number of feeding trials (N_{Trials}), total number of attacks ($N_{Attacks}$), and hours of observation conducted using bluefish and summer flounder as predators and longfin squid as prey.

Trial Type	N_{Trials}	$N_{Attacks}$	Hours of observation
Bluefish			
Size-selection	19	30	9.5
Survival	16	26	48
Preference	5	5	2.5
Total	40	61	60
Summer flounder			
Size-selection	13	30	6.5
Survival	16	57	48
Preference	5	31	2.5
Total	34	118	57

Table 3.2: Number of bluefish and summer flounder feeding trials conducted using each relative size-ratio class.

	Bluefish		Summer flounder	
Relative prey size	Size-selection	Survival	Size-selection	Survival
0.10 - 0.19	7	5	2	0
0.20 - 0.29	15	6	4	4
0.30 - 0.39	19	5	9	5
0.40 - 0.49	12	0	12	4
0.50 - 0.59	4	0	7	3
0.60 - 0.69	0	0	3	0
0.70 - 0.79	0	0	2	0
Total trials	19	16	13	16

FIGURES

Figure 3.1: Conceptual model of the limiting factors on predator selection.

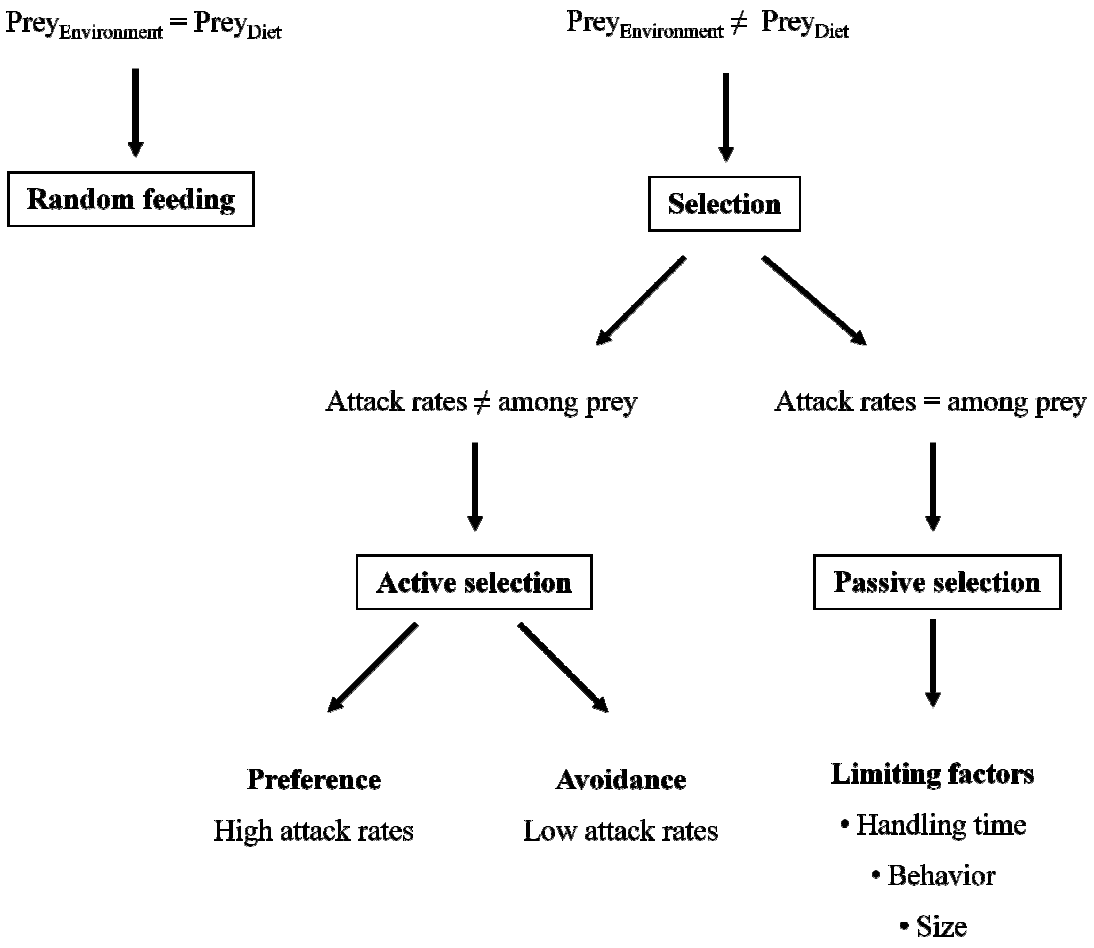


Figure 3.2: Attack rates on relative size ratios of longfin inshore squid by A) bluefish and B) summer flounder during size-selection trials. Bars show calculated standard errors (1 SE); * indicates no attacks were observed, although the size-group was offered.

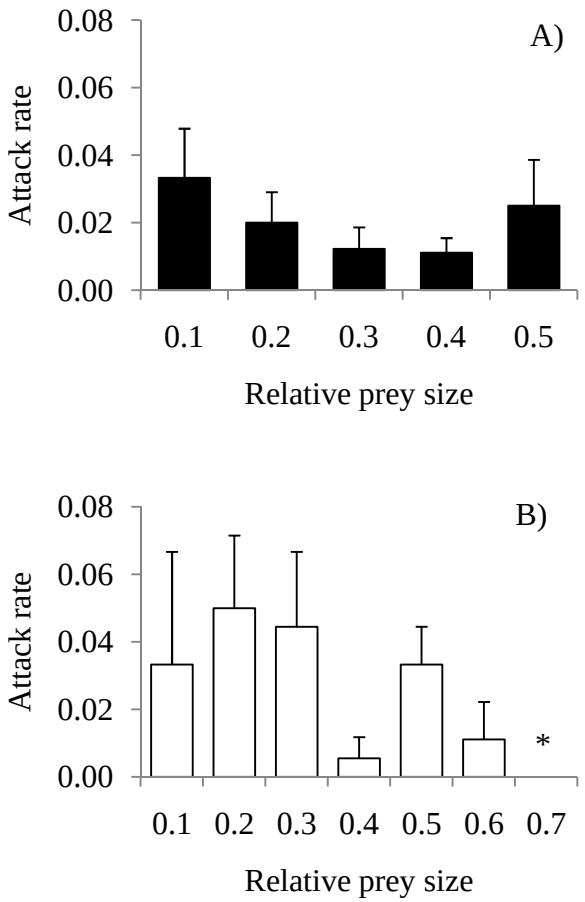


Figure 3.3: Capture success rates for A) bluefish and B) summer flounder on longfin inshore squid. Regression equations were estimated by the equations $y = -1.5x + 1.14$, $r^2 = 0.844$, $p = 0.03$ for bluefish and $y = -0.05x + 0.75$, $r^2 = 0.002$, $p = 0.94$ for summer flounder. Standard error bars (1 SE) and regression lines are shown.

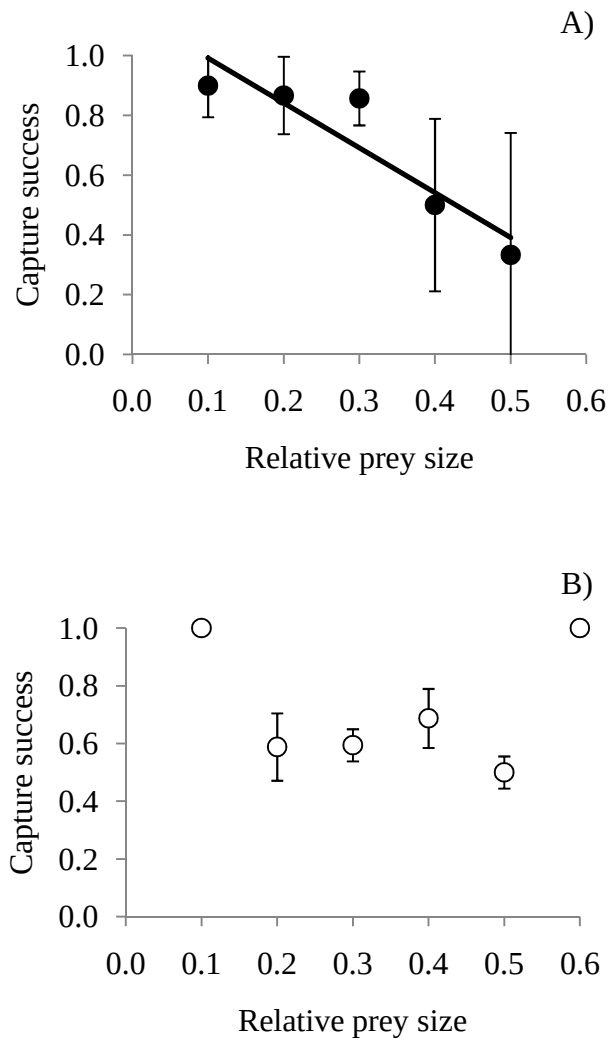


Figure 3.4: Handling times for A) bluefish and B) summer flounder. Regressions were estimated using the equations $y = 1.58e^{3.33x}$ for bluefish and $y = 0.2333e^{10.94x}$ for summer flounder. Note differences in scale in the y-axis.

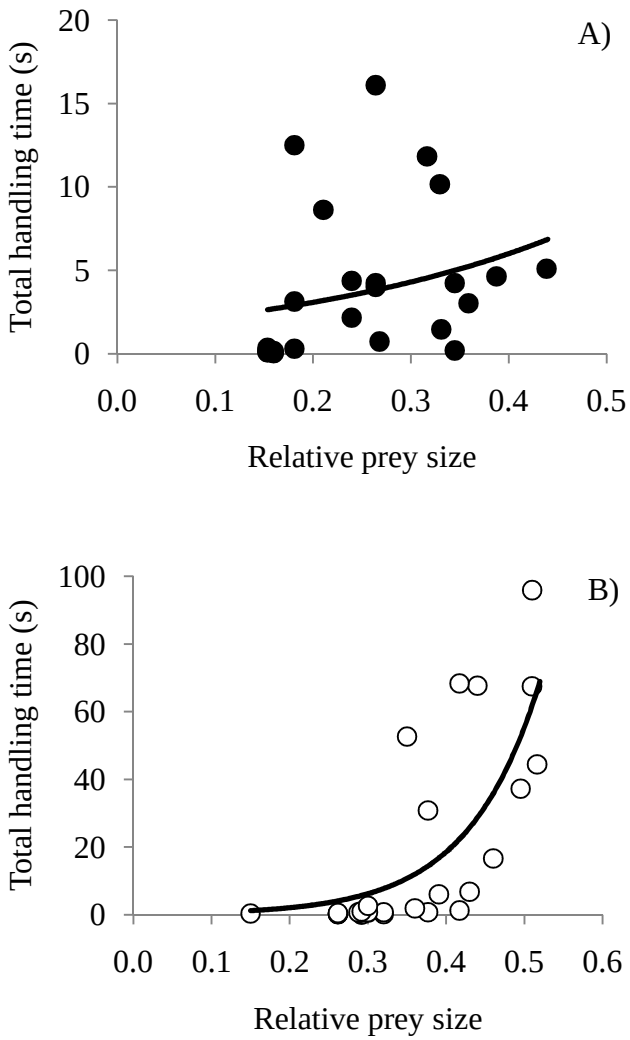


Figure 3.5: Estimated squid profitability curves for A) bluefish and B) summer flounder. Curves were estimated using (relative prey mass / handling time x capture success) for all relative predator-prey size ratios between 0.05 and 1.00. Solid lines show profitability estimations of the full model, and dashed lines show profitability with capture success removed. Note differences in scale in the y-axis.

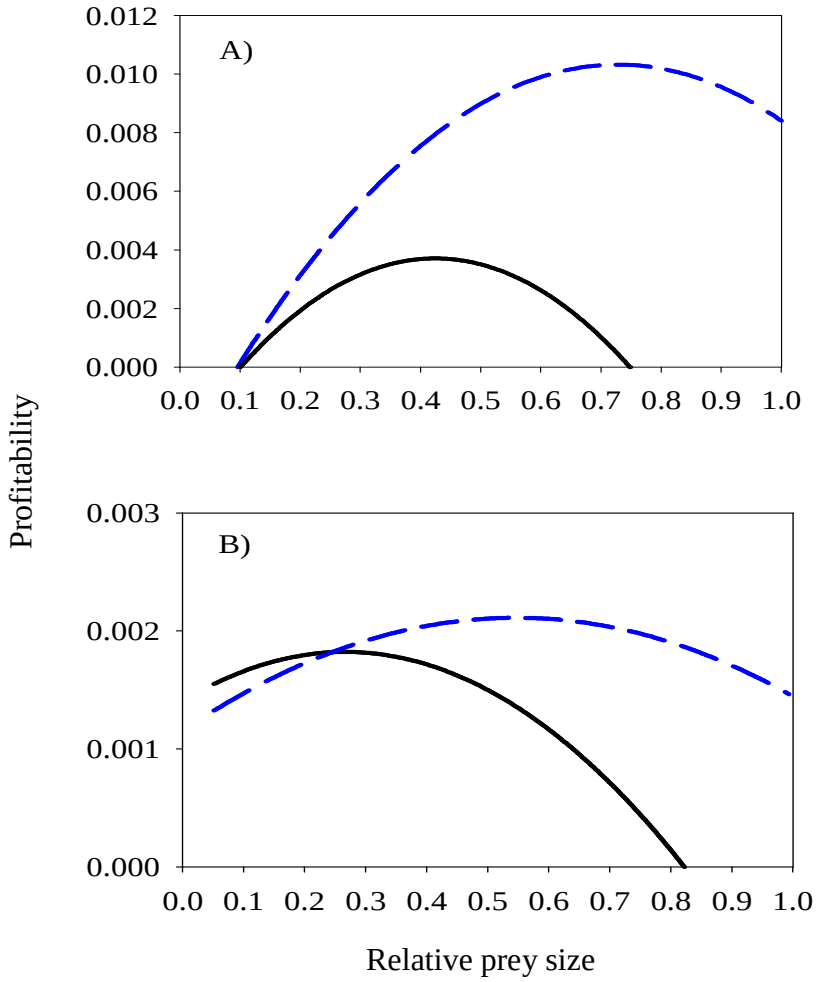


Figure 3.6: Size-dependent survival rates of longfin inshore squid in the presence of A) bluefish and B) summer flounder. Survival curves were estimated using Cox regression analysis in SAS. Significant differences were detected among survival rates of relative size groups in the presence of bluefish (X^2 = 6.90, *df* = 2, *p* = 0.009) but not flounder (X^2 = 2.02, *df* = 3, *p* = 0.16). Relative size groups tested for bluefish ranged from 0.10-0.39 and 0.20-0.59 for summer flounder and are denoted as 0.10-0.19 (solid line), 0.20-0.29 (long dash), 0.30-0.39 (dotted), 0.40-0.49 (short dash), and 0.50-0.59 (dash-dot-dot).

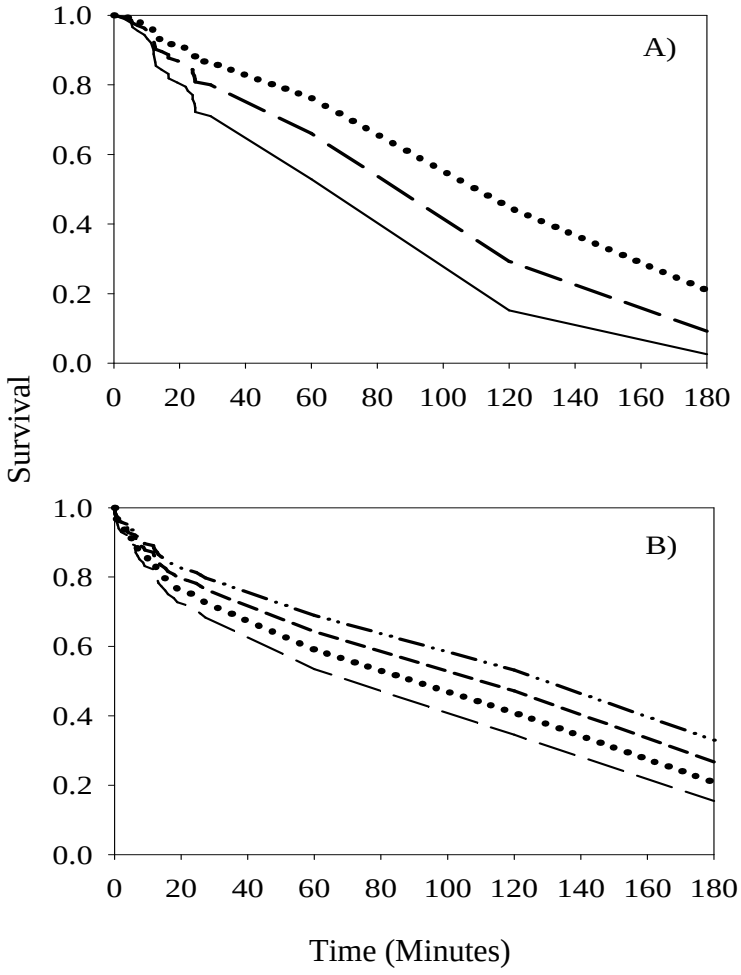
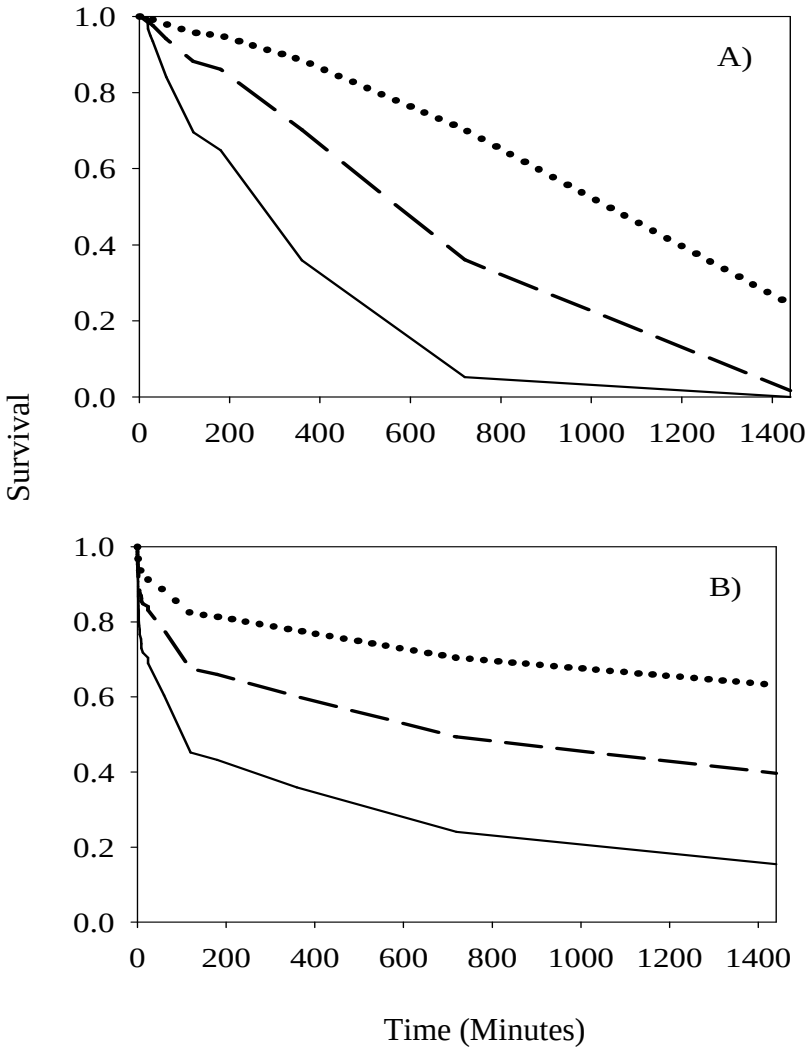


Figure 3.7: Time-dependent survival rates of longfin inshore squid (solid line), mummichogs (long dash), and Atlantic silversides (dotted line) in the presence of A) bluefish and B) summer flounder predators.



CHAPTER 4

ANTI-PREDATOR RESPONSES AND THEIR INFLUENCE ON SURVIVAL IN A COLEOID CEPHALOPOD

Abstract

In the present laboratory study, longfin inshore squid (*Loligo pealeii*) were exposed to two predators, bluefish (*Pomatomus saltatrix*) and summer flounder (*Paralichthys dentatus*), representing ambush and cruising foraging tactics respectively. Classification tree analyses were used to describe the relative influence of different anti-predator behaviors on squid survival, specifically deimatic (startle or threat), and protean (erratic) behaviors. Flight was identified as the strongest predictor of squid survival in the response to both predators. The use of other defense behaviors and their respective survival values depended on which predator was encountered. With bluefish, squid primarily used inking and a combined movement of dropping to the bottom with a disruptive body pattern to avoid predation. Reactivity, erratic jetting and scattering maneuvers were most influential in determining squid survival during interactions with flounder. Shoaling was found to increase the chances of squid survival in the presence of both predators. Results show that squid modify their behavior to different types of threats and provide the first survival values of specific anti-predator defense behaviors in a coleoid cephalopod.

Introduction

Predation is a constant source of risk for most animals. To maximize survival, prey have developed a wide repertoire of defenses ranging from physical armor, toxic chemicals, behavioral displays to evasive maneuvers (Cott 1940; Edmunds 1974; Bryan et al. 1997; Lenzi-Mattos et al. 2005; Speed and Ruxton 2005). Primary defenses are used to avoid detection and decrease encounter rates with potential predators, and are generally characterized by camouflage and cryptic behaviors (Endler 1991). When attack is imminent, secondary defenses are employed to delay, inhibit, or escape from an approaching predator and, in some cases, cause the predator to release a prey after it has been captured. The most common secondary defense is to flee (Humphries and Driver 1970; Eibl-Eibesfeldt 1975); however, direct interactions with predators are often unavoidable (Lingle and Pellis 2002; Edut and Eilam 2004). As a result, prey may attempt to startle, threaten, or confuse a predator with defensive postures and erratic behaviors (Humphries and Driver 1970; Edmunds 1974; Driver and Humphries 1988).

Deimatic defenses are sounds, displays, and postures that intimidate or bluff (Young 1950; Edmunds 1974). Defensive eyespots are a well known example of a deimatic display and are found in a variety of taxa including frogs (Lenzi-Mattos et al. 2005), cuttlefish (Hanlon and Messenger 1996), butterflies, and moths (Vallin et al. 2005, 2007). Depending on the capabilities of the prey, deimatic displays may signal a warning of true danger or attempt to deceive a predator into believing there is danger; either way, the intention of such displays is to cause predators to hesitate or abandon their attacks (Humphries and Driver 1970; Hanlon and Messenger 1996). Protean behaviors are defined as escape behaviors that are erratic and unpredictable (Humphries and Driver

1967). The term “protean” was inspired by the Greek sea-god Proteus, who was known for his abilities to change form (Chance and Russell 1959). Examples of protean defenses include use of color or body patterns to change appearance as seen in cephalopods (Hanlon and Messenger 1996), irregular movements such as freezing and fleeing in rodents (Edut and Eilam 2004), unsystematic escape trajectories in insects (Domenici et al. 2008), and similar examples in many taxa (Driver and Humphries 1988). Since predator-prey encounters may be incidental (e.g., the predator is not hungry) (Stankowitch and Coss, 2006), deimatic and protean behaviors may also be effective in assessing risk and testing predator motivation (Edmunds 1974).

Formation of large groups is another type of anti-predator defense that is used by both terrestrial and aquatic animals. In particular, fish and cephalopods often rely on schooling or shoaling to increase vigilance and confuse or overwhelm approaching predators (Shaw 1978; Pitcher and Parrish 1993; Jeschke and Tollrian 2007). Predator success generally declines with increasing prey group size (Neill and Cullen 1974; Landeau and Terborgh 1986), but recent evidence suggests that schooling and shoaling may be less effective in safeguarding against ambush predators compared to attacks from searching predators (Krause et al. 1998; Botham and Krause 2005). Many prey are likely to encounter both chasing and lie-and-wait predators, and their behavior should be flexible enough to respond appropriately to these different types of threats. The decision of which defense response to employ presumably depends on the type of predator, the severity of the threat, and the environmental factors surrounding the encounter; for example, a visual display may be less effective at night (Martins 1989; Hoverman and Relyea 2007). Since the effectiveness of anti-predator defenses may also be contingent

on whether they are performed by individuals or in groups, particular behaviors may be utilized more often depending on the context of the interaction.

Coleoid cephalopods are prey to a variety of marine vertebrates including fish, mammals, and birds. Because they are soft-bodied and lack hard protective structures such as spines and shells, coleoid cephalopods have evolved a wide range of other primary and secondary defense behaviors (Packard 1972; Hanlon and Messenger 1996). Most notable are their advanced abilities to color change and camouflage (Hanlon 2007; Barbosa et al. 2008; Mathger et al. 2008), exhibit postural displays (Hanlon et al. 1999; Bush et al. 2009), and use ink to confuse both menacing predators and conspecifics (Wood et al. 2008). While cephalopods are known to use deimatic and protean displays towards predators (Hanlon and Messenger 1996; Adamo et al. 2006; Langridge 2009), few studies have evaluated the conditions and types of predators that evoke these defenses during predator-prey interactions. Further, direct observations of predator responses to cephalopod deimatic and protean displays and the survival value of these defenses have not been measured experimentally (Hanlon and Messenger 1996).

Hanlon and Messenger (1996) hypothesized that cephalopods would principally rely on crypsis to avoid detection by predators and when primary defense failed, flight or deimatic displays would be employed followed by protean behaviors in a combined effort to prevent, misdirect, or delay an impending attack (Hanlon and Messenger 1996). In this study, I test this model by applying a laboratory-based approach using longfin inshore squid (*Loligo pealeii*) as prey, and evaluate the survival value of key defense behaviors in response to two predators representing contrasting foraging tactics. Bluefish (*Pomatomus saltatrix*) and summer flounder (*Paralichthys dentatus*) are natural predators

of longfin squid in North Atlantic waters (Staudinger 2006) and were chosen to represent cruising and ambush foraging modes, respectively. Classification tree analysis was used to 1) identify the series of anti-predator defense behaviors that best predicted squid survival in the presence of each predator, 2) assess whether deimatic or protean defenses were better predictors of squid survival, and 3) determine whether shoaling influenced survivorship. This particular analytical technique was chosen so that I could describe the influence of multiple factors on squid survival simultaneously.

Methods

Animal maintenance

Behavioral trials were conducted over a two-year period at the Marine Resources Center of the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts. Bluefish ranging in size from 31 – 63 cm total length (TL), and summer flounder 36 – 47 cm TL, were collected from local waters and transported back to the laboratory. Predators acclimated to captivity for approximately 1 month prior to use in behavioral experiments and were maintained on a diet of frozen and live fish and squid. Longfin inshore squid 2 – 21 cm Mantle length (ML) were collected from Vineyard and Nantucket Sounds using a modified trawl net. Squid were transported back to the MBL in a live-well tank and transferred either directly into the experimental tank or into a temporary holding tank. When it was necessary to hold squid overnight, they were fed live fish and small squid. Squid were handled as little as possible to avoid imposing further stress post-capture, and transferred between tanks in containers filled with seawater to minimize exposure to air. No squid were held in captivity for more than 48

hours, and only individuals that were robust and showed little to no obvious physical distress were chosen for behavioral trials.

Experimental design

All trials were conducted in a 28 x 10^3 Liter, 3.1 x 0.8 m (diameter, height) round tank filled with recirculating, and biofiltered seawater maintained between 16° and 20° Celsius. The bottom of the experimental tank was lined with a mixed gravel and sand substrate approximately 2 - 4 cm deep. This allowed squid to rest on the bottom and camouflage and also allowed flounder to bury. The area surrounding the experimental tank was lined with black plastic sheeting to prevent disturbance to acclimating animals and during filming. The tank was illuminated by natural light from adjacent windows and during filming by two 500 watt lights positioned above the tank.

Twenty-four hours prior to each trial, three predators of approximately the same size were introduced into the experimental tank and food was withheld to standardize hunger levels. Three hours prior to the start of each trial an opaque PVC cylinder, 1.5 meters in diameter and one meter in height was lowered into the experimental tank, 15 squid were then placed into this inner partitioned area and allowed to acclimate. At the start of each trial, the cylinder was hoisted above the tank using pulley system, and predators and prey began to interact. Trials lasted 30 minutes. Behavioral interactions were recorded using Panasonic miniDV PV GS500 video cameras that were manually operated at two lateral viewing windows, and from a third camera mounted above the experimental tank.

Squid that survived were not used in multiple trials; however repeated use of predators was necessary due to the difficulties of obtaining and maintaining large numbers of fish in the laboratory. To determine whether successive use of predators over the course of all trials affected feeding behavior, statistical analyses examined whether groups of predators, identified by size, demonstrated significant changes in feeding saturation (attack rates) or feeding efficiency (capture success). A Friedman's two-way ANOVA was performed to test treatment differences using a randomized complete block design (Zar 1984). Fish groups were treated as subject variables and the number of days used in trials was assigned as the treatment variable. All tests yielded p-values > 0.18, suggesting that repeated use in trials did not influence bluefish and flounder feeding behaviors.

Behavioral variables

All predator-prey behaviors were assessed using frame-by-frame analysis of video recorded during each trial. For each predator-prey interaction, the sequence of anti-predator behaviors exhibited by squid was recorded as well as the location in the tank where the attack occurred, the tactic used by each predator to attack squid, and whether the attack occurred on a solitary individual or shoals of squid. The outcome of each interaction was classified as either 1) mortality due to predation, 2) an escape whereby a predator executed an attack but the squid was successful in evading the predator, or 3) an attack abandoned by the predator. In abandoned attacks, predators oriented towards prey and initiated an aggressive movement towards prey but did not open their mouths or complete their attacks. For example, bluefish were observed to orient towards an

individual or group of squid but then turn away during the final approach. Summer flounder often reduced swimming speeds and sometimes glided through the water several body lengths away from the targeted squid (Chapter 2).

Attack locations were differentiated as occurring either on the substrate, in the lower third of the water column, mid third of the water column, or upper third of the upper water column. Attacks on the substrate were separated from lower water column attacks if squid were resting on the bottom compared to actively swimming in the water column just above the substrate. Bluefish used two general modes of attack; if a squid was on the substrate, bluefish would deeply angle their bodies downwards, and grab squid off the bottom. All other attacks were made by bluefish that were actively swimming at different depths throughout the water column. Flounder attacks were classified as ambush, active, or stalking depending on whether the fish initiated an attack from a stationary position, while actively swimming in the water column, or creeping along the substrate (Chapter 2). Shoaling indicated whether an attack was made by a predator on a solitary squid or a shoal of squid. I used the term shoal rather than school because squid did not always swim parallel to each other and were often oriented in different directions (Pitcher and Parrish 1993).

Squid behaviors were classified as either primary or secondary defenses. Squid that were camouflaging on the substrate for ≥ 1 minute were considered to be using primary defense. All other behaviors were classified as secondary defenses. Deimatic and protean displays were divided into 4 categories including body pattern, locomotor, movement, and postural (Table 4.1). Detailed descriptions of protean and deimatic body patterns were prohibitive due to the large size of the tank, obscured vantage points, and

inconsistent water clarity; therefore I only distinguish between deimatic disruptive body patterns that generally consisted of amber and pink colorations and often included banding, and protean color changes that were limited to All dark (red), or Clear (white) as seen in the blanch-ink-jet maneuver (Hanlon et al. 1999). The deimatic body pattern categorized as disruptive on the substrate was essentially the same as the body pattern used for primary defense however deimatic body patterns were displayed in concert with the protean movement of dropping to the bottom and displayed for less than a minute (Hanlon et al. 1999). Squid displaying the upwards v-curl display raised their arms in a "V" posture and exposed their beaks (Figure 4.1) (Hanlon and Messenger 1996). During vertical hanging displays squid drooped their arms below their body with their posterior end higher in the water column than their head and arms; this postural component was often exhibited with an All dark body pattern (Figure 4.1). The tentacles extended display was similar to the vertical hanging display except their tentacles drooped below their bodies in addition to the arms, and was often accompanied by some form of flight. Protean "movements" were characterized by fin swimming while protean "locomotor" behaviors involved jet propulsion.

Statistical analyses

Classification tree analysis was used to explore three questions: 1) what anti-predator defense behaviors best explain survival or mortality in the presence of each type of predator? 2) Are deimatic or protean defenses better predictors of squid survival in the presence of bluefish and flounder, and 3) does shoaling influence survival? To answer the first question, classification trees were built for each predator using all independent

variables listed in Table 4.1. Classification trees evaluating deimatic and protean behaviors were built using a subset of variables related to body pattern, postural, locomotor, and movement behaviors. The influence of shoaling was assessed by examining variable importance in the two preceding analyses and by building classification trees using a subset of contextual variables that included shoaling, location of attack, and predation tactic.

Classification tree analysis is a nonparametric, rank-based discrimination procedure that explains differences among pre-specified groups and has the ability to test the significance of overall group classification (McGarigal et al. 2000). Classification tree analysis works by recursively partitioning data into groups that are increasingly more homogeneous using split-values of the explanatory values, and that maximize within-group homogeneity and among-group heterogeneity according to a pre-specified information index. Trees are typically overgrown and then "pruned" back to a smaller tree size (according to the number of terminal nodes, also called "leaves") that has the minimum honest estimate of true (prediction) error which is determined by a cross-validation procedure.

Classification tree analyses were employed in this study for several reasons. First, the data contained both continuous and categorical explanatory variables, which many parametric statistical methods are unable to manage. Second, classification tree analyses have the ability to explain non-homogenous relationships between explanatory and response variables, which are a common occurrence in studies such as ours. Third, results from classification tree analyses are easily interpretable and can be used effectively and efficiently for predictive purposes.

In this study I conducted classification tree analysis using the “cartware” package under the “rpart” library in the R computing environment (version 2.9.2, Free Software Foundation, Inc., Boston MA). Specifically, I built trees using splits as determined by the Gini information index and pruned our final trees using a 10-fold cross-validation procedure by employing the 1-S.E. rule (De'ath and Fabricius 2000). Additionally, I assessed the statistical significance of each classification tree using a Monte Carlo permutation procedure using 100 permutations. By applying classification trees analysis to our data, I was able to describe the influence of multiple explanatory variables on squid survival from experimental trials.

Results

All behaviors

A total of 86 behavioral interactions were evaluated between squid and bluefish and 109 interactions between squid and flounder. The majority of interactions with both predators resulted in mortality (Table 4.2). During interactions with bluefish, squid survival was more often the result of abandoned attacks; conversely, squid survived more interactions with flounder by escaping attacks. In nearly a third of all interactions with flounder, squid showed no reaction and experienced high mortality during these events (Table 4.3). Although squid were more reactive to bluefish they succumbed to higher rates of predation overall. Primary defense was used more often in the presence of bluefish yet this strategy was less effective in ensuring survival in comparison to when it was used in the presence of flounder (Table 4.3).

When all behavioral and contextual variables were included in classification tree analyses, a 2 leaved tree (Kappa = 0.241, $p = 0.031$) was formed for bluefish (Figure 4.2A) and a 3 leaved tree (Kappa = 0.495, $p < 0.01$) formed respective to flounder (Figure 4.3A). In both analyses, flight was classified as the best predictor of squid survival. Squid that did not flee ≥ 10 body lengths had a 57% (40/70) probability of being eaten by bluefish (Figure 4.2A) and a 67% (45/67) probability of being eaten by flounder (Figure 4.3A). If squid fled distances ≥ 10 body lengths, there was a 69% (11/16) probability that bluefish would not pursue squid and abandon their attacks (Figure 4.2A). In the presence of flounder, squid had the highest probability 89% (8/9) of survival if they were attacked near the bottom and fled distances ≥ 10 body (Figure 4.3A).

It was suspected that flight was masking the influence of other behavioral variables of interest; consequently, this variable was removed and alternative trees were built for each predator using the remaining 13 explanatory variables. The resulting tree for bluefish formed a 3 leaved tree (Kappa = 0.337, $p = 0.012$) and inking was selected as the primary splitting variable (Figure 4.2B). When flight was not considered, squid that inked had a 69% (11/16) probability of survival during interactions with bluefish due to abandon attacks (Figure 4.2B). Squid that did not ink, had the highest probability of survival (abandoned attack = 78%, 7/9) when the extended tentacles display was performed, and the lowest probability of survival (mortality = 61%, 37/61) when upward v-curl and vertical hanging postures were displayed towards bluefish.

When flight was withheld from the flounder analysis, a tree including 6 leaves was formed (Kappa = 0.534, $p < 0.01$) predicting that when squid did not react to an

approaching flounder there was an 84% (27/32) probability of mortality (Figure 4.3B). For squid that did react, survival was predicted by the combined influence of attack location, predation tactic, and shoaling. When flounder used ambush attacks, solitary squid that were attacked in the mid to upper water column had higher chances of survival (64%, 8/12) by evading attacks than solitary squid attacked near the bottom (probability of abandoned attack = 60%, 3/5). Overall, shoaling squid had the highest chances of survival (probability of abandoned attack = 63%, 19/30) particularly when they were ambush attacked by flounder in the mid to upper water column.

Deimatic and protean defenses

Classification trees built using only deimatic and protean variables (body pattern, postural, locomotor, and movement displays) formed root-trees (size = 1) and were unable to successfully cross-validate. This result may have been due to low or disparate observations of deimatic and protean behaviors. Another possibility was that deimatic and protean behaviors were strongly linked to other factors (e.g., location of attack). To test this, alternative trees for each predator were built by interchanging shoaling, attack location, and predation tactic. Ultimately, the only trees to successfully cross-validate were those that included shoaling. The resulting classification tree for bluefish formed a 3 leaved tree (Kappa = 0.349, p = 0.042) and predicted squid survival based on shoaling and protean movement (Figure 4.4A). Overall, solitary squid were most likely to succumb to predation by bluefish (probability of mortality = 59%, 24/41). Squid in shoals that tightened their formation in response to an approaching bluefish were almost certainly eaten (100%, 7/7). In contrast, when squid moved to the surface or dropped to

the bottom in response to bluefish, there was a 63% (24/38) probability of survival due to abandoned attacks.

The classification tree for flounder formed 5 leaves (Kappa = 0.389, $p < 0.01$) corresponding to protean locomotor and body pattern displays (Figure 4.4B). Squid that used the blanch-ink-jet display had a 64% (9/14) probability of survival due to an abandoned attack (Figure 4.4B). If erratic jetting was exhibited survival was more probable (62%, 9/13) when squid also displayed rapid color changes in comparison to when erratic jetting was used alone (probability of mortality = 58%, 38/65). Conversely, squid that jetted out of the water or scattered in response to flounder were more likely to survive (67% (4/6) probability of abandoned attack, 75% (6/8) probability of escape) than squid that jetted erratically, regardless of whether they exhibited rapid color changes (Figure 4.4B).

Shoaling

Classification trees evaluating the affects of shoaling formed 4 leaves (Kappa = 0.394, $p < 0.01$) for bluefish (Figure 4.5A) and 6 leaves (Kappa = 0.435, $p < 0.01$) for flounder (Figure 4.5B). During interactions with bluefish, squid survival depended on the depth at which shoals or solitary squid were attacked (Figure 4.5A). The probability of mortality was highest (83%, 19/23) when solitary squid were attacked by bluefish on the substrate. Shoaling squid were most likely to succumb to predation (59%, 13/22) when bluefish attacked them at the surface. Results for flounder were similar to the first classification tree (Figure 4.3B); solitary squid experienced a high probability of mortality (57%, 4/7) when ambush attacked near the bottom, and shoaling squid were

likely to survive (probability of abandoned attack = 58%, 19/33) when attacked in the upper third of the water column (Figure 4.5B).

Discussion

Previous studies have shown that coleoid cephalopods respond to potential predators with a variety of anti-predator behaviors (Hanlon and Messenger 1996; Adamo et al. 2006; Langridge 2009), but to the best of our knowledge this is the first study to predict and measure the survival values of key defense behaviors based on actual predation. Our results suggest that flight was the most important anti-predator defense used by squid in response to bluefish and flounder. This was the only behavior that yielded similar outcomes of survivorship in the presence of either predator. Other defensive behaviors appeared to be adapted to each type of predator and the survival value of these behaviors was contingent on which predator was encountered.

Predator type differences

Hanlon and Messenger (1996) suggested that because coleoid cephalopods lack physical defensive structures, the primary defense of camouflage in its many manifestations is used extensively to avoid visual predators. In this study, camouflage via disruptive body patterns was used frequently by longfin squid in response to bluefish; however, because of the high mortality rates associated with this display during interactions with bluefish and the low occurrence of primary defense with flounder, camouflage was not identified as a strong predictor of survival in the presence of either predator. The restricted space and artificially high light levels of the laboratory setting

may have given bluefish a predatory advantage by facilitating detections of camouflaging squid. The experimental arena also lacked structural complexity that can affect probabilities of prey capture (Michel and Adams 2009). Under natural conditions, camouflage may be more effective in preventing detection or recognition of squid by bluefish as well as other visual predators because near field (foveal) versus far field (or peripheral) vision have different sensing capabilities.

During encounters with flounder, squid spent less time on or near the substrate and primary defenses were observed less often than in trials using bluefish. Squid behavior was likely influenced by flounders' strong association with the bottom and the high predicted risk of mortality when attacked in the lowest portion of the water column. Contextual factors were better predictors of squid survival than anti-predator behaviors during encounters with flounder. Of paramount importance was whether squid reacted at all to an approach by flounder, and whether flounder used ambush or active attack tactics. This suggests that predator behavior, specifically the tactic used to hunt prey, may be more influential on squid survival than anti-predator behaviors used during interactions with ambush predators.

Squid reacting to disturbances under more natural conditions showed similar reactions to those observed in the present study. Stevenson (1934) reported that *L. pealeii* scattered or jetted away from the source of a threat or in more extreme situations dropped to the bottom, camouflaged in a disruptive pattern (with transverse dark bars), and remained motionless. Hurley (1978) observed *L. opalescens* to tighten their school formation and move a short distance away from potential predators. The two accounts took place in shallow (Stevenson 1934) and deep water environments (Hurley 1978)

respectively, and provide evidence that squid respond differently to threats of predation in part based on the environmental conditions surrounding the interaction (Martins 1989; Hoverman and Relyea 2007).

Deimatic and protean defenses

Deimatic behaviors are thought to startle, threaten, or frighten predators into hesitating or abandoning their attacks (Edmunds 1974). Recent studies have shown that prey do not always perform deimatic displays to all types of predators (Sherbrooke 2008), and displays vary in their effectiveness against different predators (Vallin et al. 2005, 2007). Deimatic displays were more important during encounters with bluefish but did not always guarantee survival. Upward v-curl and vertical hanging postures were less effective than the tentacles extended display in intimidating bluefish. The tentacles extended posture was often used in conjunction with flight and may explain the higher probabilities of survival associated with this behavior. In comparison, upwards v-curl and vertical hanging postures were primarily displayed from stationary positions near the surface. Arm postures were successful in causing a percentage of attacks to be aborted and suggest that deimatic displays are an effective anti-predator strategy in longfin squid. Our findings contrast with the assertion made by Langridge (2009) that coleoid cephalopods do not display deimatic responses towards highly dangerous predators.

Protean behaviors function by confusing approaching predators and impairing their ability to predict prey escape trajectories or positions (Humphries and Driver 1967; Driver and Humphries 1988). During high-intensity interactions with flounder, protean locomotor behaviors were more important in predicting squid survival than during

encounters with bluefish. Squid that jetted out of the water, scattered, or used the blanch-ink-jet maneuver in response to flounder had higher probabilities of survival than mortality. These behaviors were often performed near the surface where flounder were generally less efficient at capturing squid. In continental shelf waters of the northwest Atlantic, squid and flounder have the highest spatial overlap during the day-time when squid are very near or resting on the bottom (Macy 1982; Staudinger 2006). Consequently, reaction distances and times may be shorter and affect escape probabilities, particularly to ambush attacks by flounder.

Swimming speed, directness of approach, and predator eye movements were not measured in this study but have been shown to affect anti-predator tactics and flight initiation distances (Stankowich and Blumstein 2005; Stankowich and Coss 2006). Evaluations of predator-prey responses were limited to interactions where fish exhibited some form of orientation or approach towards squid and behaviors could be interpreted as reliable signals of predatory intent. In addition to behaviors shown in direct response to an approaching predator, deimatic postures and body patterns were also displayed towards predators that demonstrated no immediate threat and were swimming or resting on the substrate below squid. Since deimatic displays were successful in deterring direct attacks, it is possible that these behaviors may also have influenced predators at times that were not obvious to us. In previous studies, cuttlefish have also shown deimatic displays to non-predators that swim nearby (Hanlon and Messenger 1988; Langridge 2009). Alternatively, squid may have used deimatic and protean behaviors to test the motivational state of predators and assess risk (Edmunds 1974).

The influence of shoaling

Increasing group size has been shown to decrease the risk of predation by increased vigilance and decreasing predator capture success in a wide range of taxa (Neill and Cullen 1974; Krause and Ruxton 2002). Shoaling may be less effective against ambush or stalking predators (Krause et al. 1998), and some predators may be attracted to large groups more than solitary individuals due to their conspicuousness especially in environments where visibility is poor (Botham and Krause 2005). Our results suggest that solitary squid experienced higher probabilities of mortality in comparison to shoaling squid in the presence of both active and ambush predators. Furthermore, anti-predator behaviors that increased survivorship for groups of squid did not necessarily provide the same protection for solitary individuals. Solitary squid that dropped to the substrate and camouflaged in the presence of bluefish were extremely vulnerable to predation. In contrast, shoaling squid had the highest probabilities of survival when attacked at the same locations. When predators did attack shoals, bluefish appeared to target individuals on the peripheries and flounder often attacked the leading squid as a shoal moved above the predator lying in wait. These observations agree with conclusions of previous studies that although groups provide protection from predation, the benefits are not necessarily equal for all members (Krause et al. 1998; Stankowich 2003).

Other defenses

In cephalopods that inhabit shallow-water environments, inking is thought to function primarily as a visual display to predators and as an alarm cue to conspecifics (Hanlon and Messenger 1996; Wood et al. 2008). Ejecting ink increased the probability

of survival in the presence of bluefish but was less effective during interactions with flounder. Bluefish were observed to turn at acute angles away from ink plumes ejected by retreating squid and abandon their approach; conversely, flounder appeared relatively unaffected. Investigations using the squid *Sepioteuthis sepioidea* demonstrated that the chemical properties of ink were important in evoking alarm behaviors in conspecifics (Wood et al. 2008). It was beyond the scope of this study to determine whether squid ink functioned primarily as a chemical or visual defense against predators; however, our results suggest that the effectiveness of inking as an anti-predator defense differs between predator species.

Although flounder did not appear to be inhibited by inking, acute body patterns used in combination with protean locomotor behaviors increased squid's probabilities of survival. Cuttlefish are known to display acute body patterns with deimatic postures while feeding and in response to model predators (Adamo et al. 2006), yet this is the first direct evidence that these behaviors may also enhance survival during predator-prey interactions. Specific body patterns were not evaluated in this study due to inconsistent water clarity and obscured views of squid behaviors; however, it is noteworthy to mention that ventral banding and lateral stripe displays were observed in combination with upwards v-curl and vertical hanging postures (Figure 4.1). Countershading has been observed during feeding (Ferguson and Messenger 1991) and mating (Hanlon et al. 1999) but its function is largely unknown and requires further investigation. Because ventral body patterns would be most visible to predators swimming below squid, it seems likely that benthic predators are the targeted recipient of these displays. It is also possible that acute body patterns exhibited during predator-prey interactions served as alarm signals to

conspecifics as well as deterrents to approaching predators (Hanlon and Messenger 1996).

Aggressive defense has never been directly observed in cephalopods, but accounts of scarring found around the jaws and mouths of marine mammals have provided evidence of struggles with cephalopods (Lockyer and Morris 1985; Clarke 1986b). There has been speculation as to whether these wounds were inflicted by the cephalopod in an effort to escape or due to an aggressive attack (Roper and Boss 1982). During several high-intensity interactions with flounder, squid wrapped their arms around the operculum or head of the attacking flounder, displayed All dark body patterns, and often ejected copious amounts of ink. The flounder prevailed in all cases and squid were consumed; however, this appeared to require a great energetic cost to the fish. To complete the capture cycle, flounder thrashed and shook the squid violently, perhaps to stun the prey. Squid were clearly attacked first and retaliation was used as a last attempt to force a predator to release them. This behavior may be rare or not possible against all predators since no occurrences were observed during interactions with bluefish; however these few instances suggest that squid will continue to display anti-predator defenses even when death is imminent.

Conclusions

Squids and other soft-bodied coleoid cephalopods have evolved a wide range of behaviors to protect themselves against predators. Although no single defense behavior guaranteed survival, there were clear advantages of using certain behaviors over others during confrontations with each type of predator. Flight substantially improved overall

survival rates and is likely the most frequent response when primary defense fails (Driver and Humphries 1988; Eibl-Eibesfeldt 1975). Because flight may not be possible in all situations (e.g., predator is detected too late to escape), squid may instead use behaviors that attempt to startle or confuse predators and deter repeated attacks. Hanlon and Messenger (1996) hypothesized that when primary defense fails, cephalopods will either flee, or show deimatic displays and then use protean escape maneuvers. Strong links between deimatic and protean behaviors were not found in the present study and suggest that squid do not necessarily use these behaviors together or in succession. Deimatic displays may be used alone, with protean behaviors, or in combination with other behaviors such as inking and flight. Many of the anti-predator behaviors evaluated in this study could be classified into one or more categories (Hanlon and Messenger 1996); for example, inking could be classified on its own (as was done here) or as a protean defense. Thus, the category of defense behavior used for assessing squid survival may be less important than the specific behavior identified by the analyses. The defense behavior or combination of behaviors elicited by squid in response to a threat was flexible and will likely fluctuate depending on a variety of factors including predator type and the surrounding environmental conditions. It is uncertain if the anti-predator responses shown towards bluefish and summer flounder are species-specific or are typical to other demersal and pelagic predators. Future studies that test additional squid-predator combinations are needed to resolve this question. Laboratory experiments may provide an oversimplified view of species interactions and are only a first step to understanding the survival value of cephalopod defense tactics. Additional studies conducted in natural

habitats are necessary to gain further insight into anti-predator behaviors as well as predator responses.

TABLES

Table 4.1: List of independent variables used to predict longfin squid mortality (1) and survival (0, 2) in classification tree analyses. Variable types are denoted by C = categorical and N = numeric. Abbreviations listed in variable descriptions correspond to classification trees in Figures 2 - 5.

Independent variables		Type	Description
Location of attack		C	Substrate (sub), lower water column (lo), mid-water column (mid), upper water column (up)
Predation tactic		C	*Bluefish*: Grab, active *Summer flounder*: Ambush (amb), active (act), intermediate (int)
Shoaling		C	Solitary (solo), shoaling (shoal)
Squid behavioral defenses			
No defenses observed		N	No Reaction
Primary defense		N	Camouflage on substrate
Secondary Defenses			
Deimatic	Body pattern	C	Disruptive in the water column, disruptive on substrate
Deimatic	Locomotor	N	Orient towards predator
Deimatic	Postural	C	Tentacles extended (tent), upward v-curl (upv), vertical hanging (verthg.)
Protean	Movement	C	Drop to substrate (drop), move to surface (mvsu), tighten schooling (tight)
Protean	Locomotor	C	School scatter (scatt), erratic jetting (err), jet out of water (oow), ink-blanch-jet (blnch)
Protean	Body pattern	N	Rapid color change to All dark
Flight		N	Short distance (short), slow short distance (slow), long distance (flight)
Inking		N	Ink
Retaliation		N	Retaliation

Table 4.2: Outcomes of predator-prey interactions between longfin inshore squid, bluefish, and summer flounder. N = the number of observations and % = percentage of all observations resulting in squid survival and mortality.

Bluefish	N	%
Escapes	10	11.6
Mortalities	41	47.7
Abandoned attacks	35	40.7
Total interactions	86	

Summer flounder		
Escapes	35	32.1
Mortalities	46	42.2
Abandoned attacks	28	25.7
Total interactions	109	

Table 4.3: Results of predator-prey interactions between squid, bluefish, and flounder. N = occurrence of each behavior, % all interactions = percentage of all interactions where each behavior was observed, % mortality = percentage of interactions resulting in predation; WC = water column.

	Bluefish			Flounder		
Behavior	N	% all interactions	% mortality	N	% all interactions	% mortality
Location of attack						
Substrate	40	46.5	55.0	11	10.1	54.5
Lower-WC	0	0.0	0.0	26	23.9	53.8
Mid-WC	14	16.3	21.4	18	16.5	55.6
Up-WC	32	37.2	50.0	51	46.8	27.5
Predation tactic						
Active	45	52.3	42.2	61	56.0	31.1
Ambush				38	34.9	60.5
Grab; Stalking	40	46.5	55.0	6	5.5	33.3
Shoaling						
Solitary	41	47.7	58.5	39	35.8	46.2
Shoaling	44	51.2	38.6	66	60.6	39.4
No reaction to attack	13	15.1	69.2	30	27.5	86.7
Defenses exhibited	68	79.1	41.2	70	64.2	24.3
Primary defense	15	17.4	66.7	8	7.3	37.5
Deimatic defenses						
Body pattern	33	38.4	48.48	18	16.5	44.44
Locomotor	2	2.3	0.0	5	4.6	0.0
Postural	21	24.4	38.10	14	12.8	28.57
Protean defenses						
Body pattern	5	5.8	0.0	23	21.1	21.7
Movement	43	50.0	51.16	15	13.8	46.67
Locomotor	6	7.0	16.67	28	25.7	17.86
Flight	33	38.4	18.18	67	61.5	7.46
Inking	16	18.6	12.5	38	34.9	10.5
Retaliation	0	0.0	0.0	8	7.3	100.0

FIGURES

Figure 4.1: Deimatic postural and body pattern displays shown by longfin squid during predator-prey trials. A) Upwards v-curl with ventral banding B) vertical hanging with ventral stripe C) vertical hanging and All Dark body pattern. Image stills were taken from video recorded during behavioral trials.

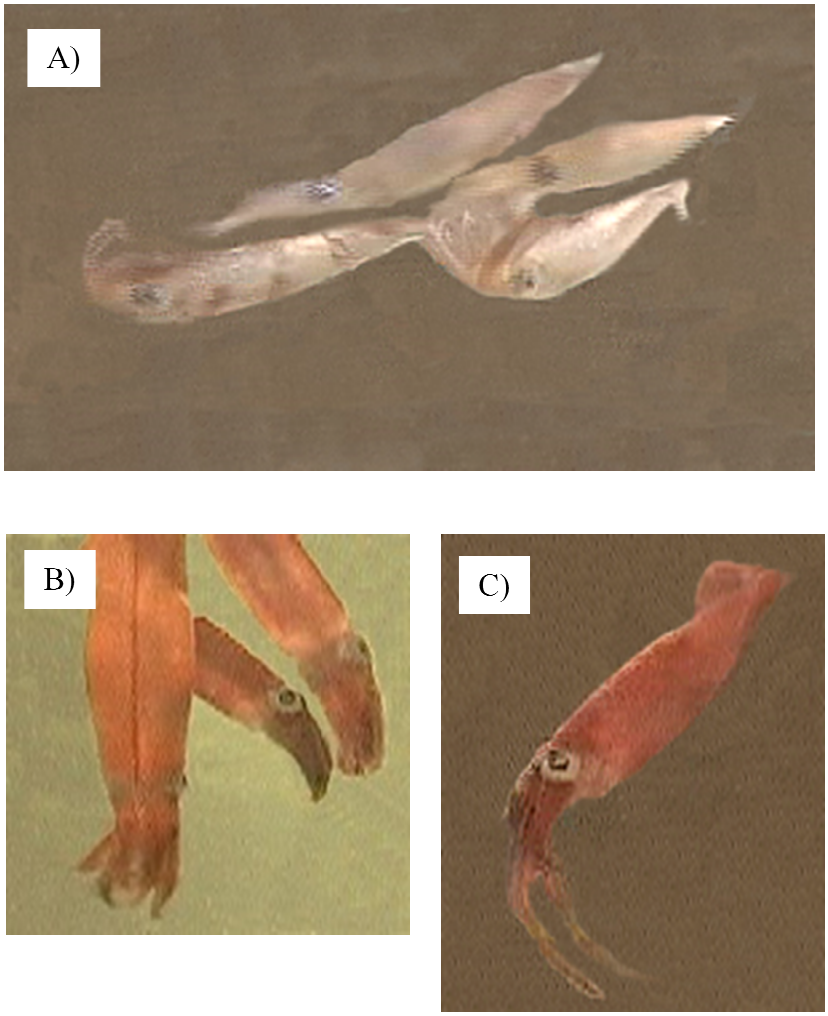


Figure 4.2: Classification trees including all behavioral and contextual variables used in response to bluefish; A) all variables listed in Table 4.1 and B) flight removed. Observations that are "true" for each splitting variable go to the left branch; for all other responses go to the right branch. "= yes" indicates that behavior was present, "= no" indicates the behavior was not present. Values located at the base of each leaf correspond to the response variables (0 = escape, 1 = mortality, 2 = abandoned attacks), the proportion of observations that were classified as the dominant response in each leaf, and the total number of observations in each leaf. All abbreviations are listed in Table 4.1.

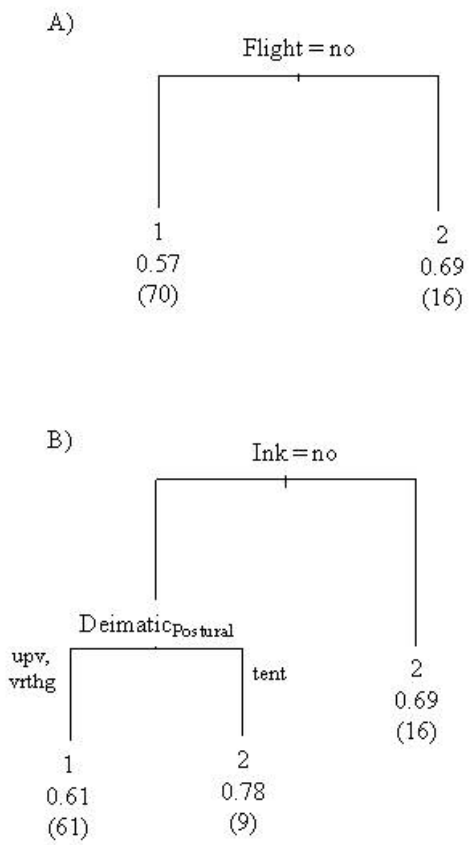


Figure 4.3: Classification trees including all behavioral and contextual variables used in response to flounder; A) all variables listed in Table 4.1 and B) flight removed. Observations that are "true" for each splitting variable go to the left branch; for all other responses go to the right branch. "= yes" indicates that behavior was present, "= no" indicates the behavior was not present. Values located at the base of each leaf correspond to the response variable (0 = escape, 1 = mortality, 2 = abandoned attacks), the proportion of observations that were classified as the dominant response in each leaf, and the total number of observations in each leaf. Group = prey group size, Tactic = predation tactic, Location = location of attack, Reaction = indicates whether squid reacted to a predator approach prior to an attack. All other abbreviations are listed in Table 4.1.

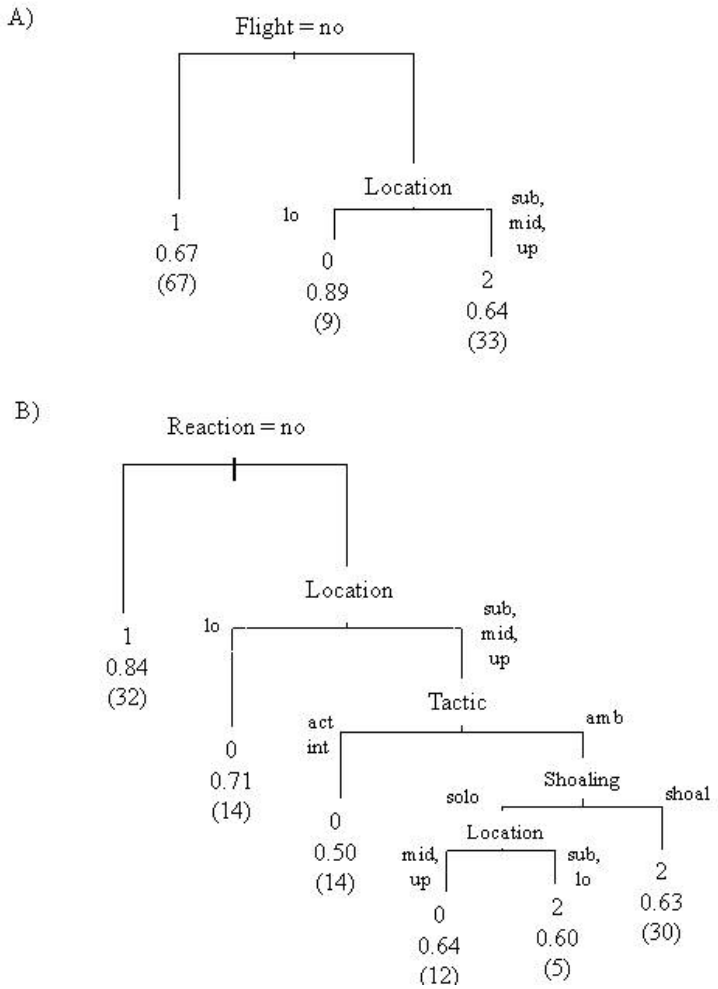


Figure 4.4: Classification tree describing the influence of deimatic, protean, and shoaling behaviors on squid survival when displayed towards A) bluefish and B) flounder. Observations that are "true" for each splitting variable go to the left branch; for all other responses go to the right branch. "= yes" indicates that behavior was present, "= no" indicates the behavior was not present. Values located at the base of each leaf correspond to the response variable (0 = escape, 1 = mortality, 2 = abandoned attacks), the proportion of observations that were classified as the dominant response in each leaf, and the total number of observations in each leaf. All abbreviations are listed in Table 4.1.

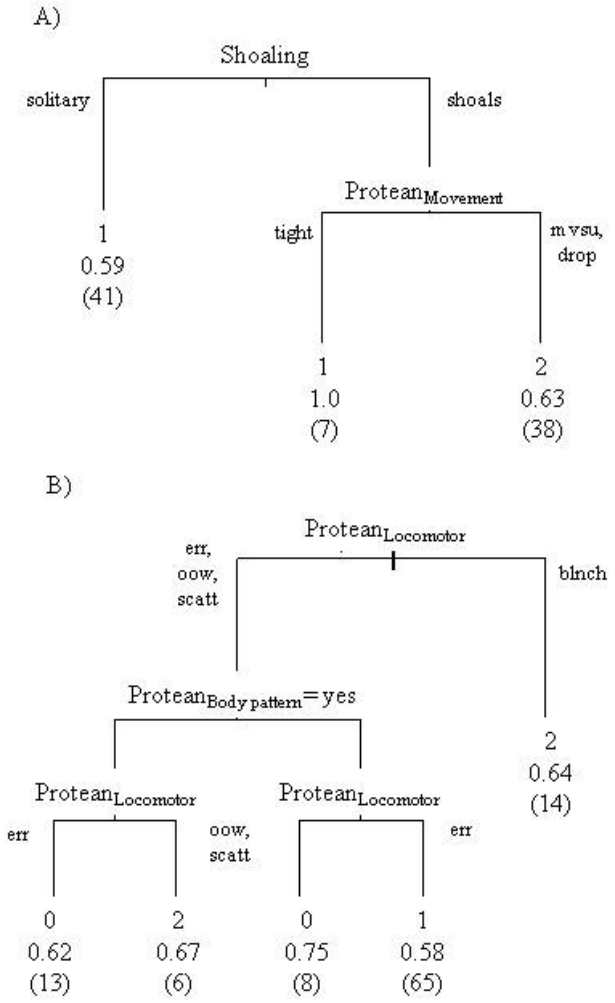
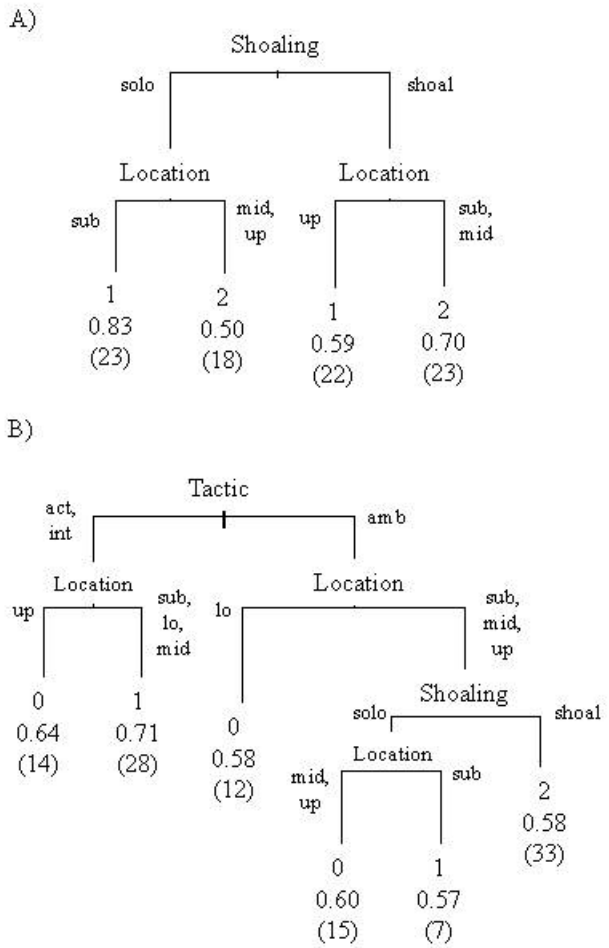


Figure 4.5: Classification tree describing the influence of attack location, shoaling, and predation tactic on squid survival by A) bluefish and B) flounder. Observations that are "true" for each splitting variable go to the left branch; for all other responses go to the right branch. "= yes" indicates that behavior was present, "= no" indicates the behavior was not present. Values located at the base of each leaf correspond to the response variable (0, 1, 2), the proportion of observations that were classified as the dominant response in each leaf, and the total number of observations in each leaf. Group = prey group size, Tactic = predation tactic, and Location = location of attack. All other abbreviations are listed in Table 4.1.



CONCLUDING REMARKS

This dissertation presents an overview of size-based predation on longfin inshore squid (*Loligo pealeii*). In Chapter 1 predator-prey body size data were used to construct a community view of size-based predation on longfin squid populations in the northwest Atlantic Ocean. Chapter 2 described feeding tactics used by summer flounder to capture squid and evaluated predator preference for squid and fish prey. Chapter 3 quantified behavioral factors influencing size-based predation on squid. Attack and capture behaviors were compared between bluefish and summer flounder and the influence of different foraging tactics were discussed. Chapter 4 evaluated whether squid modified anti-predator behaviors in response to bluefish and flounder and measured the effectiveness of these defenses in ensuring squid survival. Together, these studies outline how predators use squid as prey, identified behavioral and morphological limitations on predator selection, and evaluated how anti-predator defenses used by squid influence their vulnerability to predation.

Squid play a vital role in the northwest Atlantic ecosystem, serving as prey to a wide range of species, and also as predators on larval fish, conspecifics, and secondary consumers (Link et al. 2006). Knowledge of predator-squid relationships have been limited to quantifications of the amounts of squid consumed, while size-based consumption rates have largely been overlooked. This information is crucial to understanding what portion of the prey resource is being utilized by predators and to assess whether humans are competing directly or indirectly for similar squid resources through commercial exploitation (Livingston 1993). This dissertation provides a first step to resolving these shortcomings.

The original aim of Chapter 1 was to provide a review of size-based predation on longfin inshore squid as well as other ecologically and commercially important cephalopod species in the northwest Atlantic, particularly the northern shortfin squid (*Illex illecebrosus*); however, data on this species were scarce. Shortfin squid inhabit pelagic waters of the outer continental shelf and slope (Hendrickson 2004) and have been reported in the diets of large pelagic predators including tunas (Barr 1991), sharks (Kohler 1987), and billfishes (Stillwell and Kohler 1985). Knowledge of the trophic ecology of pelagic species is surprisingly poor. Ongoing efforts to collect new diet data from large pelagic predators are providing needed information on predator foraging habits and natural mortality rates on key prey species including shortfin squid. Preliminary data suggest that predators are consuming significantly larger shortfin squid than longfin squid (Staudinger, *unpublished data*) and size-based patterns of predation among cephalopod prey differ within predator species diets.

In Chapters 2 and 3, I demonstrated that there was a strong relationship between relative prey size and feeding success when squid were targeted as prey. Summer flounder were also found to show strong preferences for demersal prey types in comparison to pelagic species. Although the factors limiting each predator were different (capture success in bluefish and handling times in flounder), the results provide insight into why and how consumption patterns on squid and other forage species fluctuate with predator behavior and prey population abundance.

Chapter 4 provided the first evaluations of the survival values of anti-predator defense behaviors in longfin squid. Results add to mounting evidence that although squid are invertebrates, they are capable of complex behaviors and have the ability to adapt

their behavior to different predators. Deimatic and protean defense behaviors were successful in deterring a proportion of bluefish and flounder attacks and indicated that squid are capable of intimidating dangerous predators; however, squid mortality rates were generally high in the presence of bluefish and summer flounder. Overall, the results of Chapters 2, 3, and 4 suggest that squid survival and vulnerability were largely shaped by predator behavior and seemingly less so by the behavior of prey. Bluefish and summer flounder are particularly voracious and dangerous predators, therefore squid defenses may be more effective against other, less aggressive predators which they encounter in the northwest Atlantic, including cannibalistic conspecifics.

Previous studies have shown squid defense behaviors also function as alarm cues and antagonistic displays towards conspecifics (Hanlon et al. 1999; Wood et al. 2008). It is possible that behaviors observed in the present study could have a dual function when roles are reversed and squid become the predators (Vovk 1985; Rodhouse and Nigmatullin 1996). The limits of predation on squid were explored in this dissertation, but to fully comprehend squid's trophic role in the northwest Atlantic, additional studies are needed to determine the relative size window when predator-prey role switching occurs between squid and fish. This dissertation may provide a framework for future studies seeking to evaluate predator-prey relationships between additional species of predators and cephalopods, as well as in other marine systems.

Harvesting marine populations that occupy lower trophic levels may have unintended consequences and result in trade-offs between ecological and economic goals (Pauly et al. 1998). Further, recovery of top predators may be hindered if food-web structure has been degraded by overexploitation (Okey and Wright 2004). Therefore,

perhaps the greatest challenge currently facing fishery management is trying to answer the questions: are there enough resources to go around and how will human exploitation affect what is available in the future? The first step towards resolving these uncertainties is to have a firm understanding of basic underlying processes mediating natural mortality due to predation.

The results of this dissertation can be used to improve the quality of management of squid and their predators by providing information on the interspecific relationships that ultimately regulate population dynamics in the ecosystems which they inhabit. The scope of the present study shifts from the broad perspective of ecosystem all the way down to organismal level. Taken as a whole or in parts this information can be incorporated into single-species or more holistic models. Examples of appropriate applications include investigating how a highly successful year-class of finfish (e.g., bluefish) would impact squid populations as it progressed ontogenetically. How do natural cycles (seasonal, decadal) in squid abundance impact the timetable and feasibility of management goals to rebuild predator biomass in a specific age group or assemblage? Lastly, improved data on natural mortality rates may be useful in adjusting biological reference points to maintain sustainable yields of squid (Moustahfid et al. 2009).

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