THE METABOLIC COST OF BEHAVIOURAL TRADEOFFS DURING THE EARLY LIFE STAGES OF THREE NORTH-ATLANTIC MARINE FISHES

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by

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Abstract: This thesis uses studies of physiology and behaviour to better understand the foraging and predator-avoidance tradeoffs experienced by young marine fishes. It has previously been speculated that, due to their small size and high growth rates, larval and juvenile fish may have a limited aerobic capacity compared to adult individuals. This could be an important constraint influencing the behavioural ecology of young fishes, but has been difficult to evaluate because there has been no single study to examine changes in metabolic rate or aerobic scope for any fish species over their entire life history. Thus, an important first step in this thesis was to examine the standard (minimal) metabolism, maximal metabolism, and aerobic scope for three species of north Atlantic marine fish (shorthorn sculpin *Myoxocephalus scorpius*, ocean pout *Zoarces americanus*, and lumpfish *Cyclopterus lumpus*) from the larval stage until adulthood. This research showed that young fish have a greatly increased mass-specific metabolic demand compared to adults, and that their aerobic scope is vastly diminished. The results also suggest that the relationship between aerobic scope and life history stage is greatly influenced by a species' developmental trajectory.

A second group of projects then investigated the effects of increased mass-specific metabolic demand on anti-predator behaviour in young marine fishes. It is known that most animals will reduce foraging while in the presence of predatory threat, but that individuals are often more risky (i.e. they will continue to forage) during times when they are hungry or have increased energetic requirements. However, despite their high mass-specific energy demands, it was

observed that young ocean pout and larval shorthorn sculpin both display large foraging reductions when exposed to predators. Due to the high energetic requirements of these fishes, these results suggest that these foraging interruptions could be very costly. To support this view, these studies also found that frequent, prolonged exposures to a predatory threat caused reduced growth, decreased lipid stores, and reduced ability to acquire certain essential dietary fatty acids that are crucial for normal development.

A final study then examined how a limited aerobic scope may affect the choice of foraging strategy in young lumpfish – a species that can either actively forage by swimming, or use a "sit-and-wait" foraging strategy while clinging to the substrate using a ventral adhesive disc. Behavioural observations showed that lumpfish predominantly use the 'cling' foraging strategy when prey is abundant, but resort to the more costly 'swim' strategy to seek out food when prey is scarce. The metabolic cost of active foraging was also quantified using swim-tunnel respirometry, and a model was devised to predict the prey density at which lumpfish should switch between foraging modes to maximize energy intake. The results of this model do not agree with previous observations of lumpfish behaviour, and thus it appears that juvenile lumpfish do not try to maximize their net energetic gain. Instead, the data suggest that juvenile lumpfish forage in a manner that reduces activity and conserves space in their limited aerobic scope.

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Shaun Killen

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List of Abbreviations and Symbols

±	plus or minus
%	percent
α	alpha
ω	omega
µm	micrometer
AA	arachidonic acid
ANCOVA	analysis of covariance
ANOVA	analysis of variance
b	scaling exponent
b_b	scaling exponent, basal metabolism
b_m	scaling exponent, maximal metabolism
b_s	scaling exponent, standard metabolism
BF_3	boron triflouride
BL	body length
BMR	basal metabolic rate
°C	degrees Celsius
cal	calories
cm	centimeter
Co.	company
DHA	docosahexaenoic acid
e.g.	*exempli gratia*
EPA	eicosapentaenoic acid

EPOC	excess post-exercise oxygen consumption
F	F-ratio
FAME	fatty acid methyl ester
FAS	factorial aerobic scope
FID	flame ionization detection
g	gram
GLM	general linear model
h	hour
HSI	hepatosomatic index
HUFA	highly unsaturated fatty acid
i.d.	inside diameter
i.e.	*id est*
Inc.	incorporated
kg	kilogram
L	liter
m	meter
MAP	modal action pattern
mg	milligram
min	minute
ml	milliliter
mm	millimeter
MMR	maximal metabolic rate
MS-222	3-aminobenzoic acid ethyl ester

MTE	Metabolic Theory of Ecology
n	number
O_2	oxygen
OSC	Ocean Sciences Centre
p	probability
PL	phospholipids
PUFA	polyunsaturated fatty acid
PVC	polyvinyl chloride
RMR	routine metabolic rate
S	second
SCUBA	self-contained underwater breathing apparatus
SEM	standard error of the mean
SMR	standard metabolic rate
sp.	species
ST	sterols
TAG	triacylglycerols
TLC	thin-layer chromatography
U_{crit}	critical swim speed
wt.	weight

Co-Authorship Statement

The experiments described in this thesis were designed and conceptualized by S. S. Killen, with assistance from J. A. Brown and A. K. Gamperl. S. S. Killen was responsible for all data collection and analysis, with the exception the data for ocean pout *Zoarces americanus* presented in Chapter Two, which was collected in collaboration with I. Costa. All manuscripts resulting from this thesis were prepared by S. S. Killen, with editing assistance and creative input from J. A. Brown and A. K. Gamperl.

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Chapter One

General Introduction

This thesis examines the physiological cost of several behavioural tradeoffs experienced during the early life stages of three marine fish species, and provides novel information on inter-specific variation in metabolic scaling amongst larval marine fishes and its effects on metabolic scope. This introductory chapter begins with a brief discussion of behavioural tradeoffs and their relationship to animal metabolism (with specific reference to young fishes). Next, I focus on two specific types of behavioural tradeoff that are examined in this thesis: (1) the tradeoff between foraging and predator avoidance; and (2) the choice of foraging mode. Finally, this chapter concludes with an outline of the thesis objectives.

1.1 Tradeoffs and Energetics

Like all organisms, fish have a finite amount of both time and energy. The decisions on how to spend these resources come in the form of tradeoffs, that is, when an organism spends time or energy on one activity or function, it has a diminished capacity to perform others (Stearns 1992). To survive and pass their genes on to the next generation, animals must perform multiple activities including foraging, predator avoidance, and reproductive behaviours. Any time devoted to any one of these behaviours cannot be used to perform the others.

In this manner, behavioural tradeoffs are capable of affecting energy acquisition, since both reproduction and predator-avoidance can reduce the time available for foraging activity. However, there are also physiological tradeoffs that can directly affect energy *allocation* within an animal, and thus the function, survival, or fitness of the animal. Only once an animal has acquired energy in the form of food can it then allocate this energy among its behavioural and physiological processes in a manner that maximizes its survival and reproductive success. To better illustrate the concept of tradeoffs in energy allocation within an animal, it is useful to conceptualize energy expenditure in the following manner (Sibly and Calow 1986):

$$R = R_m + R_g + R_a + R_f + R_e \qquad \text{(Eq. 1.1)}$$

Where R is the total amount of energy obtained from food resources; R_m is the metabolism required for maintenance ('standard' metabolism in ectotherms and 'basal' metabolism in endotherms); R_g is the energy devoted to growth (biosynthesis of both somatic and reproductive tissues); R_a is the energy used during activity; R_f is the energy used during feeding, digestion, and assimilation of food resources; and R_e is energy excreted in waste products (feces and urine). Based on this equation, the allocation of energy to any one function (e.g. maintenance, growth, or activity) results in a physiological tradeoff. For example, any energy directed towards physical activity decreases the amount available for growth. Ideally, an animal would spend no energy on movement, but in reality

this is not possible because most animals need to perform at least some physical activity to both avoid predators and obtain food energy. As a result, they are often forced to prioritize certain activities according to environmental conditions (e.g. in the presence of predators they may prioritize physical activity for escape) or their own physiological state (e.g. if they are in reproductive condition they may direct energy to reproductive tissues instead of somatic growth).

Another important consideration is that animals have an upper limit on their metabolic rate (due to a finite capacity for oxygen delivery and cellular ATP production), and that this upper limit influences their total scope for aerobic activity (aerobic scope = the difference between the minimal and maximal metabolic rates; Priede 1985). Thus, at any given time, an animal must fit the demands of all oxygen-consuming physiological functions within the bounds of this aerobic scope. This can lead to important physiological tradeoffs that are distinct from the energy acquisition and allocation tradeoffs mentioned earlier. For example, an individual that is directing a large proportion of circulating oxygen towards muscular activity will have a reduced capacity to simultaneously perform other aerobic processes such as growth or digestion. Conversely, fast-growing individuals may have less scope available for physical activity. For example, Arnott et al. (2006) observed that fast-growing populations of Atlantic silversides *Menidia menidia* have a decreased aerobic scope compared to slower-growing populations, and that this diminished scope is attributable to increased levels of minimal metabolism associated with growth. These fast-growing fish consequently have inferior swimming abilities (decreased

endurance), and also eat larger meals, which leads to an increase in the portion of available scope subsequently required for digestion (Arnott et al. 2006)

The partitioning of energy between physiological functions should be an important determinant of individual fitness, because processes that affect growth may be critical in determining survival and reproductive potential. This generalization is especially true for fishes, a group in which growth is highly correlated with survival during the early life stages (mainly due to size-dependent predation; Bailey and Houde 1989), and fecundity during the adult life stage (Gross and Sargent 1985). The amount of time and energy allocated to predator-avoidance behaviours will also be crucial in determining survival in fish species, not only because predation is regarded as being the main source of mortality for larval and juvenile fishes (Bailey and Houde 1989), but because it can also impact the time available for foraging (discussed in detail below). Thus, there is a complex scenario for larval and juvenile fishes in which numerous physiological and behavioural tradeoffs may be interconnected and also highly sensitive to natural selection. Young fish are under pressure to grow quickly, but must also expend energy while foraging and avoiding their many predators. Furthermore, young fishes may be forced to spend large amounts of time performing predator avoidance behaviours (such as hiding, freezing, or fleeing), which detract from the time available for acquiring food energy.

Over the last century, a large body of research has focused on factors affecting the survival of larval marine fishes, with the main goal of establishing a link between environmental variability and survival during the early life stages

and recruitment to adult populations (see Trippel and Chambers 1997). Also important is that differential survival during the early life stages may be a crucial source of natural selection for many marine fish species. For example, the extremely high rates of mortality during the larval stage of many marine fishes could cause important shifts in character traits within a cohort (Conover and Schultz 1997). In this regard, it is largely unknown whether the phenotypes of larvae that survive differ from those which do not. Interestingly, the ability to balance physiological and behavioural tradeoffs could be one factor affecting survival during the early life stages, especially since several authors have noted (e.g. Weiser 1991, Weiser 1995, Post and Lee 1996) that young fish seem to have a restricted energy budget compared to adult individuals. Due to the effects of metabolic scaling, the extremely small body size of larval fish results in rates of mass-specific standard metabolism substantially greater than that of adults. Moreover, it is thought that rapid growth and development during early ontogeny may compete with physical activity for oxygen supply, thus causing a greatly reduced scope for routine activity. The strongest evidence for this interpretation comes from Post and Lee (1996), who compiled data from numerous studies on routine and active metabolism throughout the ontogeny of fishes, and speculated that the aerobic scope of fishes is probably limited early in life. Unfortunately, there are limited data with which to draw comparisons of aerobic scope between life stages, and it has therefore been difficult to understand the potential importance of these differences. However, metabolic challenges such as increased mass-specific metabolism and a reduced aerobic scope could impose

physiological constraints with significant consequences for the behavioural ecology of young fishes.

1.2 Predator-Avoidance and Foraging

One aspect of ecology that plays a large role in behavioural tradeoffs is the interaction between predators and their prey. Predators can directly reduce prey populations through consumption, but the non-lethal effects of predators on prey behaviour are often overlooked (but see Lima 1998a). For example, predatory presence can affect energy acquisition in animals by influencing their behaviour. Most animals cannot consume food and watch for predators at the same time, and an animal's adaptations for avoiding predation may often restrict its foraging ability (e.g. remaining motionless during crypsis). In the presence of predators, individual prey will usually reduce foraging activities in favour of predator avoidance behaviours (e.g. hiding, freezing; Lima and Dill 1990; Houston et al. 1993; Lima 1998a; Lima 1998b). By reducing foraging activity, prey become less conspicuous and can also direct more attention toward the activity of the predator. However, every instance of predator avoidance comes at the cost of lost foraging opportunities and is therefore a behavioural tradeoff.

Despite the potentially considerable effects of predation on energy intake, there has been little attempt to examine the costs of reduced foraging under predation threat from a physiological perspective. However, such costs are likely to be severe for young fishes because of the importance of rapid growth during early life. For example, many marine fish species produce large numbers of very

small larvae that possess limited energy stores and, as previously mentioned, very high mass-specific metabolic rates (Post and Lee 1996). These larvae need to acquire external energy almost constantly for their growth, maintenance, and activity. As a consequence, compared to adults, larval fish may suffer a disproportionately high cost for every instance of predator-related reduced foraging.

In other animal taxa, the physiological effects of reduced foraging under predation threat (in terms of reduced energy acquisition), have been inferred by measuring growth rates. This metric is a gross measure of incurred costs, and does not provide detailed information regarding metabolic demands or nutritional requirements. Nonetheless, rapid growth is crucial during the larval and juvenile stages of species, and frequent interruptions in foraging that result from the presence of predators are known to decrease growth rates during these life stages. For example, the rearing of larval amphibians in the presence of caged predators decreases foraging activity and growth (Werner and Anholt 1996; Peacor and Werner 2000), and Skelly (1992) implicated reduced foraging activity in the reduced growth rates of grey treefrog tadpoles (*Hyla versicolor)* that were reared in natural pond enclosures with non-lethal exposure to predators. Rapid growth during early life is important for survival in multiple ways, including the need to avoid size-dependent predation (Fuiman and Magurran 1994). Increased size also confers increased competitive ability and the ability to eat larger food items, which leads to further growth (Werner and Gilliam 1984). It is also important to note that the juveniles of many temperate fish species reduce

foraging during the winter and must accumulate body mass and lipid reserves prior to this over-wintering period in order to survive (Post and Evans 1989). Interruptions in foraging that result from frequent predation threat may affect the amount of lipids they can acquire before this over-wintering period.

There have been few attempts to examine the tradeoff between foraging and predator avoidance behaviour during the early life stages of marine fishes, especially among those species that possess a distinct larval stage. However, among the few studies that have been done in this area, it has been shown that the larvae of Atlantic cod *Gadus morhua* (Skajaa et al. 2003), lumpfish *Cyclopterus lumpus* (Williams and Brown 1991), and three-spine stickleback *Gasterosteus aculeatus* (Bishop and Brown 1992), will all reduce foraging in the presence of a predatory threat. Unfortunately, there has been no work to examine the potential effects of this reduced foraging behaviour on the growth and survival of young marine fishes. Moreover, little is known about the development of this decision-making ability during early ontogeny, and whether the tendency to reduce foraging under predation threat is affected by changes in metabolic demand as individuals develop. Typically, early survival in these species is extremely low and the two main causes of mortality in the wild appear to be predation and starvation (Bailey and Houde 1989). Thus, a tendency to reduce foraging while in the presence of predators may represent an important interaction between these two factors, given that frequent exposure to predators may decrease the rates of energy acquisition in larvae that have a high metabolic demand.

1.3 Foraging Modes

Foraging activity is necessary for free-living organisms to acquire food energy from their environment. Food acquisition comes at a cost, however, because foraging generally requires some amount of energy expenditure on physical activity. The balance between energy used and energy gained is important because it would not be advantageous for animal to spend more energy on foraging than they are able to consume. Furthermore, any energy spent on foraging cannot be used for reproduction or growth and therefore constitutes a tradeoff in energy allocation.

For these reasons it should be in an animal's best interest to reduce the time and/or energy devoted to food acquisition beyond that which is necessary and forage as efficiently as possible. This concept forms the basis of optimality theory and has been applied in the study of numerous aspects of foraging behaviour. Most work in this area has focused on prey choice (i.e. *what* to forage), and patch or habitat selection (*where* to forage). Interestingly, however, there has been relatively little attention paid to *how* animals forage. In many optimal foraging models (e.g. those that examine patch residence time or prey item selection), the act of foraging is implicitly assumed to be costly, but this cost is rarely defined. Instead, it is generally ignored or assumed to remain constant as the animal searches for prey. However, there is evidence that under certain conditions, animals can alter the manner in which they search for prey and thereby change the amount of energy spent on activity (O'Brien et al.1989; Bautista et al. 2001). For example, some bird species will alternate between

search tactics that require relatively little energy expenditure (e.g. searching while perched or walking), and those that are more costly (searching while flying; Rudolph 1982; Bautista et al. 2001). Similarly, several species of insects (Formanowicz 1982), amphibians (Jaeger and Bernard 1981), and reptiles (Huey and Pianka 1981) switch between relatively passive search strategies (e.g. sit-and-wait, ambush), and tactics that involve more activity (e.g. cruise or pursuit foraging, wide-ranging searches). This body of research suggests that while foraging actively, individuals spend more energy on movement but capture increased numbers of prey, whereas ambush foragers capture less prey but spend less energy in doing so.

Although switching between foraging modes has not been studied as extensively in teleost fishes as it has in most other animal groups, there have been some advances in this area. Crowder (1985) observed that depending on prey density, many planktivorous fishes will alternate between particulate and filter feeding. White crappie *Pomoxis annularis* increase their search speeds when feeding on small prey as compared to large prey (O'Brien et al. 1989). Studies focusing on alternate foraging modes in young fishes are even fewer, but it has been observed that juvenile brook charr *Salvelinus fontinalis,* and Dolly Varden *Salvelinus malma,* will both switch from active to ambush foraging with increased stream flow rates (Grant and Noakes 1987; Fausch et al. 1997). Finally, larval lumpfish *Cyclopterus lumpus*, which possess a ventral adhesive disc, switch between ambush foraging while adhering to a substrate and cruise

foraging while searching for prey while swimming (Brown 1986; Williams and Brown 1991).

The ability to switch between foraging modes could be very beneficial for larval and juvenile fishes. First, the energy required for locomotory activity can be substantial (Bishop 1999; Wiebel and Hoppeler 2005), and thus the ability to decrease energy expenditure on this activity would allow young fish to direct more energy towards growth and development. This is especially true considering that the mass-specific cost of transport is much higher in smaller organisms compared to large individuals (Tucker 1975). Secondly, young fish could theoretically approach the upper bounds of their limited aerobic scope if they are devoting a large proportion of their energy budget to locomotory activity. Aside from diverting energy from growth, this allocation could have serious consequence because fish face an increased risk of death when operating near their maximum aerobic capacity (Wood et al. 1983; Calow 1984; Priede 1985). For these reasons, young fish should possess the behavioural flexibility to alter foraging tactics in response to certain environmental cues (e.g. prey density), and this is an area that warrants further study.

1.4 Summary of Thesis Objectives

The overall goal of this thesis is to combine physiological measurements with behavioural observations of young marine fishes, because this approach will provide a more complete understanding of how physiological costs associated

with behavioural tradeoffs impact marine teleosts during the early life stages. To accomplish this goal, several related studies were conducted.

The study described in Chapter Two examined changes in standard metabolism, maximal metabolism, and aerobic scope in three species of marine teleosts (ocean pout *Zoarces americanus*; shorthorn sculpin *Myoxocephalus scorpius*; and lumpfish *Cyclopterus lumpus*) from hatching to adulthood. This was the first study to measure these variables directly over the entire life history of any teleost species, thereby allowing a direct comparison of metabolism and aerobic scope among various life stages. Furthermore, this work examines the metabolic challenges experienced by fishes during the early life stages and how differences in developmental trajectory affect mass-dependent metabolism in larval marine fishes. This chapter subsequently provided a foundation for the hypotheses tested in the remainder of the thesis.

Chapter Three examines the long-term cost of reduced foraging under predation threat in young ocean pout. It was expected that because young ocean pout possess high rates of mass-specific metabolism, frequent foraging reductions would be very costly and affect growth and energy storage. Chapter Four expands on this concept by examining the long-term costs of predator-related foraging reductions in larval shorthorn sculpin, a species that is much smaller and more delicate at hatch than ocean pout, and examining how fine-scale changes in metabolic demand through early ontogeny affect the decisions to forage under predation threat (i.e. if mass-specific metabolic rate increases as

individuals approach metamorphosis, are they less likely to reduce foraging under the threat of predation?).

Finally, the experiments described in Chapter Five examine the foraging mode selection of juvenile lumpfish in response to changes in prey density. Specifically, direct measures of active metabolism were used to understand how switching between foraging modes may allow lumpfish to balance food intake with the metabolic cost of foraging activity. In addition, this study investigated the extent to which mode-switching may be an energy-saving mechanism in young lumpfish, and how it may allow individuals to conserve space in their limited aerobic scope.

Chapter Two

Metabolic scaling during ontogeny in three species of marine teleosts and its implications for aerobic scope

2.1 INTRODUCTION

The metabolic rate of organisms increases with body mass according to the equation:

$$Y = aM^{b}, \qquad \text{(Eq. 2.1)}$$

where Y can be basal or maximal metabolic rate, *a* is the species-specific scaling constant, M is body mass and *b* is the scaling exponent (b_b and b_m, for the scaling of basal and maximal metabolic rates, respectively). This relationship forms the basis of a widely accepted tenet in biology, known as 'Kleiber's law', which states that the minimal metabolic rate of organisms scales to the three-quarter power of body mass ($b_b = 0.75$). However, after a century of research, there are many unresolved issues regarding the effects of body size on metabolic rate. For example, significant controversy still surrounds the precise value of b_b (specifically, is $b_b = 0.75$ or 0.67? Dodds et al. 2001; White and Seymour 2003, 2005; Savage et al. 2004), and whether or not this value is 'universal' across all taxa (Bokma 2004; Glazier 2005). Furthermore, there are sparse data on maximal metabolic rate (MMR) in ectotherms, and it is unclear whether the variation present in the allometry of MMR in endotherms also exists

in other animal groups (e.g. in birds and mammals, b_m is elevated in 'athletic' species; Bishop 1999; Glazier 2005). Understanding the allometry of MMR, in combination with basal metabolic rate (BMR), provides insight into the aerobic scope of organisms at various sizes or stages of development (e.g. factorial aerobic scope; FAS = MMR/ BMR), and is critical to know because aerobic scope reflects the capacity of organisms to perform all oxygen-consuming functions above minimal metabolic requirements and, potentially, the ability of organisms to respond to environmental extremes or other challenges (Djawdan et al. 1997; Bochdansky et al. 2005).

Fishes are an integral part of aquatic ecosystems, yet compared with mammals and birds, relatively little is known about the scaling of their basal and active metabolism. Further investigation is warranted, however, because the limited data available for fishes (e.g. Post and Lee 1996; Clark and Johnston 1999; Bokma 2004) suggest that the scaling of standard metabolic rate (SMR; analogous to BMR in endotherms; referred to as 'standard' because it is specific for a given temperature) in this group differs from either the 0.67 or 0.75 'scaling laws' that have been proposed for endotherms. Furthermore, fishes are the only vertebrate group in which an individual's life history may span a range of wet mass of up to eight orders of magnitude, and larval fishes are believed to have reduced aerobic scope compared with adults (Weiser 1995; Glazier 2005). However, it has been difficult to grasp the magnitude and importance of mass-dependent changes in metabolism in this group, because the few studies on this

subject vary greatly in their methodology, making it difficult to compare larval data with that obtained for juveniles and adults. Diminished aerobic scope could be an important constraint for larval fishes because it would limit the energy available for important behaviours and physiological functions. Ten years ago, Post and Lee (1996) emphasized the need for a comprehensive examination of SMR and MMR that spans the complete ontogeny of any teleost species, yet to date, no single study has addressed this issue. As a result, we currently have a limited understanding of how aerobic scope in fishes changes through ontogeny.

In this study, I measured SMR and MMR in three species of marine teleost fishes (ocean pout *Zoarces americanus,* lumpfish *Cyclopterus lumpus,* and shorthorn sculpin *Myoxocephalus scorpius*) over their life history using similar methodologies for all developmental periods. My goal was to produce accurate intraspecific estimates of b_s and b_m (scaling exponents for standard and maximal metabolism, respectively) for these species over several orders of magnitude of body mass and to calculate changes in aerobic scope over different life history stages. The species used in this study are unique, because unlike most fishes, they show no activity in respirometers while at rest, and therefore measures of routine metabolic rate (RMR) closely approximate SMR (i.e. they are directly comparable to measures of BMR in endotherms). This characteristic largely eliminates some of the criticisms of earlier studies on metabolic scaling in teleosts, where baseline metabolic measurements were more characteristic of RMR than SMR, and thus measures of aerobic scope were likely underestimates (Post and Lee 1996). In addition, these species show differing patterns of

development during their early life stages, and comparisons between these species therefore allow a better understanding of the effects of developmental trajectory on aerobic scope during ontogeny.

2.2 MATERIAL AND METHODS

2.2.1 Animals

Masses of fertilized eggs of *Z. americanus*, *C. lumpus* and *M. scorpius* were collected by scuba divers from shallow-water habitat in coastal Newfoundland and maintained in the laboratory in aerated incubators supplied with a constant flow of seawater. After hatching, larvae were maintained on a diet of enriched *Artemia* nauplii (1000–1500 *Artemia* L^{-1}; 3–4 times per day), and holding temperatures (3°C for *M. scorpius*, 8°C for *Z. americanus* and 11°C for *C. lumpus*) were chosen to approximate those experienced by the early life stages of each species in the wild. These same temperatures were also used when measuring the metabolic rate of each species and were consistent across all life stages. Fish used in this study which were hatched from eggs in the laboratory were a mixture of individuals from 3-4 families for each species. Juveniles included a combination of wild fishes captured by scuba divers and individuals that had been raised in the laboratory after hatching from eggs obtained from the wild. All adult fishes were collected from the wild by scuba divers.

2.2.2 Respirometers

Three separate Blazka-style respirometers were used: a 57 ml respirometer was used for fishes less than 1 g; a 6.8 L respirometer was used for individuals 15–80 g; and an 81 L respirometer was used for individuals greater than 120 g. The methods for measuring the water oxygen concentration in the 6.8 and 81 L Blazka-style respirometers have been described previously (Rodnick et al. 2004). Briefly, oxygen concentration and temperature were measured within each respirometer by pumping water through an external circuit containing a custom flow chamber and a galvanic oxygen electrode equipped with a thermal sensor (Model CellOx 325; WTW, Inc.,Welheim, Germany). This oxygen electrode was connected to an oxygen meter (Model Oxi 340; WTW, Inc.) equipped with automatic temperature compensation. Measurements of oxygen consumption were initiated after the flow of water from an external reservoir of aerated, temperature-controlled water was terminated and the respirometer had been sealed.

To measure the oxygen consumption of fishes in the 57 ml respirometer, I used a fibre-optic flow-through oxygen sensor (Presens, Germany), because this type of sensor does not consume oxygen. Data obtained from this sensor were recorded directly using the accompanying computer software (OXYVIEW v. 4.16). A peristaltic pump (Masterflex; Cole-Parmer Instrument Co.) and tubing with low oxygen permeability (Masterflex Tygon Food for the majority of the circuit and a small section of Masterflex Tygon LFL at the pump-head) moved water from the respirometer and past the sensor via a short external circuit and.

Preliminary blank experiments with hypoxic seawater (30–40% oxygen saturation) confirmed that there was no oxygen gain in the closed system after 1.5 h of monitoring (noting that measurements of oxygen consumption in this study lasted for a maximum of 40 min, see below). To maintain the temperature of the water in the respirometer and oxygen concentrations in the water following transfer of the larvae to the respirometer, a second external circuit supplied aerated seawater from a reservoir in a water bath set at a water temperature appropriate for each species. This circuit was closed when measurements of oxygen consumption were made, and therefore to maintain water temperature within the respirometer, the entire set-up was located in a cold-room set at the desired experimental temperature. To reduce background bacterial contamination, seawater used for the 57 ml respirometer was sterilized with ultraviolet radiation and the system was cleansed daily with absolute ethanol. In addition, blank measurements were performed after each trial to quantify any possible bacterial oxygen consumption. In the majority of cases (over 85% of trials), there was negligible background oxygen consumption. However, in cases where a background rate of oxygen consumption was observed, this value was subtracted from the experimental data.

2.2.3 Determination of standard metabolic rate

Fishes were fasted (48 h for juveniles and adults, overnight for larvae), and then carefully placed into the respirometers and allowed an appropriate acclimatization period (24 h for juveniles and adults, 4 h for larvae; determined in

preliminary experiments). To reduce light levels and external disturbance, black plastic was wrapped around the respirometers during the acclimatization period and left in place during measurements of SMR. The behaviour of the fishes was monitored during measurements via a mirror either below (for the two larger respirometers) or behind (for the 57 ml respirometer) the respirometers. Because of the inactive nature of the species used in this study (during all life stages), I was easily able to measure oxygen consumption of fishes under conditions of zero swimming activity, thus approximating SMR. An exception was larval *M. scorpius*, which swim constantly even under low lighting conditions. For this reason, measures of SMR in *M. scorpius* larvae were performed in complete darkness (larval *M. scorpius* stop swimming after acclimatization to darkness and rest motionless in the respirometer). For larval *M. scorpius* and *C. lumpus*, multiple individuals were placed into the respirometer simultaneously so that a measurable decrease in oxygen could be obtained. The number of individuals varied depending on the size of the larvae, but represented at least 70 mg of biomass. Resting measurements of oxygen consumption were made over 20 min for larval fishes, and 20–40 min for juveniles and adults. Water oxygen content was measured once every minute for the 57 ml respirometer, and once every 2 min for the two larger respirometers. After the measurements of oxygen consumption were completed (either SMR alone, or SMR and MMR—see below), the juvenile and adult fishes were removed from the respirometer and weighed on an electronic scale accurate to 0.1 g sensitivity. Larval fishes were quickly rinsed with freshwater (to remove external salts), blotted with a paper towel, and

weighed on a microbalance (APX-60; Denver Instrument Co.). In cases where multiple larvae were necessary for one trial, their total wet mass was divided by the number of individuals to obtain an average value (this value was subsequently used on the log-plots of oxygen consumption versus wet mass—see *Data and statistical analyses*).

2.2.4 Determination of maximal metabolic rate

For juvenile and adult fishes, MMR was determined after individuals were exhaustively exercised using a burst swimming protocol similar to that described by Reidy et al. (1995). Briefly, the water current speed was gradually increased until the fishes began to perform burst-type swimming and the fishes were then allowed to swim at this speed until exhaustion (as indicated by an inability to maintain swim speed and a tendency to rest against the bottom or back of the respirometer; typically after approximately 3–5 min of burst swimming). This protocol was initiated immediately following the measurement of SMR. The measurements of MMR began immediately after the fishes reached exhaustion and continued for the first 10 min of recovery. During this time, a constant decrease in water oxygen content was observed. This protocol estimates MMR based on excess post-exercise oxygen consumption (EPOC; Lee et al. 2003), and was used instead of a critical swimming speed (U_{crit}) protocol because some of the study species display aberrant sustained swimming behaviour in the respirometers. An exception to this protocol were some adult *M. scorpius* that would not swim at all in the respirometer. These fish were exercised to

exhaustion by manual chasing and immediately placed in the respirometer for measurement. Measures of MMR collected via this method were within the range obtained for adult *M. scorpius* whose MMR was measured using the burst-swimming protocol. For juvenile and adult *C. lumpus*, I measured MMR under conditions of both sustained maximal swimming and after exhaustion via burst swimming (only data obtained after burst swimming are reported in this study). Importantly, there was no significant difference in the values for MMR obtained via either method for *C. lumpus*, and I therefore believe that my protocol was a valid method for estimating MMR in *Z. americanus* and *M. scorpius*.

C. lumpus possess a ventral adhesive disk, and to prevent individuals from adhering to the inside of the respirometers a thin film of Parafilm (American National Can) was carefully glued to the disk of anaesthesized (MS-222) individuals. For juveniles less than 0.5 g, only a thin film of adhesive (VetBond 3M Tissue Adhesive) was applied to the disk (without the addition of a plastic layer) with the aid of a dissecting microscope. The resulting layer of dried adhesive served the same function as the Parafilm that was applied in the case of larger fishes.

For larval *M. scorpius*, current speeds in the respirometer were gradually increased until larvae began to swim vigorously and rely on burst-type swimming to maintain their position (approximately 5 body lengths s^{-1}). The larvae were allowed to swim in this manner for approximately 2 min, after which time the flow speeds were decreased. After a rest period of approximately 30 s, the current speed was again increased to a level that would force burst-type swimming. This

protocol was repeated for a 12-min period, over which time water oxygen content was measured every minute. This protocol was used because some previous studies have found that larval fishes have a low anaerobic capacity (Forstner et al. 1983), and it was uncertain whether EPOC could be measured for the larval fishes in this study. This method was not possible for larval *C. lumpus*, however, because they adhere to the respirometer with their ventral adhesive disk, which is too small to cover in order to prevent adhesion. Instead, larval *C. lumpus* were manually exercised to exhaustion prior to placement in the respirometer. Oxygen consumption was measured for 10 minutes after quickly placing the larvae into the respirometer. Using this protocol, larval *C. lumpus* showed large increases in oxygen consumption post-exercise that were comparable in magnitude to those observed for larval *M. scorpius*. Therefore, I believe my measures provide an accurate estimate of MMR in larval *C. lumpus*.

2.2.5 Data and statistical analysis

Data analyses were performed using MINITAB statistical software (v. 13.0; Minitab, Inc.). Rates of oxygen consumption (mg O_2 h^{-1}) were calculated for each trial using linear regression. Measures of oxygen consumption for each species were then plotted on log plots against wet mass, and scaling exponents were estimated by applying power curves to the data. Although wet mass was carefully measured for all animals in my study, errors in the measurement of wet mass are probably greater for larvae than for juvenile and adult individuals. However, this source of error, and its affect on estimates of scaling exponents,

was not examined in this thesis. FAS (factorial aerobic scope) through ontogeny was calculated as the ratio of MMR to SMR (MMR: SMR) for each species, using the equations for the power curves for standard and maximal metabolism. I chose to examine factorial scope instead of absolute aerobic scope because: (i) absolute aerobic scope expressed on a non-mass-specific basis (e.g. mg O_2 h^{-1}; Weiser 1985) provides little insight into comparisons across large size ranges because small fishes have comparatively minute increases in oxygen consumption with increased aerobic activity simply because they have less biomass; and (ii) absolute aerobic scope expressed on a mass-specific basis (in units of mg O_2 kg^{-1} h^{-1}) is a misleading measure because small animals require more energy (per unit mass) to perform aerobic functions other than those contributing to SMR. For example, the minimum cost of transport (COT; mg O_2 kg^{-1} km^{-1}) is known to be much higher for smaller animals (Tucker 1975), and net COT is 5–10-fold higher for juvenile *C. lumpus* (approx. 600 mg) than for adult individuals (> 500 g) at swimming speeds appropriate for both life stages (S. S. Killen and A. K. Gamperl 2006, unpublished data). For these reasons, factorial metabolic scope, where maximum metabolism is expressed relative to the fish's standard metabolic costs, is a more suitable measure for comparing the aerobic capacity of animals across large size ranges.

Intraspecific estimates of b_s or b_b are advantageous because they do not depend on the phylogeny of species or the evolution of metabolic rates (Bokma 2004; Martin et al. 2005). However, there is concern that early development could affect intraspecific metabolic scaling in fishes. For example, there is

evidence that the SMR of larval fish scales differently from that of juveniles and adults, possibly scaling isometrically or with positive allometry ($b > 1$), and that MMR in fishes may also scale in a biphasic manner (Post and Lee 1996). To account for this possibility, I used two separate methods to estimate both b_s and b_m. In the first analysis, I determined b_s and b_m over the entire life history of each species. In the second, I accounted for early ontogenetic effects on metabolic scaling by performing a biphasic analysis. In this analysis, the two scaling phases were separated in *M. scorpius* and *C. lumpus* using a quantifiable, biological criterion—the end of metamorphosis. Metamorphosis was defined by the appearance of juvenile pigmentation, fully developed fins and fin rays, and flexion of the notochord, and occurs at approximately 25 mg wet mass for *M. scorpius* and 150 mg for *C. lumpus*). I did not conduct a biphasic analysis on *Z. americanus* because individuals of this species are large at hatching (3–4 cm total length, 100–200 mg), and are considered to be fully formed juveniles in terms of behaviour and morphology (Methven and Brown 1991). Power curves were then fitted to the data to obtain the scaling exponents. Estimates of b are presented ±SEM, and with 95% confidence limits.

2.3 RESULTS

2.3.1 Standard metabolism

Estimates of b_s ranged from 0.82 to 0.84 when calculated across the life history of each species (Figures 2.1, 2.2). When post-metamorphic scaling

Figure 2.1 Oxygen consumption versus body mass for three species of fishes. (A) *Myoxocephalus scorpius*; (B) *Cyclopterus lumpus* and (C) *Zoarces americanus*. The filled circles represent maximal metabolic rate (MMR) and the open circles represent standard metabolic rate (SMR). For *M. scorpius* and *C. lumpus*, a biphasic analysis (see text) was performed to obtain estimates of both SMR and MMR, where separate regressions were determined before ('premet') and after ('postmet') the end of the metamorphic period (see Materials and Methods for description). A biphasic analysis was not performed for *Z. americanus* because this species lacks a larval period. Dashed lines represent estimates of scaling for SMR and MMR in *M. scorpius* and *C. lumpus* without accounting for ontogenetic effects (no biphasic analysis). Regression equations for these combined estimates of *b* are as follows: for *M. scorpius*, $VO_{2stan} = 0.147^{0.833\pm0.01}$ (95% CL on b = 0.818, 0.842); $VO_{2max} = 0.260^{0.878\pm0.01}$ (95% CL on b = 0.865, 0.891); for *C. lumpus*, $VO_{2stan} = 0.194^{0.844\pm0.01}$ (95% CL on b = 0.837, 0.852); $VO_{2max} = 0.342^{0.883\pm0.01}$ (95% CL on b = 0.871, 0.894). For reference, theoretical representations of b = 0.75 and 0.67 are also shown on the panel for (C) *Z. americanus*.

FIGURE 2.1

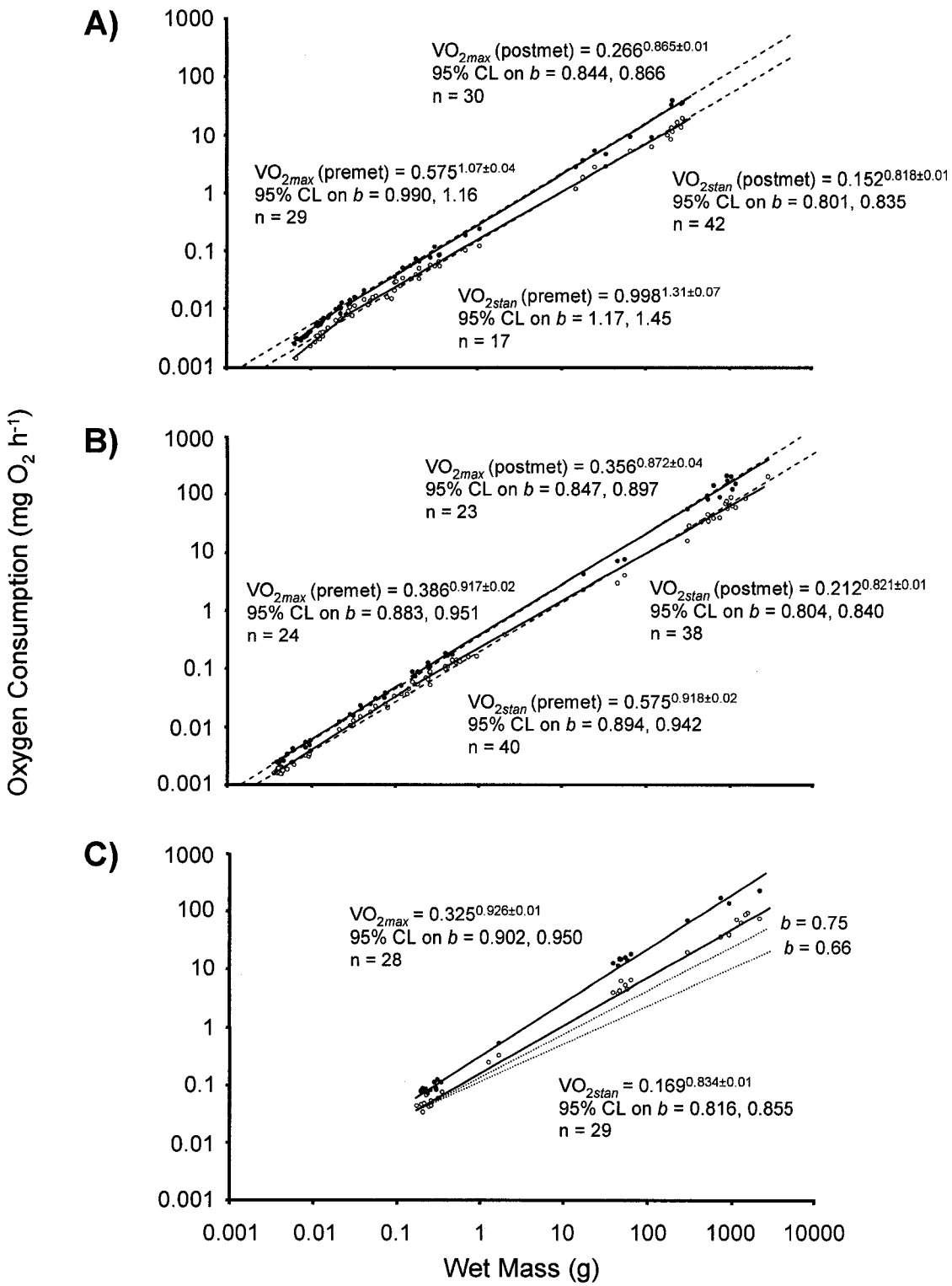
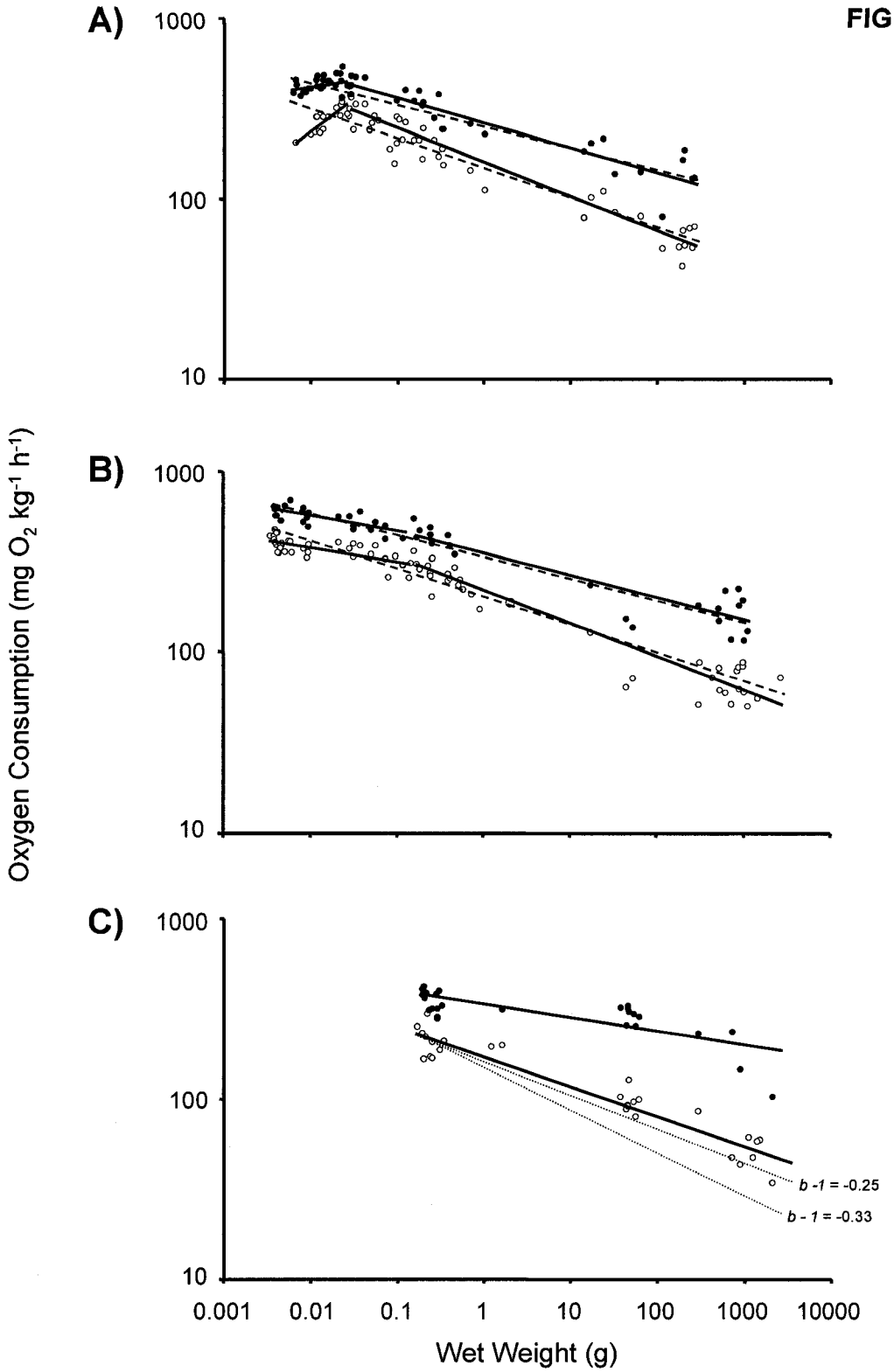

A)
B)
C)
Oxygen Consumption (mg O_2 h^{-1})
Wet Mass (g)
1000
100
10
1
0.1
0.01
0.001
0.001
0.01
0.1
1
10
100
1000
10000
VO_{2max} (postmet) = $0.266^{0.865\pm0.01}$
95% CL on b = 0.844, 0.866
n = 30
VO_{2max} (premet) = $0.575^{1.07\pm0.04}$
95% CL on b = 0.990, 1.16
n = 29
VO_{2stan} (postmet) = $0.152^{0.818\pm0.01}$
95% CL on b = 0.801, 0.835
n = 42
VO_{2stan} (premet) = $0.998^{1.31\pm0.07}$
95% CL on b = 1.17, 1.45
n = 17
VO_{2max} (postmet) = $0.356^{0.872\pm0.04}$
95% CL on b = 0.847, 0.897
n = 23
VO_{2max} (premet) = $0.386^{0.917\pm0.02}$
95% CL on b = 0.883, 0.951
n = 24
VO_{2stan} (postmet) = $0.212^{0.821\pm0.01}$
95% CL on b = 0.804, 0.840
n = 38
VO_{2stan} (premet) = $0.575^{0.918\pm0.02}$
95% CL on b = 0.894, 0.942
n = 40
VO_{2max} = $0.325^{0.926\pm0.01}$
95% CL on b = 0.902, 0.950
n = 28
b = 0.75
b = 0.66
VO_{2stan} = $0.169^{0.834\pm0.01}$
95% CL on b = 0.816, 0.855
n = 29

Figure 2.2 Mass-specific oxygen consumption (metabolic intensity) versus body mass for three species of teleost fishes. (A) *Myoxocephalus scorpius*; (B) *Cyclopterus lumpus* and (C) *Zoarces americanus*. The filled circles represent maximal metabolic intensity and the open circles represent standard metabolic intensity. Metabolic intensity was calculated using the data in Figure 2.1. Dashed lines represent estimates of scaling for standard metabolic intensity and maximal metabolic intensity in *M. scorpius* and *C. lumpus* without accounting for ontogenetic effects (no biphasic analysis). For reference, theoretical representations of b -1 = -0.25 and -0.33 are also shown on the panel for (C) *Z. americanus*. The current Figure is for illustrative purposes only – statistical analyses were not performed because wet mass is a component of both the dependent and independent variables.

FIGURE 2.2


A)
B)
C)
1000
100
10
Oxygen Consumption (mg O_2 kg^{-1} h^{-1})
b -1 = -0.25
b - 1 = -0.33
0.001
0.01
0.1
1
10
100
1000
10000
Wet Weight (g)

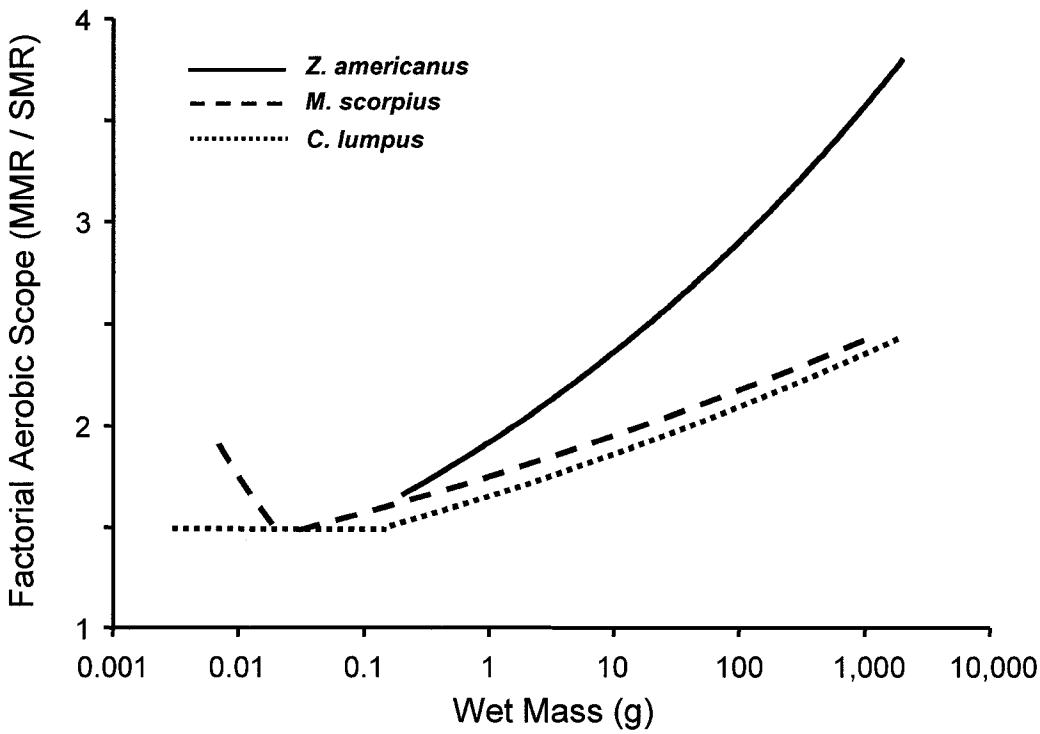
patterns were analysed separately from the larval stage (using the biphasic analysis), post-metamorphic estimates of b_s in *C. lumpus* and *M. scorpius* (the two study species with a larval stage) decreased slightly, but were still within this general range. Premetamorphosis, b_s during the larval stage for *C. lumpus* and *M. scorpius* was greatly elevated compared with post-metamorphosis. This pattern was especially true for larval *M. scorpius*, which showed positive allometry for SMR during this life stage (pre-metamorphic b_s = 1.34).

2.3.2 Maximal metabolism

Estimates of b_m were generally higher than for b_s, and ranged from 0.88 to 0.93 when calculated across the entire life history of each species (Figures 2.1, 2.2). As was the case with SMR, post-metamorphic estimates of b_m in *C. lumpus* and *M. scorpius* (species with a larval stage) were slightly decreased compared with the values obtained without the biphasic analysis, but were not significantly different (95% confidence limits showed overlap). Premetamorphosis, b_m during the larval stage for *C. lumpus* and *M. scorpius* was higher than post-metamorphosis. Once again, this trend was especially pronounced for *M. scorpius* larvae, which showed pre-metamorphic scaling that was close to isometric (pre-metamorphic b_m = 1.07).

Figure 2.3 Factorial aerobic scope (FAS) in three species of fishes for different life history stages. Values were calculated from the fitted curves in Figures 2.1 and 2.2 (FAS =MMR/SMR).

FIGURE 2.3


Factorial Aerobic Scope (MMR / SMR)
4
3
2
1
Z. americanus
M. scorpius
C. lumpus
0.001
0.01
0.1
1
10
100
1,000
10,000
Wet Mass (g)

2.3.3 Aerobic scope

In general, the FAS for each species was low early in life, gradually increased with ontogeny, and was highest in adults (Figure 2.3). For example, FAS in adult *C. lumpus* was 79% higher than at the lowest point during the larval stage, the FAS of adult *M. scorpius* increased 72%, and FAS in *Z. americanus* increased 2.7-fold between newly-hatched individuals and adults.

Within this general trend of increasing factorial scope with ontogeny, there were differences between species with regard to changes in FAS during early development. For *C. lumpus*, FAS was relatively constant pre-metamorphosis (approximately 1.49), whereas it decreased throughout the larval stage for *M. scorpius* and reached its lowest point at the end of metamorphosis (approximately 1.41).

2.4 DISCUSSION

2.4.1 Standard metabolism

The present analysis is the only single-study dataset for SMR over the complete life history of any fish species. My results clearly show that the intraspecific scaling of SMR in fishes differs significantly from either the 0.67 or the 0.75 scaling exponents that are commonly reported for endotherms. Instead, using both types of analyses (i.e. examining b_s over the entire life history of each species, or examining the pre- and post-metamorphic periods separately), estimates of post-larval b_s were between 0.82 and 0.84 in the three species examined. Thus, even when the potential biases of early developmental effects

on SMR are eliminated, b_s in the fishes studied is substantially different from b_b in endotherms. Although my results may not be directly comparable to previous fish studies where RMR was measured, when combined, the data strongly suggest that the scaling exponent for SMR in fishes is 0.80–0.85. For example, Bokma (2004) performed an intraspecific analysis of fish RMR by compiling data from various life stages for 113 species. In that analysis, the largest dataset compiled was for the sea trout, *Salmo trutta trutta,* where multiple studies included data that spanned a size range of 0.1–600 g; *b* for that species was approximately 0.86. In the only three species for which Post and Lee (1996) found sufficient data to conduct an analysis of RMR over full life histories (the common carp *Cyprinus carpio,* rainbow trout *Oncorhynchus mykiss,* and sea bream *Pagrus major*), *b*-values of the second scaling phase were all between 0.82 and 0.84. Clark and Johnston (1999) performed an interspecific analysis of RMR in post-larval teleosts and found that *b* was approximately 0.80. Most recently, White et al. (2006) surveyed 82 fish species and found an overall b_s value of approximately 0.88.

These findings for b_s in fishes may have important implications for the bioenergetics models which are commonly used to investigate trophic relationships. Such models have been used to predict the potential impact of predators on larval fish populations, and to estimate the prey requirements (e.g. zooplankton biomass) of larval fish populations (e.g. Rand et al. 1995). These models often rely on scaling exponents to adjust for differences in body size when estimating the energy requirements of populations, and these estimates of

metabolic demand can be off by several orders of magnitude if inappropriate scaling exponents are used (Klumb et al. 2003). Recently, bioenergetics models have been used in attempts to extrapolate the metabolic rate of individuals to broad-scale ecosystem effects (Enquist et al. 2003; Ernest et al. 2003; Brown et al. 2004; West and Brown 2005; Woodward et al. 2005). The application of metabolism to the study of ecology has great potential, and in their 'Metabolic Theory of Ecology' (MTE), Brown et al. (2004) state that BMR can be used to predict diverse ecological phenomena including rates of predation, patterns of species diversity, and rates of biomass production. Their theory also uses the 0.75 scaling exponent to correct for the effects of body mass on BMR, and thus isolate the effects of temperature on ecosystems. However, if the scaling exponent for teleost fishes is closer to 0.85, the role of body size in determining the effects that fishes exert on ecosystems may differ from that observed for endothermic species. Clearly, the MTE should account for potential differences in the scaling of minimal metabolic rate among taxa rather than assuming a universal 0.75 scaling exponent.

Early development had an effect on standard metabolic scaling in *C. lumpus* and *M. scorpius*, as b_s was elevated in both species during the larval stage compared with the juvenile and adult stages. This biphasic scaling pattern supports the general model for RMR through ontogeny in teleosts as proposed by Post and Lee (1996). However, although both *C. lumpus* and *M. scorpius* showed elevated values for b_s during the larval stage, the exact value for b_s was very different between the two species. Specifically, *M. scorpius* showed strong

positive allometry during the larval stage (b_s = 1.31), while b_s for *C. lumpus* was much lower (b_s = 0.92). This difference in scaling between the two species could reflect differences in developmental trajectories. *C. lumpus* larvae progress gradually to the juvenile stage, whereas larval *M. scorpius* metamorphose abruptly with rapid changes in morphology and behaviour. Elevated scaling exponents for RMR have previously been observed during the larval stage of fishes (Post and Lee 1996; Giguere et al. 1988), and have generally been attributed to either: (i) increased rates of growth or protein turnover during the larval stage (Weiser 1991, 1995; Glazier 2005), (ii) the differential emergence of metabolically active tissues and organs during larval development (Oikawa et al. 1991), or (iii) increases in respiratory surface area as larvae switch from cutaneous respiration to the use of gills (Kamler 1992; Post and Lee 1996). A more abrupt change in any (or all) of these factors in *M. scorpius* compared with *C. lumpus* during development could cause a proportionally greater increase in absolute metabolic rate beyond that which would be predicted based on body size alone. Although further research is required to determine the role that these factors play in elevating the scaling exponent for RMR in larval fishes, my results suggest that differing patterns of development during metamorphosis can cause variation in larval metabolic scaling among species.

Regardless of the exact scaling pattern during early development, metabolic scaling through ontogeny causes mass-specific metabolic demand to be much greater early in the life of fishes. Indeed, metamorphosis is a particularly demanding period for some species. Considering that energy is the

main 'currency' in most foraging models, it is likely that these increased requirements could not only increase the likelihood of starvation during the early life stages, but also affect the behavioural ecology of larval and juvenile fishes. For example, for adult fishes, increases in energetic demand (as a result of factors such as parasitic infection) can increase feeding activity and cause individuals to take greater risks when foraging under predation threat (Godin and Sproul 1988;Godin and Crossman 1994). Conversely, although young *Z. americanus* are known to reduce foraging in the presence of a predator (Killen and Brown 2006), their high mass-specific metabolism suggests that such foraging interruptions are energetically very costly. Clearly, additional research should examine how the increased mass-specific metabolic requirements of young fishes impact their foraging decisions and survivability.

2.4.2 Maximal metabolism

With regard to my analysis of MMR, I found that the post-metamorphic b_m for all three species ranged from 0.87 to 0.93. This result is in contrast with the traditional view that MMR in teleost fish scales isometrically when examined intraspecifically (Brett and Glass 1973; Weiser 1985; Goolish 1991; Blier et al. 1997). Nonetheless, this difference is not surprising given that previous studies on the scaling of MMR in fishes have concentrated on relatively athletic salmonid species, whereas none of the species examined here can be considered athletic. *Z. americanus* and *M. scorpius* are benthic, whereas *C. lumpus* are semi-pelagic.

Previous research on birds and mammals has shown that b_m is consistently higher than b_b and varies substantially between species (Bishop 1999; Glazier 2005). Furthermore, Weibel et al. (2004) state that MMR should vary substantially among species because it depends on factors such as mitochondrial and capillary volumes (factors that vary with a species' athleticism but may not be directly related to body size). They also report that the b_m for 'athletic' mammalian species was about 0.942, while the b_m of 'normal' species was lower at around 0.849. My results, when compared with the data available for salmonids (Brett and Glass 1973; Weiser 1985), support the findings of Weibel et al. (2004) and provide further evidence that the only quality that is 'universal' with respect to MMR allometry is that b_m tends to be higher than b_s, although the degree to which it is elevated is probably dependent on a species' lifestyle ('athletic' versus 'sedentary').

2.4.3 Aerobic scope

The ratio of MMR to minimal metabolic rate (SMR or BMR) is an individual's FAS. This measure represents the factor by which an organism can increase its metabolic activity above maintenance levels, and therefore represents an animal's capacity to support various oxygen-consuming physiological functions (e.g. activity, digestion, response to stressors, etc.; Jobling 1983; Priede 1985; Bishop 1999). The differential effects of scaling on MMR and SMR in the three species examined results in an overall increase in FAS through ontogeny. In endotherms, it has also been noted that MMR is less

dependent on body mass than SMR, and that larger animals therefore have a greater factorial scope compared with smaller animals (Bishop 1999; Weibel et al. 2004). For fishes, a limited aerobic capacity could be an important constraint during the early life stages, especially since many fish species possess a larval stage of exceedingly small body size compared with other vertebrates. My results confirm that the FAS of fishes is extremely limited early in life (Figure 2.3). Furthermore, these results demonstrate that the early relationship between FAS and body mass can differ in species with a distinct larval period (i.e. *C. lumpus* and *M. scorpius*) compared with species that are well developed at hatch (*Z. americanus*). For example, the positive allometry displayed for SMR in larval *M. scorpius* results in a decline in FAS during this life stage, whereas Z. *americanus* simply display a gradual increase in FAS throughout their life history.

Such a limited FAS early in life could affect the ability of young fishes to 'multitask' physiologically demanding processes. For example, the energetic demands of rapid growth, when combined with the high energetic cost of locomotion needed for foraging and predator evasion (i.e. large increases in aerobic metabolism occur while recovering from burst-type anaerobic activity such as when escaping predators), may leave little room for homeostatic maintenance during times of environmental or nutritional stress. This may contribute to the enormous rates of mortality (often greater than 90%) often observed for larval marine fishes (Bailey and Houde 1989). Although no single factor (temperature variation, prey availability, etc.) has consistently been linked to recruitment variability (Dower et al. 2002; Pepin 2004), a decreased aerobic

scope could reduce the ability of larval fish to respond to environmental stressors, thus making them very sensitive to fluctuating environmental conditions. While it is clear that the life history strategies utilized by fish species with small larvae are successful at producing adult individuals, the metabolic challenges imposed by scaling result in physiological tradeoffs early in life that may be important for the ecology of these animals. For example, it is often assumed that increased SMR in larval fishes is advantageous because it is associated with rapid growth and thus decreased size-dependent mortality to predation (Glazier 2005), but recent work with fish larvae has shown that there can be selection against individuals with high SMR when food supply is limited or variable (which is common for larval fishes; Bochdansky et al. 2005). Interestingly, lowered SMR in fishes correlates with increased FAS (Cutts et al. 2002), and therefore reduced SMR could be beneficial for larvae because it lowers the 'floor' on the bounds of aerobic metabolism and allows greater room for the simultaneous performance of important physiological functions. However, additional work is required to specifically examine individual variability in the aerobic scope of young fishes, and whether a decreased SMR and/or increased metabolic scope are critical factors that determine which young fishes are among the small number that reach adulthood.

In conclusion, my results provide further evidence that ecologists must consider variations in metabolic scaling across taxa if they are to incorporate aspects of metabolism into their work. Furthermore, because the metabolic capacity of species or individuals probably influences many ecological processes,

future research should consider including data on b_m or aerobic scope when extrapolating metabolic physiology to broad-scale ecosystem effects. This approach may be especially important for fishes, given that the observed scaling patterns for SMR and MMR result in an extremely limited aerobic scope during early life history stages.

Chapter Three

Energetic cost of reduced foraging under predation threat in newly hatched ocean pout

3.1 INTRODUCTION

Consumption by predators is a major source of mortality in fish populations, especially during the early life stages (Bailey and Houde 1989). However, predators can also influence prey behaviour. For example, in the presence of predators, individual prey will often reduce foraging activity in exchange for predator avoidance behaviours (e.g. hiding, freezing; Lima and Dill 1990, Houston et al. 1993, Lima 1998a,b). By reducing foraging activity prey become less obvious to predators and they may be more vigilant against potential attacks.

It is widely assumed that this sort of anti-predator behaviour comes at the cost of reduced energy intake (Abrahams and Dill 1989, Fuiman and Magurran 1994, Lima 1998a,b). Indeed, energy is often used as a common 'currency' for evaluating lost foraging opportunities in foraging models or experiments that examine foraging behaviour. In support of this assumption, individuals are less likely to reduce foraging activity in the presence of a predatory threat if they are hungry or have increased energetic demands (Dill and Fraser 1984, Godin and Crossman 1994, Lima 1998a,b). However, there has been little attempt to quantify the actual energetic cost of predator-related foraging reductions, or the long-term effects of frequent interruptions to regular foraging behaviour. From

the few studies that have been performed in this area, it is known that the costs of reduced foraging may accumulate over time and cause reduced growth in individuals that are frequently exposed to predation threat (amphibians — Skelly 1992, Werner and Anholt 1996, Peacor and Werner 2000; aquatic insect larvae — Dixon and Baker 1988, Ball and Baker 1996). Aside from analysis of growth rates, however, the effects of predator-related foraging reductions on other aspects of energy acquisition and nutrition are largely unexplored.

For fishes, most studies on the effects of predatory presence on growth rates have examined predator-induced shifts by prey species to energy-poor habitats (e.g. Fraser and Gilliam 1992, Tonn et al. 1992, Diehl and Eklov 1995, Perrson and Eklov 1995). For this reason, the long-term effects of predator-related foraging reductions on growth and energy storage in fishes remains unknown. Furthermore, studies of predator-induced habitat shifts have usually focused on juvenile or adult individuals. However, reduced growth may be especially significant for newly-hatched fishes because this is a delicate life stage in which rapid growth and development is extremely important (Bailey and Houde 1989, Fuiman 1994, Fuiman and Magurran 1994, Leggett and Deblois 1994). Individuals that grow slowly are at a disadvantage because they spend more time as a potential food source for predators (Fuiman and Magurran 1994). It is known that very young individuals, and even larval fishes, will respond to predators and forage in a threat-sensitive manner (Williams and Brown 1991, Bishop and Brown 1992, Skajaa et al. 2003). Currently, the only studies on reduced foraging under predation threat in newly-hatched or larval fishes have

involved exposing individual prey to acute pulses of predatory threat of very short duration (Williams and Brown 1991, Bishop and Brown 1992, Skajaa et al. 2003, Engstrom-Ost and Lehtiniemi 2004). For these reasons, the long-term effects of frequent foraging reductions due to predation threat on the growth and survival of young fishes are not known.

To evaluate the long-term fitness consequences of reduced foraging under predation threat, I reared newly-hatched ocean pout *Zoarces americanus* with and without the presence of frequent predatory threat. I hypothesized that frequent interruptions to regular foraging behaviour in the presence of predators would negatively affect the growth of young fish. Furthermore, I hypothesized that reduced energy intake due to a reduction in foraging activity would have consequences for the lipid composition of the individual prey given that lipids are an important metabolic fuel in young fishes (Sargent 1995). I also performed the experiments at 3 and 8°C to investigate how water temperature affects the extent of the tradeoff between foraging and predator avoidance and the cost of reduced foraging opportunities. It was expected that because of increased energetic demands, ocean pout acclimated to the higher temperature would be more motivated to forage under the threat of predation, and would therefore show a less drastic cost in terms of reduced growth rates.

3.2 MATERIALS AND METHODS

3.2.1 Animals

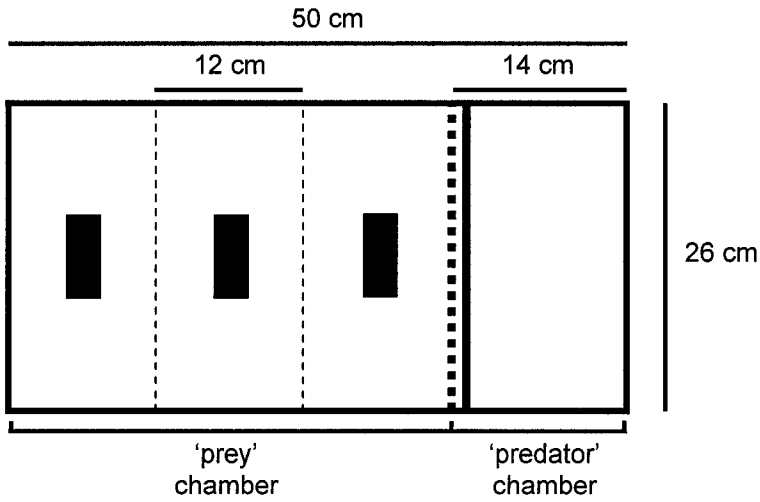
Eggs of ocean pout *Zoarces americanus* were collected by SCUBA divers and transported to the laboratory and placed in incubators with aerated, flowing seawater at ambient temperatures. Following hatching (in early January), approximately half of the ocean pout were gradually acclimated to 8°C over the course of 1 week, whereas others were held at ambient temperatures (3°C). After this acclimation period, arbitrarily-selected groups of ocean pout were transferred into eight experimental tanks (mean wet mass ±SEM: 3°C = 270.4 ± 6.47 mg, 8°C = 278.1 ± 5.32 mg [no significant difference between temperatures; unpaired t-test, p = 0.395]; mean standard length ±SEM: 3°C = 42.41 ± 0.17 mm, 8°C = 43.49 ± 0.20 mm [significant difference; unpaired t-test, p = 0.002]). All fish used in experiments were of the same age, and were a mixture of individuals from three families. Half of the tanks were maintained at 8°C whereas the others were kept at 3°C (the predator and control treatments within each temperature regime are described below). At the time of this study, the ambient water temperature was 3°C, but Newfoundland inshore water temperatures can be as high as 8°C in the late-spring and summer, when ocean pout similar to the sizes used in this study may still be found in the wild (in contrast to the pelagic larvae of many marine fishes that remain larvae for a short duration and therefore do not occur over a wide temperature range). In the current study, behavioural observations (see description below) began after transfer to the experimental tanks and continued until the fish were eight weeks of age.

3.2.2 Experimental tanks

The experimental tanks were 40 L flow-through aquaria which were divided into 2 separate chambers — a 'predator' chamber measuring 14 × 26 × 30 cm (length × width × height), and a 'prey' chamber measuring 36 × 26 × 30 cm (Figure 3.1). The chambers were separated by two adjacent partitions, one of which was transparent and non-removable and the other removable and opaque. These partitions allowed visual exposure of the pout to the predators by raising the opaque partition, while preventing physical contact between the predators and larvae. Water flowed continuously into each tank, starting at the prey chamber, passing through a small opening into the predator chamber, and then out of the tank (the direction of flow was toward the predators). For this reason I believe the effects of olfactory cues originating from the predator were minimal. In each tank, the prey chamber contained 25 ocean pout. I randomly assigned two tanks at each temperature to contain two predatory juvenile cod *Gadus morhua* (mean total length 13.76 ± 0.36 cm) in the predator chamber. The remaining tanks were the control treatment and contained larval ocean pout but no predators. To analyze prey behaviour in relation to proximity to the predators and to ensure that equal numbers of behavioural observations were made for all tank locations, the prey chamber was further subdivided into three grid sections using markings on the outside of the tanks. In addition, each grid section contained a small section of PVC pipe that could potentially be used for cover by the ocean pout. This pipe was added to reduce any stress response elicited by observing the predators, and because individuals in the wild would

Figure 3.1 Top view diagram of a 40 L experimental tank (top view), showing locations of prey chamber (36 × 26 × 30 cm: length, width, height; containing 25 newly-hatched ocean pout *Zoarces americanus*), and the predator chamber (14 × 26 ×30 cm: containing two juvenile Atlantic cod *Gadus morhua* in predator treatments but empty in control treatments). Heavy dashed line represents permanent transparent partition; heavy continuous line represents removable opaque partition. Light dashed lines represent grid sections within prey chamber (used to quantify proximity of pout to predator chamber). Each grid section contained a small section of PVC pipe for cover (filled rectangles).

FIGURE 3.1



likely have the opportunity to hide while under predation threat. All experimental tanks were exposed to a 16:8 h light:dark photoperiod.

Ocean pout in all treatments were fed daily by adding live enriched *Artemia* nauplii to each tank four times per day to maintain prey densities at approximately 1000 *Artemia* L^{-1}. The *Artemia* were actively swimming in the water column at all times of the day (at each temperature), and were mixed in the water column with the aid of a small airstone placed into each tank. Any excess prey and fecal matter at the bottom of the tanks were removed by siphoning at the end of each day.

3.2.3 Behavioural observations

Each day, pout in the predator treatments were visually exposed to the predatory cod for 6 h by removing the opaque partition. The partitions in control tanks were also removed at this time, but the ocean pout were not exposed to predators. Behavioural observations of foraging and predator avoidance were made three times a week beginning after the fishes were transferred to the experimental aquaria. Following removal of the opaque partition, *Artemia* were added to the prey chamber of the tanks. After a 2 min acclimation period, the focal animal technique (Altmann 1974) was used to observe ocean pout behaviour. With this technique, the behaviour of one individual is recorded over a specific length of time (one min in the present study). Different behaviours were recorded and tabulated using a hand-held Psion event recorder and associated software (The Observer 3.0, Noldus Information Technology). The

behavioural repertoire of the ocean pout included 3 easily observable modal action patterns (MAPs; Barlow 1968). These actions were 'crawl', 'orient', and 'lunge' (Table 3.1).

During each observational period, behavioural observations were conducted on four pout in each grid section of the prey chamber, with two ocean pout that were exposed and two ocean pout that were hiding under cover (for a total of 12 observations per tank). Differences were found in the foraging activity and response to predatory exposure among pout that were hiding and pout that were exposed, but for this study, behavioural observations were only analyzed if the ocean pout were judged to be able to observe the predatory chamber. Over the course of a given observation, it was common for individual ocean pout to move between being open areas and hiding, or to change grid sections within the tanks (this was more common at 8°C because of the increased activity of individuals at this temperature). In these cases, observations were divided into time intervals that represented the time spent in each location. The frequencies of the observed behaviours were then converted to counts min^{-1}. To avoid biases caused by disproportionate counts of behaviours while in a location for a short amount of time, intervals were not included in the final analysis if the focal ocean pout spent less than 5 s in that location.

3.2.4 Growth measurements

At the beginning of the experiment and at the end of each week, five ocean pout were arbitrarily selected from each tank, anaesthetized using MS-

Table 3.1 Description of Modal Action Patterns (MAPs) observed for newly hatched ocean pout reared with and without frequent predatory exposure.

MAP	Description
Crawl	Main form of locomotion in the young ocean pout. Short forward movement (less than one-second in duration) while remaining in contact with the bottom while being supported by pectoral fins.
Orient	Turning of the eyes, head, or body towards passing *Artemia* in an attempt to focus in preparation for an Attempt.
Attempt	An effort to capture a passing *Artemia* in mouth by rapidly lunging forward. Usually preceded by at least one Orient.

222, and measured for standard length using Matrox Inspector 3.0 image analysis software on images captured on a digital camera (Pixera PVC 100C). Following image capture, the fish were allowed to recover from the anaesthesia and were returned to the appropriate tanks.

At the beginning and end of the experiment, seven fish from each tank were collected for analysis of wet mass. Individual ocean pout were quickly dried with a paper towel and then placed on dry, tared weigh containers. Their mass was then measured on a microbalance. In addition, the livers were removed with the aid of a dissecting microscope from individuals collected at the end of the experiment. The livers were then weighed on a microbalance and expressed as a percent of total wet mass to obtain the hepatosomatic index (HSI) for each individual.

3.2.5 Lipid analysis

After the temperature acclimation period (and prior to the transfer of individuals into the experimental aquaria), arbitrarily-selected individuals were captured from their holding tanks at each temperature and immediately flash frozen in liquid nitrogen for later lipid analysis (n = 8 for each temperature). At the end of the experiment, five ocean pout from each tank (n = 10 per treatment) were captured in an identical manner. These samples were then frozen at –80°C for approximately three months until the lipid analysis could be performed.

In preparation for lipid analysis, individual ocean pout were thawed, quickly rinsed with distilled water, and carefully measured for wet weight using

lipid-clean instruments and weigh-foils. Lipids were then extracted using a variation of the Folch procedure (Folch et al. 1957) as described by Parrish (1998). Briefly, individual pout were homogenized in 7.5 ml of 2:1 chloroform–methanol and washed with water to remove non-lipid contaminants. The entire lower organic phase was then collected and washed four times using 2:1 chloroform–methanol.

Quantitative determination of lipid classes was performed on these extracts with rod thin-layer chromatography with flame ionization detection with a MARK V Iatroscan (Iatron Laboratories). The extracts were spotted on silica gel coated chromarods and a three-stage development system was used to separate lipid classes (described in detail by Copeman et al. 2002). After each separation, the rods were scanned and the three resulting chromatograms were combined using T-scan data scan software (RSS). The signal (detected in millivolts) was quantified using lipid standards (Sigma).

Fatty acid methyl esters (FAME) were prepared by transesterfication with 10% BF_3 in methanol at 85°C for 1 h (Morrison and Smith 1964, Copeman et al. 2002). A Varian Model 3400 GC equipped with a Varian 8100 autosampler was used for qualitative fatty acid analysis (Varian). The column was an Omegawax 320 column, 30 m, 0.32 mm i.d., 0.25 µm film thickness (Supelco). Hydrogen was used as the carrier gas and the flow rate was set at 2 ml min^{-1}. The column temperature profile was 65°C for 0.5 min, hold at 195°C for 15 min after ramping at 40°C min^{-1}, and hold at 220°C for 0.75 min after ramping at 2°C min^{-1}. The injector temperature increased from 150 to 250°C at 200°C min^{-1}. Peaks were

detected by flame ionization with the detector held at 260°C. Fatty acids peaks were integrated using Varian Star chromatography software (Version 4.02) and identification was made with reference to known standards (PUFA 1 and 37 Component FAME Mix, Supelco). Based on whole-body lipid concentrations, the qualitative (proportional) measures of fatty acid content were then used to calculate the concentrations of each identified fatty acid (mg g^{-1} wet mass).

3.2.6 Data and statistical analyses

All statistical analyses were performed with Minitab Version 13.1. The level of significance for all tests was $\alpha = 0.05$. Data are presented as means ± SEM. All data were tested for the assumptions of the statistical tests used (normality, homogeneity and independence of residuals; Sokal and Rohlf 1995).

I recognize the potentially confounding factor of tank effects on my treatments and, for behavioural observations and growth measurements, I performed tests for tank effects by performing an ANCOVA (within each predator–temperature treatment combination) with the factors of tank and time (the duration of the experiment in weeks was used as a covariate). In all cases, the effects of the rearing tanks and the time × tank interactions were insignificant ($p > 0.60$ in all cases). Because of the high number of observations performed for each tank during this study, this method is a highly-sensitive test for tank effects. For this reason, the effects of rearing tanks were not included in subsequent analysis.

For behavioural observations, a 3-way ANCOVA was originally performed using the variables of temperature, treatment (predator-exposed versus control), tank grid section, and fish age (used as a covariate). The covariate of age is indistinguishable from time in the experiment, and so this analysis accounts for the repeated nature of my sampling (in a repeated measures analysis, time can be used as a categorical variable or a covariate). This model had significant 3-way interactions, all of which involved the explanatory variable 'temperature'. Due to the presence of these significant interaction terms, lower-order terms within the models could not be independently evaluated. To allow for a closer evaluation of the other explanatory variables, 'temperature' was removed from the model, and a revised 2-way ANCOVA was performed for each category within 'temperature'. This analysis revealed that tank grid section (proximity to the predator) had no significant affect, and so data from all sections were therefore pooled and a final ANCOVA was performed within each experimental temperature. The effects of age, predators, and the interaction of these factors were of interest in the present study. The interaction term was significant in some cases and prevented the interpretation of the lower-order terms. To allow for further interpretation, unpaired t-tests were performed for each week to compare differences in the response variables between the treatments.

For changes in standard length over time, an ANCOVA was performed within each temperature treatment with the factors of predator treatment and fish age (used as a covariate) to examine the effects of predator presence on growth rates (by comparing the slopes of the regression equations). For final wet

weights, hepatosomatic indices, and lipid class and fatty acid profiles, a 2-way ANOVA was performed (using the factors of predator treatment and temperature), followed by a post-hoc Tukey-Kramer test to examine differences across all temperature–predator treatment combinations.

3.3 RESULTS

3.3.1 Behavioural observations

The main form of locomotion in the young ocean pout was to 'crawl' along the bottom of the tank using their pectoral fins for support. Only rarely did individuals leave the bottom and swim in the water column. Over the course of the experiment, the number of crawls performed by individuals during the observation periods increased significantly, particularly for ocean pout at 8°C (Figure 3.2, Table 3.2). At both temperatures, this rate of increase was greater for ocean pout reared in the absence of predators. These pout had significantly more crawls by the middle and late stages of the study (there were significant age–predator interactions indicating that the slopes between the predator and control treatments were different within each temperature).

At both temperatures, pout decreased foraging activity while visually exposed to the predators (fewer orients and lunges; Figure 3.2, Table 3.2), although the tendency to reduce foraging was not influenced by proximity to the predator (as indicated by the analysis of data from the different grid sections of the prey chamber). The frequency of orients increased with increasing age at both temperatures, but at 8°C, the rate of this increase over time was lower in the

Figure 3.2 Frequencies (mean ± SEM) of behavioural modal action patterns for young ocean pout *Zoarces americanus* reared at 3° or 8°C, with or without visual exposure to predators (juvenile Atlantic cod *Gadus morhua*). *Significant difference between predator treatment and control ($p < 0.05$).

FIGURE 3.2

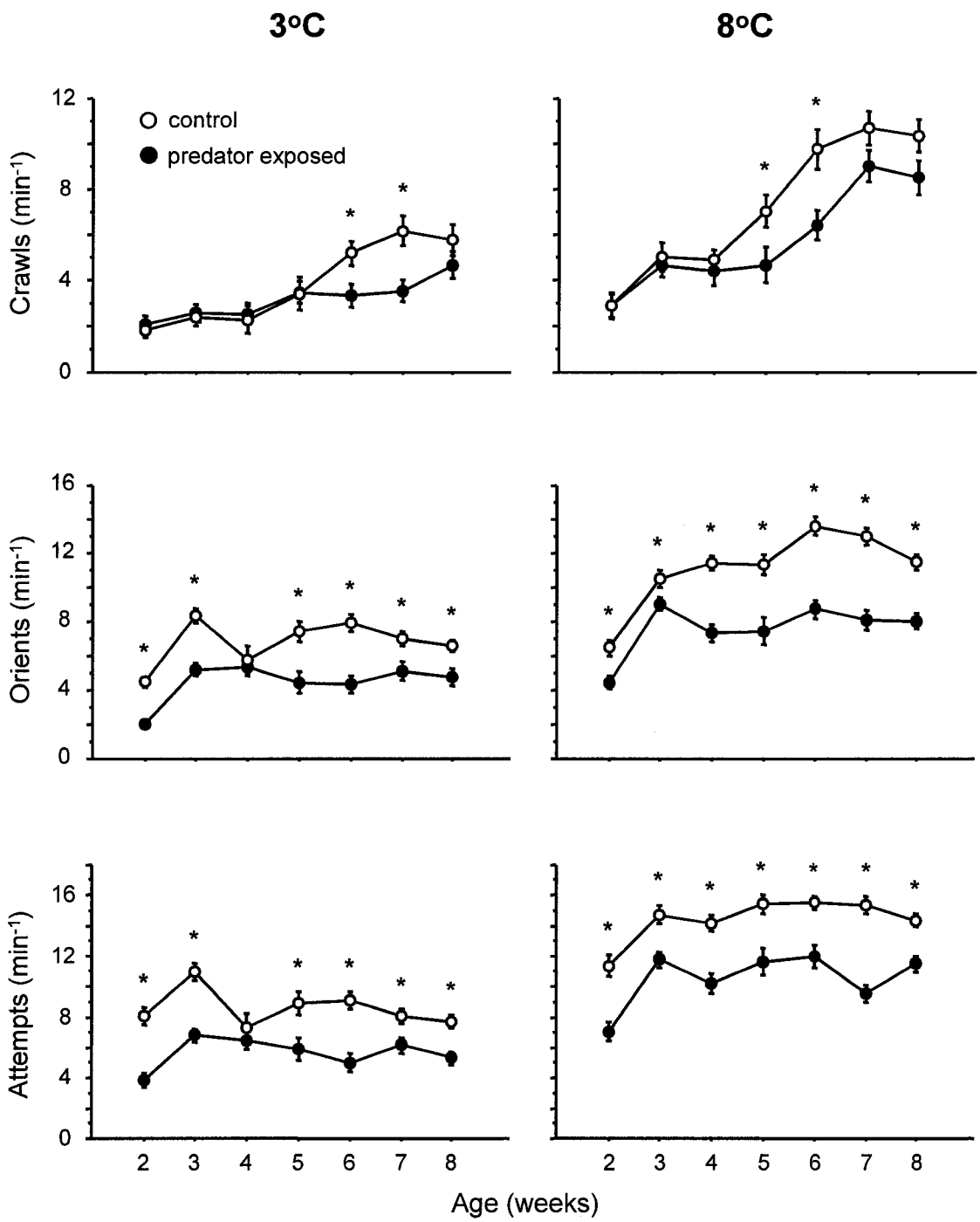

3°C
8°C
control
predator exposed
Crawls (min-1)
Orients (min-1)
Attempts (min-1)
0
4
8
12
16
2
3
4
5
6
7
8
Age (weeks)

Table 3.2 General Linear Model results for observations of modal action patterns (MAPs) for young ocean pout *Zoarces americanus* reared with frequent predatory exposures compared to a control, at 3°C and 8°C. Linear regression equations were calculated using the age of the pout (in weeks; a) as a covariate. The mean value for each MAP (counts min^{-1}) represents the overall mean over the entire experimental period for each treatment. * indicates a significant difference ($p<0.05$). ^ indicates a lower order term that cannot be evaluated independently because the interaction term is significant.

MAP	Treatment	Regression	Mean (± SEM) (counts/min)	Term	*p*	F
3°C (df = 546)						
Crawls	control	y = 0.76 + 0.80a	3.80 (± 0.23)	age	<0.001*^	77.50
	predator	y = 1.65 + 0.33a	3.15 (± 0.19)	predator	0.121^	2.29
				age x predator	0.002*	9.97
Orients	control	y = 6.18 + 0.20a	6.95 (± 0.20)	age	<0.001*	11.42
	predator	y = 3.47 + 0.24a	4.43 (± 0.18)	predator	<0.001*	21.90
				age x predator	0.759	0.09
Attempts	control	y = 9.70 - 0.22a	8.86 (± 0.24)	age	0.276	1.19
	predator	y = 5.41 + 0.05a	5.62 (± 0.21)	predator	<0.001*	39.89
				age x predator	0.077	3.13
8°C (df = 752)						
Crawls	control	y = 1.76 + 1.37a	7.45 (± 0.29)	age	<0.001*^	167.58
	predator	y = 2.08 + 0.96a	5.78 (± 0.27)	predator	0.689^	0.16
				age x predator	0.023*	5.16
Orients	control	y = 8.54 + 0.67a	11.34 (± 0.20)	age	<0.001*^	48.44
	predator	y = 6.48 + 0.31a	7.66 (± 0.20)	predator	<0.001*^	10.52
				age x predator	0.023*	6.80
Attempts	control	y = 13.2 + 0.32a	14.55 (± 0.21)	age	<0.001*	17.23
	predator	y = 9.31 + 0.33a	10.57 (± 0.25)	predator	<0.001*	30.51
				age x predator	0.988	0.00

predator treatment compared to the control (there was a significant age–predator interaction). Regardless of predator treatment, the frequency of lunges increased significantly with increasing age at 8°C, but did not increase at 3°C. On average, for pout at 3°C, the number of orients and lunges in the predator treatment were both reduced to approximately 63% of the control frequency. At 8°C, the mean number of orients in the presence of predators was reduced to 67% of control rates, while the frequency of lunges was reduced to 73% of the control rate.

3.3.2 Growth

Within the 3°C treatments, pout raised without predators had a significantly higher wet mass at the end of the experiment (control: 490.60 ± 6.64 mg; predator treatment: 426.61 ± 13.75 mg; ANOVA, Tukey-Kramer, $p = 0.0003$). In addition, they had a significantly higher growth rate in terms of standard length (Figure 3.3; control: 1.40 ± 0.05 mm week^{-1}; predator treatment: 1.06 ± 0.05 mm week^{-1}; ANCOVA, $p < 0.0001$). Pout in the 8°C treatments did not differ significantly in final wet mass (control: 568.20 ± 13.67 mg; predator treatment: 544.59 ± 26.84 mg; ANOVA, Tukey-Kramer, $p = 0.44$), and although the growth rate of the predator-exposed pout was decreased (Figure 3.3; control: 1.80 ± 0.05 mm week^{-1}; predator treatment: 1.60 ± 0.09 mm week^{-1}), this difference was not significant (ANCOVA, $p = 0.061$, $F = 3.54$).

Figure 3.3 Increases in standard length (SL) with age (*a)* in young ocean pout *Zoarces americanus* reared at 3°C (■, □) or 8°C (●, ○). Filled symbols represent treatments in which ocean pout were visually exposed to predators (juvenile Atlantic cod *Gadus morhua*); open symbols represent control treatments (no exposure to predators). Regression equations are as follows: control at 3°C: SL = 41.42 + 1.41*a*; predator treatment at 3°C: SL = 41.38 + 1.06*a*; control at 8°C: SL = 42.09 + 1.80*a*; predator treatment at 8°C: SL = 42.34 + 1.60*a*. Growth rates (regression slopes) are shown beside each regression line (±SE), and were significantly different between predator treatment and control at 3°C (ANCOVA, $p < 0.0001$, $F = 23.71$), but not at 8°C (ANCOVA, $p = 0.061$, $F = 3.54$). Data points are mean ± SEM; n = 10 for each data point.

FIGURE 3.3

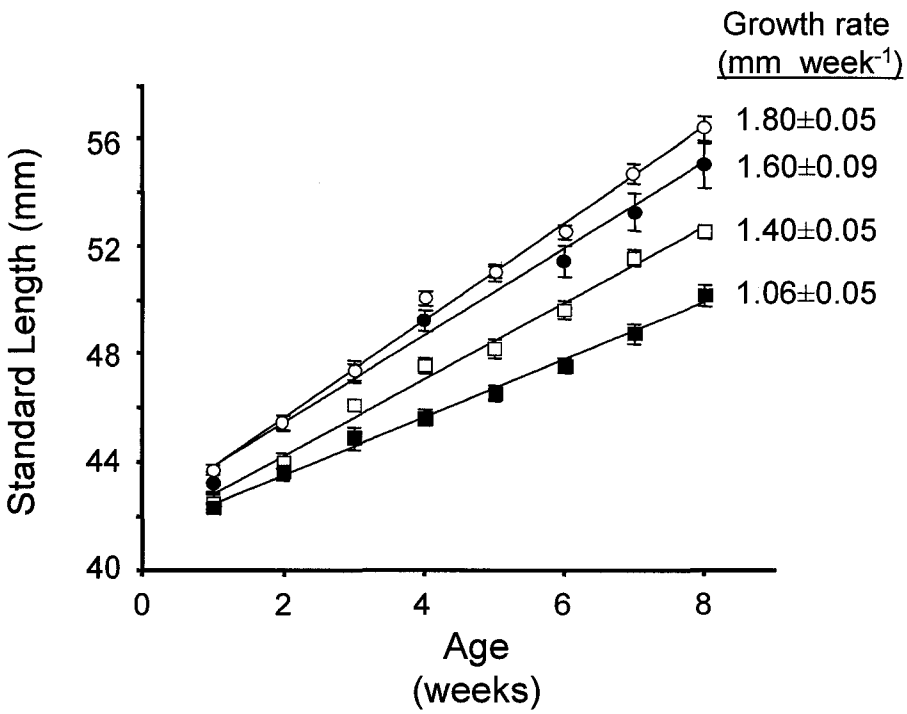

Growth rate
(mm week-1)
1.80±0.05
1.60±0.09
1.40±0.05
1.06±0.05
Standard Length (mm)
56
52
48
44
40
0
2
4
6
8
Age
(weeks)

Table 3.3 Mean (±SEM) initial lipid and fatty acid profiles for ocean pout *Zoarces americanus* used in this study. Samples were taken one week post-hatch, after pout in the 8°C treatments were allowed to acclimate to this temperature. Only essential fatty acids (AA, EPA, DHA), and fatty acids which were present in abundances >5% of total identified fatty acids are shown. n = 8 for each temperature

	3°C	8°C
Total lipids (mg g^{-1} wet mass)	35.23 ± 2.79	38.52 ± 3.46
Lipid classes (mg g^{-1} wet mass)		
Phospholipids	13.97 ± 2.00	15.47 ± 1.21
Sterols	2.95 ± 0.27	3.42 ± 0.19
Triacylglycerols	14.34 ± 0.93	16.3 ± 2.06
Fatty Acids (mg g^{-1} wet mass)		
16:0	3.60 ± 0.33	3.85 ± 0.25
16:1ω7	1.89 ± 0.30	0.90 ± 0.26
18:0	0.87 ± 0.08	0.93 ± 0.09
18:1ω9	4.52 ± 0.44	4.70 ± 0.30
18:1ω7	1.34 ± 0.27	1.28 ± 0.06
18:3ω3	0.15 ± 0.04	0.21 ± 0.45
20:4ω6 (AA)	0.46 ± 0.10	0.51 ± 0.09
20:5ω3 (EPA)	4.51 ± 0.60	5.02 ± 0.72
22:6ω3 (DHA)	4.33 ± 0.94	6.26 ± 0.96

3.3.3 Lipid classes

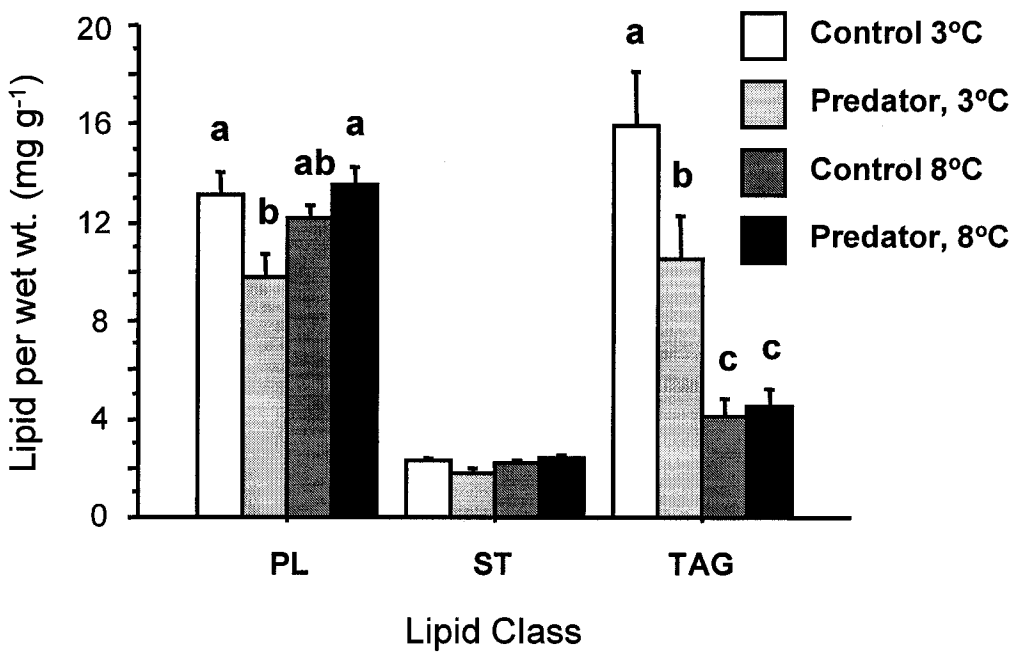
Initial lipid class and fatty acid levels (collected at the beginning of the study) are listed in Table 3.3. By the end of the study, hepatosomatic indices (HSI) in individuals reared in the presence of predators at 3°C were about 45% lower than pout that were not exposed to predators (Table 3.4). Furthermore, pout exposed to predators at 3°C had 30% less whole-body lipid content by the end of the study (Table 3.4). At 8°C, there were no significant differences in HIS or whole-body lipid content. However, the HSI and whole-body lipid levels in either treatment at 8°C were similar to those in the predator treatment at 3°C. The most abundant lipid classes were phospholipids (PL), sterols (ST), and triacylglycerols (TAG). At 3°C, pout exposed to predators had about 27% less PL at the end of the study and 36% less TAG compared to pout that were not exposed to predators (Figure 3.4). At 8°C, however, there were no significant differences between the predator treatments for either PL or TAG. Overall, pout at 8°C had final TAG concentrations that were significantly lower compared to ocean pout at 3°C, regardless of predator treatment. Rearing temperature did not have a significant effect on PL. Neither predators nor temperature had a significant affect on ST concentrations. Lipid data were also analyzed to examine whether there was the dependence of lipid content on body size (data not shown). Only levels of phospholipids and sterols were dependent on body size (i.e. both scaled with positive allometry). However, the inclusion of scaling factors to account for this dependence on body size did not change any of the

Table 3.4 Final hepatosomatic index and whole-body lipid levels for young ocean pout *Zoarces americanus* reared in the presence of frequent predation threat compared to control group (n = 14 per treatment). The experiment was performed at 3°C and 8°C. Significant differences between predator-temperature combinations are indicated by differences in lettering (ANOVA, Tukey-Kramer, $p < 0.05$).

	3°C		8°C	
	Control	Predator	Control	Predator
Hepatosomatic Index (%)	3.5 ± 0.2^{a}	1.9 ± 0.1^{b}	2.2 ± 0.1^{b}	2.3 ± 0.2^{b}
Whole Body Lipid (mg / g)	34.4 ± 3.2^{a}	24.8 ± 2.8^{b}	20.4 ± 0.9^{b}	22.6 ± 1.5^{b}

Figure 3.4 Final whole-body concentrations (mean ± SEM) of phospholipids (PL), sterols (ST), and triacylglcerols (TAG) for young ocean pout *Zoarces americanus* reared at 3 or 8°C, with and without presence of predators. In predator treatments, ocean pout were visually exposed to predators (juvenile Atlantic cod *Gadus morhua*), while in controls they were not. Significant differences between predator and temperature combinations are indicated by different letters (ANOVA, Tukey-Kramer, $p < 0.05$, n = 10 for each bar). There were no significant differences found across treatments for ST.

FIGURE 3.4


Lipid per wet wt. (mg g-1)
20
16
12
8
4
0
a
b
ab
a
a
b
c
c
PL
ST
TAG
Lipid Class
Control 3°C
Predator, 3°C
Control 8°C
Predator, 8°C

results described above. Absolute triacylglycerol content and total lipid content were not related to body size.

3.3.4 Fatty acids

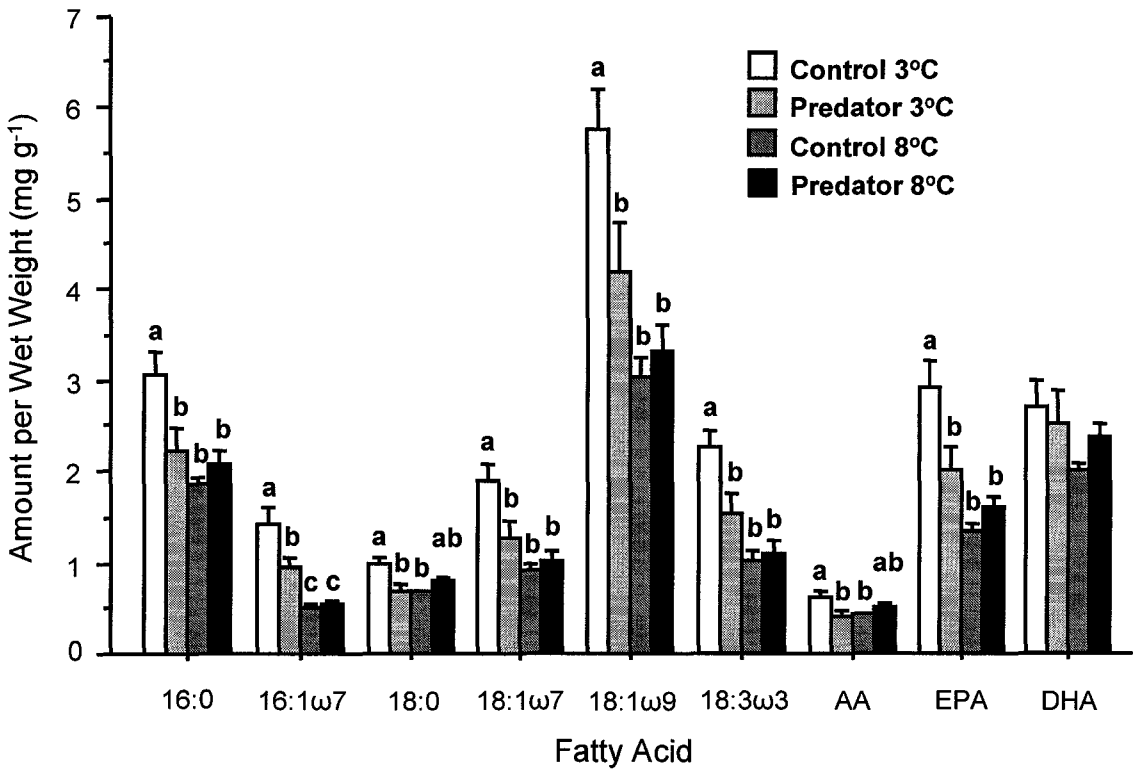
Initial fatty acid profiles (from samples collected at the beginning of the study) are listed in Table 3.3. By the end of the study, individuals exposed to predators at 3°C had lower levels of most identified fatty acids compared to pout not exposed to predators at the same temperature (Figure 3.5). This trend includes significant decreases in the essential fatty acids arachidonic acid (AA) and eicosapentaenoic acid (EPA), although there was no difference in the levels of docosahexaenoic acid (DHA) between the predator treatments at this temperature. At 8°C, there were no significant differences observed between the predator treatments for final levels of the identified fatty acids (including AA, EPA and DHA; Figure 3.5). Regardless of predator treatment, pout reared at 8°C had final levels of most fatty acids that were similar to those in pout raised with predators at 3°C.

3.4 DISCUSSION

Despite the potentially significant consequences of predator-related foraging reductions for individual growth and survival, there have been few studies that examine the energetic cost of reduced foraging rates in response to predatory exposure. This is the first study to examine the costs of reduced

Figure 3.5 Final fatty acid profiles (means ± SEM) for young ocean pout *Zoarces americanus* reared at 3 or 8°C, with and without presence of predators. In predator treatments, ocean pout were visually exposed to predators (juvenile Atlantic cod *Gadus morhua*), while in controls they were not. Only essential fatty acids (arachidonic acid, AA; eicosapentaenoic acid, EPA; and docosahexaenoic acid, DHA), and fatty acids which were present in abundances >5% of the total identified fatty acids are shown. Significant differences between predator and temperature combinations are indicated by different letters (ANOVA, Tukey-Kramer, $p < 0.05$, n = 10 for each bar). There were no significant differences across treatments for DHA.

FIGURE 3.5


Amount per Wet Weight (mg g-1)
7
6
5
4
3
2
1
0
Control 3°C
Predator 3°C
Control 8°C
Predator 8°C
a
b
b
b
a
b
c c
a
b b
ab
a
b
b
b
a
b
b
b
a
b
b
b
a
b b
ab
a
b
b
b
16:0
16:1ω7
18:0
18:1ω7
18:1ω9
18:3ω3
AA
EPA
DHA
Fatty Acid

foraging under predation threat in terms of growth and energy storage in newly hatched fishes.

3.4.1 Behavioural observations

To test the effects of exposure to predators on physiological status of larval fish, my aim was to cause frequent, prolonged interruptions to regular foraging behaviour. One concern prior to this study was that fish in the predator treatments would habituate to the presence of the predators (during the 6 h exposure and also over the course of the study). However, there was no evidence of habituation over the course of the experiments; there were always differences in foraging activity between the predator treatments and the control tanks, and counts of MAPs in the predator treatments did not increase over time. Moreover, at the end of the 6 h predatory exposure, most pout in the predator treatments were either hiding in the provided cover or had moved to the end of the tanks furthest from the predator chamber (pout in control treatments were evenly distributed throughout the aquaria). This pattern suggests that individuals were still responding to the predators given that they continued to be vigilant even after 6 h of exposure.

When visually exposed to predators, the young ocean pout did not appear to display the freezing or predator inspection behaviour that is commonly observed in many species of teleost fishes (Williams and Brown 1991). However, these behaviours may not be obvious in young ocean pout because they do not actively swim in the water column. Individuals in control and predator

treatments remained in contact with the tank bottom almost constantly, and moved only occasionally with short forward bursts (crawls). Therefore, the frequency of crawls may be used as an index of activity in young ocean pout. At both experimental temperatures, ocean pout in the control group crawled with higher frequency after 3 to 4 weeks compared to pout in the predator treatment (until Week 7 at 3°C and Week 6 at 8°C). This decreased level of activity in the presence of the predatory threat probably makes individuals less obvious to predators, and in this regard is analogous to the freezing behaviour observed for actively-swimming fishes. Many of the crawls while in the presence of predators were attempts to gradually move away from the predator or move into the provided cover. 'Fleeing' by rapidly swimming away from the predator chamber was occasionally observed in response to sudden movements by the predators.

At both temperatures, ocean pout showed reduced numbers of orients and lunges to capture *Artemia* while in the presence of predators. It is well known that fishes will decrease foraging while under a perceived threat of predation, and my observations are therefore consistent with previous studies (e.g. Helfman 1989, Williams and Brown 1991, Bishop and Brown 1992, Engstrom-Ost and Lehtiniemi 2004). The decreases in movement associated with foraging are thought to make individual prey more difficult for predators to detect, and decreased rates of foraging may also allow the prey to be more vigilant of the behaviour of the predator (Godin and Smith 1988). Indeed, it has been shown that even very young fishes can assess the level of predatory threat and subsequently make the appropriate foraging decision (Williams and Brown 1991,

Bishop and Brown 1992, Leader 1994, Engstrom-Ost and Lehtiniemi 2004). The ocean pout of the present study reduced foraging activity in the presence of a predatory threat at 1 week post-hatch (as soon as the experiment was initiated). This finding is in contrast to larval Atlantic cod and the threespine stickleback *Gasterosteus aculeatus*, which are unresponsive to the presence of predators during the first few weeks post-hatch (Bishop and Brown 1992, Leader 1994). This discrepancy is likely to be due to the fact that ocean pout are relatively large and well developed at hatch, and possess sensory capabilities that allow them to detect the presence of predators from a very early age. In the present study, the fact that the magnitude of the response to the predators was not dependent on proximity to the predator (within the confines of the experimental tanks) supports this theory. In previous studies on species with less-developed larvae, individuals will only respond to predators that are visually exposed within a distance of just a few centimeters (Bishop and Brown 1992, Leader 1994).

It was thought that a higher rearing temperature might have necessitated continued foraging at 8°C, even while under the threat of predation. Fishes have previously been observed to make state-dependent foraging decisions, with 'hungry' individuals engaging in more risky behaviour (Dill and Fraser 1984, Gotceitas and Godin 1991, Godin and Crossman 1994). Presumably, increased metabolic rate in the pout at 8°C would have increased levels of hunger and motivation to feed. The pout at 8°C also appeared to expend more energy through activity as measured by the frequency of crawls. It was therefore surprising that pout exposed to predators at 8°C showed large reductions in

foraging activity that were similar in magnitude to those observed at 3°C. Similarly, Williams and Brown (1991) did not observe a 'hunger' response when they observed no relation between the *Artemia* ration fed to larval lumpfish *Cyclopterus lumpus* and their willingness to engage in risky behaviour. These authors suggested that even the lowest *Artemia* ration in their study was probably too high to elicit this hunger response. A similar scenario may have occurred in the present study. There was no mortality observed in this study, which suggests that although the 8°C pout may have been 'energetically stressed' (see discussion on lipid levels below), they were not starved to the point where feeding was prioritized over the more immediate threat of predatory presence.

3.4.2 Growth

The consequences of reduced foraging activity were most serious for pout reared at 3°C. At this temperature, pout exposed to predators had reduced length-specific growth rates compared to pout in the control treatment, and had a lower wet mass at the end of the experiment. Surprisingly, the differences in growth rate at 8°C were not statistically significant, despite reduced foraging in the presence of predators at this temperature. The reason for this discrepancy is not known but potential explanations are discussed later. Nonetheless, my findings at 3°C suggest that it is possible for the costs of reduced foraging under predation threat to accumulate and affect growth rates over time. Previous studies have also found that the presence of predators can have profound indirect effects on prey fish populations, including reductions in growth rate

(Fraser and Gilliam 1992, Tonn et al. 1992, Diehl and Eklov 1995, Persson and Eklov 1995). However, in these studies, reductions in growth were the result of prey switching to habitats that were less energetically profitable (either due to decreased prey abundance or quality, or through increased competition due to high population densities). In contrast, the differences in growth found in the present study appear to be attributable to reduced foraging rates caused by frequent predatory exposure. The limited energy stores of young fishes, coupled with high metabolic rates associated with small body size (Killen et al. 2007), probably make reductions in feeding especially costly during early life stages. In addition, because of their small size young fishes in the wild have many potential predators and their frequency of predatory encounters will be relatively high, even while occupying 'refuges' such as vegetation. Although it is important to understand the effects of predator-induced habitat restriction on the size-structure of prey populations, it is also necessary to examine the costs of reduced foraging activity when encounters with predators occur.

Size-dependent predation is generally regarded as being an important source of mortality during the early life stages (Bailey an Houde 1989). Fast growth may be a means of avoiding consumption, because individuals that grow slowly spend more time as a potential food source for predators (Fuiman and Magurran 1994). Further, larger fish can achieve higher escape speeds (Williams et al. 1996), and also have a decreased vulnerability to gape-limited predators (Nilsson and Bronmark 2000). However, the importance of growth in avoiding predation during the early life stages has been somewhat controversial.

While increased swim speeds may be beneficial for escaping some types of predators (e.g. gelatinous predators), it may be of little advantage for evading others (e.g. pelagic fishes; Paradis et al. 1996). However, even if their absolute swim-speeds are lower, smaller fish may be able out-maneuver larger predators during a chase sequence due to their decreased turning radius (Domenici 2001). Furthermore, larger larvae may be preferentially consumed by predators that are physically able to consume them (i.e. are not gape-limited; Litvak and Leggett 1992). In fact, the potential benefits of decreased growth may help to explain why the ocean pout displayed such a strong tendency to reduce foraging under predation threat. Compared to many marine fishes, ocean pout are relatively large at hatch and do not possess a distinct 'larval' stage (Methven and Brown 1991). Therefore, trends observed with young ocean pout are probably most transferable to the early juvenile stage of species that hatch as larvae. The costs of reduced foraging may be greater for larvae, however, because slowed growth may be accompanied by disruptions to normal development. Although no mortality was observed in the present study, larval fishes can suffer severe consequences if nutritional requirements are not met at key stages of ontogeny, and could experience increased rates of indirect mortality if frequently exposed to predation threat during this delicate life stage.

3.4.3 Lipid classes

Lipid analyses were performed to examine how energy storage is affected by foraging reductions under predation threat. Pout exposed to predators at 3°C

had significantly reduced final whole-body lipid content compared to the control group. Furthermore, pout in the predator treatment had smaller livers (as measured by HSI), an organ that is a primary storage site for lipids in young fishes. Of the lipid classes analyzed, the most pronounced effects of predator presence were observed in the levels of PL and TAG. Both types of lipid were greatly reduced in pout exposed to predators at 3°C. PL are a main constituent of cellular membranes and are important as young fishes grow and develop new tissue. TAG are mainly used for energy storage, demonstrating that the control group were better able to maintain energy reserves compared to pout in the predator treatment. The potential impacts of this reduction in energy reserves are not known, but it is generally accepted that lipids are an important metabolic fuel in young fishes (Sargent 1995). TAG reserves are catabolized when feeding rates are not sufficient to support the metabolic demands of individuals, and TAG content has been shown to have a positive correlation with physiological condition in fish larvae (Fraser 1989). Furthermore, juveniles of many fish species reduce foraging during colder winter months and must accumulate lipid reserves prior to over-wintering in order to survive (Post and Evans 1989). It is not known if young ocean pout decrease foraging during the winter, but this would be another important consequence of decreases in lipid levels as a result of frequent predatory encounters.

As was the case with growth comparisons between predator and control treatments at 8°C, there were also no differences in HSI, whole-body lipid levels, or PL and TAG at this temperature. These results are surprising, but there are at

least two lines of reasoning that may explain these findings. First, compared to ocean pout at 3°C, individuals exposed to predators at 8°C may have been better able to compensate for lost feeding opportunities once the exposure period had concluded (and regular feeding recommenced), thus reducing differences in growth and lipid storage. Foraging behaviour was not quantified after the period of predatory exposure, but this scenario was possible given that pout in the 8°C treatment were generally more active and had higher rates of feeding than pout at 3°C (Table 2; mean frequencies for crawls, orients and lunges). Second, the effects of temperature on growth and metabolism at 8°C may have overwhelmed the capacity to detect any effects of predatory exposure at this temperature. For example, pout at 8°C may allocate most ingested energy toward activity, growth, and maintenance, and very little to energy storage — thus reducing detectibility of differences in lipid levels. Supporting this view, TAG levels in both treatments at 8°C were greatly reduced compared to either treatment at 3°C, despite the fact that pout at 8°C foraged nearly twice as much as pout at 3°C (Table 2 , mean counts for lunges). Furthermore, even without additional stressors, young fishes are believed to have a diminished factorial aerobic scope that may limit growth rates (factorial aerobic scope = maximal metabolic rate/standard metabolic rate; Post and Lee 1996, Blier and Pelletier 1997; Killen et al. 2007; Chapter 2 of this thesis). An increased energetic cost of maintenance at higher temperatures could further decrease the metabolic scope available for growth if maximal aerobic capabilities do not also increase with increasing temperature. In this regard, the routine metabolic rate of young ocean pout increases substantially

with increasing temperature (Killen et al. in press). The change in maximal aerobic metabolism with temperature in young ocean pout is not known, but aerobic scope in adult Norwegian eelpout *Zoarces viviparous* decreases considerably with increases in temperature and growth rate declines at temperatures above their optimum (Zakhartsev et al. 2003). Based on this evidence, I speculate that growth rates of the ocean pout at 8°C may have been close to the physiological maximum, even during periods with relatively low foraging rates when ocean pout were exposed to predators. This scenario could make it difficult to observe any reductions in growth at 8°C. Additional research into the thermal physiology of young ocean pout is needed to resolve these issues.

Regardless of the mechanisms responsible for the unexpected results at 8°C, the fact that different conclusions were reached when the same experiment was performed at two different temperatures points to the importance of temperature selection in experiments on foraging and energy acquisition in ectotherms. This consideration is particularly true for young or larval fishes, for which relatively little is known about the effects of environmental conditions on metabolism. In the wild, young ocean pout occur at both of the water temperatures used in this study, and either temperature choice would have been justifiable. However, if the study had been performed only at 8°C, I would have found no accumulated cost associated with reduced foraging under predation threat. I therefore recommend that studies on the costs of predator-related

foraging reductions in fishes be performed at multiple temperatures (within the range that is normally experienced in the wild).

3.4.4 Fatty acids

To better understand the biochemical changes in young fishes exposed to frequent predation threat, I examined the fatty acid compositions of pout from the different treatments. At 3°C, the predator treatment had lower levels of most fatty acids identified in my analyses. For example, large differences were observed for various saturated and monounsaturated fatty acids that are readily metabolized for energy. Once again, however, there were no differences between the treatments at 8°C. The 8°C pout in both treatments also had levels of most fatty acids that were similar to the 3°C pout from the predator treatment, providing further evidence that pout at 8°C had limited energy stores.

Adequate lipid nutrition is crucial for young marine fishes not only in providing energy, but to also in supplying essential highly unsaturated fatty acids (HUFAs) that are important for regular development. These fatty acids — arachidonic acid (AA; 20:4ω-6), eicosapentaenoic acid (EPA; 20:5ω-3), and docosahexaenoic acid (DHA; 22:6ω-3) — must be obtained from the diet because they cannot be synthesized by marine fishes (Sargent et al. 1999, Bell et al. 2003). Deficiencies of essential HUFAs cause reductions in growth, survival, and resistance to stress in larval fishes (Watanabe 1993, Bell et al. 2003). Ocean pout exposed to predators at 3°C showed decreased levels of AA and EPA compared to the control, and it appears that frequent reduced foraging

resulting from predatory threat can affect the intake of essential fatty acids. Although levels of DHA were not different among any of the treatments, differences resulting from decreased foraging might be difficult to distinguish because starved larvae preferentially conserve DHA (Rainuzzo et al. 1997). Deficiencies of essential HUFAs may have significant consequences during the early life stages of fishes, especially for the development of neural tissue and visual systems (Tocher 2003). Such effects would be very serious for young fishes because reduced sensory capabilities may lead to an inability to avoid predation or to capture prey themselves. For example, larval herring deprived of DHA have a reduced ability to capture prey at low light intensities (Bell et al. 1995). Reduced concentrations of essential HUFAs could also alter behaviours associated with neuroendocrine function, but this area requires further study.

In summary, this study has shown that frequent interruptions to foraging as a result of predation threat can cause reduced growth rates in young marine fishes. It is also clear that water temperature choice can have a profound effect on experiments that examine the costs of reduced foraging under predation threat in ectotherms. Furthermore, predation and water temperature can both directly impact lipid composition and energy storage abilities of young fish. If energy is viewed as a currency in the tradeoff between foraging and predator avoidance, the individuals exposed to predators at the colder temperature in this study clearly suffered a heavy cost as a result of frequent foraging disruptions. Future studies should address how such reductions in growth and lipid content may affect competitive ability or survival under adverse environmental conditions.

Chapter Four

Ontogeny of routine metabolism and foraging behaviour in larval shorthorn sculpin: evidence for increased costs of reduced foraging under predation threat

4.1 INTRODUCTION

The performance of a behaviour or physiological function by an animal requires the allocation of resources. However, due to limited time and energy, the performance of one activity often diminishes the capacity to perform others (Stearns 1992). An example of such a behavioural tradeoff is that which occurs between foraging and predator avoidance. Animals must feed to obtain energy, but foraging can make an individual prone to predation by: 1) making them more conspicuous; and 2) reducing their vigilance towards potential attacks. As a consequence, in the presence of a predatory threat, most animals will reduce their foraging activity in exchange for predator-avoidance behaviours (e.g. hiding, freezing, fleeing; Lima and Dill 1990; Lima 1998). This behavioural choice makes an animal less vulnerable to predation, but comes at the cost of reduced energy acquisition.

In the wild, consumption by predators is a major source of mortality during the early life stages of fish, and may even be important in mediating year-class strength (Folkvord and Hunter 1986; Bailey and Houde 1989). Further, it is known that juvenile and adult fish can assess the magnitude of a predatory threat and adjust their foraging behaviour accordingly (e.g. Helfman 1989; Godin and

Smith 1988), and that this behaviour is also exhibited by larval fish (Williams and Brown 1991; Bishop and Brown 1992; Skajaa et al. 2003). However, little is known about the development of this decision-making ability during the early ontogeny of teleosts. Most larvae are poorly developed at hatch, and may initially possess limited sensory capabilities that could reduce their ability to detect predators. Moreover, even if they are able to perceive a predator, the larvae of some fish species may not need to alter their foraging behaviour if the risk of being predated is low during early development (e.g. newly hatched larvae may escape detection by being transparent and thus difficult to detect). For these reasons, the interactions between larvae and their predators may be complex.

Due to the associated reduced energy intake, the decision to decrease foraging under predation threat should also be influenced by the physiological state of an individual. For example, hungry or food-deprived fishes tend to occupy more dangerous habitats (Pettersson and Bronmark 1993), display higher foraging rates while in the presence of a predatory threat (Godin and Crossman 1994), and will resume foraging sooner after an attack (Gotceitas and Godin 1991). In a similar manner, the foraging decisions of larval fishes may be influenced by changes in metabolic demand during early ontogeny. Larval fishes have a higher mass-specific metabolic rate than juvenile or adult fishes (Weiser 1995; Post and Lee 1996). Moreover, metabolic scaling is not constant throughout ontogeny, and routine metabolism within the larval stage is thought to scale either isometrically (i.e. b = 1.0; in direct proportion to body mass; Giguere

et al. 1988; Post and Lee 1996; Wuenschel et al. 2004; Killen et al. 2007) or with positive allometry (i.e. $b > 1.0$; mass-specific metabolism increasing with body mass; Almatar 1984; Killen et al. 2007). These findings suggest that although the metabolic demand of larvae may already be very high, this demand may increase even further at certain points during early development.

This scenario presents an interesting foraging dilemma for larval fishes. On one hand, the small size of larvae makes them extremely prone to predation, and so they should be cautious while in the presence of a threat. However, their increased mass-specific metabolism, combined with limited energy stores when exogenously feeding, may make each instance of reduced foraging especially costly. Work with other animals has shown that the costs of reduced foraging may accumulate over time to cause reduced growth in individuals that are frequently exposed to predation threat (amphibians – Skelly 1992; aquatic insect larvae – Ball & Baker 1996). Although fish studies in this area are rare (especially during the early life stages), Killen and Brown (2006; see Chapter Three) found that decreased foraging rates under predation threat led to reductions in growth and lipid storage in young ocean pout *Zoarces americanus*. Lipids are a primary metabolic fuel in larval fishes, and deficiencies in lipids and certain fatty acids may impair normal development and reduce the capacity for important behaviours involved in foraging and predator avoidance (Adams 1999; Tocher 2003). However, ocean pout do not posses a distinct larval phase (they are regarded as being fully formed juveniles at hatch; Methven and Brown 1991),

and thus no information exists on the long-term cost of reduced foraging under predation threat during larval stage of fishes.

In this study, we examined the foraging behaviour of larval shorthorn sculpin *Myoxocephalus scorpius* from hatch until settling, with and without the presence of a predatory threat. Individuals of this species are small at hatch (approximately 7-9 mm total length; Scott and Scott 1988) but, unlike the larvae of many other marine species, possess significant pigmentation that may make them conspicuous to predators. This species is thus well-suited for studying the effects of predatory presence on foraging behaviour in marine teleost larvae, because larval transparency can be eliminated as a possible means of predator avoidance. Specifically, there were three main goals in this study: 1) to determine if larval shorthorn sculpin alter foraging activity in the presence of a predator, and examine if this tendency changes during early development; 2) relate the observed patterns of behaviour to changes in routine metabolic demand throughout ontogeny; and 3) determine the effects of frequent reduced foraging under predation threat on growth, nutrition (in terms of lipid and fatty acid acquisition), and mortality. It was hypothesized that foraging under predatory threat would decrease as larvae developed (i.e. as they acquired the ability to visually detect predators), and that this behaviour would be associated with significant costs as the larvae approach metamorphosis.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Masses of shorthorn sculpin eggs (*Myoxocephalus scorpius*) were collected by SCUBA divers and transported to the Ocean Sciences Centre (OSC), Memorial University of Newfoundland. Once at the OSC, the eggs were placed in aerated incubators supplied with flow-through seawater and maintained at 3°C. Immediately following hatching, larvae were carefully transferred to experimental tanks that were also maintained at 3°C throughout the study (initial stocking density ~ 20 larvae l^{-1}). Larvae used in each experiment (Acute and Chronic Exposures – see below) were a mixture of individuals from four separate egg masses.

Larvae in all treatments were fed four times daily (starting one day after hatching) by adding live *Artemia* nauplii to each tank (approximately 1500 L-1). *Artemia* were enriched using DC DHA Selco (INVE, Belgium), Algamac 3050 (Aquafauna Bio-Marine, California), and freeze-dried krill (*Euphausia* sp.) on a three-day cycle. Feedings were performed at 9:00, 13:00, 17:00, and 20:00 h. *Artemia* were observed to be actively swimming in the water column at all times of the day, and were mixed with the aide of a small air stone placed into each tank. Any excess *Artemia* or fecal matter at the bottom of the tanks was removed by siphoning at the end of each day. Mortalities were also removed daily, and counted during the Chronic Exposure (see description of experiments below). All experimental tanks were exposed to a 16 h light: 8 h dark photoperiod.

4.2.2 Behavioural Observations

Two separate sets of experiments were performed in which the foraging behaviour of larval shorthorn sculpin was observed in the presence of a predatory threat (details summarized in Table 4.1). In the first experiments (March-April, 2004), the larvae were observed during an acute visual exposure to a predator ('Acute Exposure'). For these experiments, four 68 l flow-through glass aquaria with darkened sides were used, each tank divided into two separate chambers: a 'predator' chamber measuring 30 (L) X 30 (W) X 30 (H) cm, and a 'larval' chamber measuring 45 X 30 X 30 cm. The chambers were separated by two adjacent partitions, one being transparent and non-removable, and the other removable and opaque. These partitions allowed visual exposure of the larvae to the predators, while preventing physical contact between the predators and larvae. Two of the tanks were randomly designated to contain larval shorthorn sculpin in the larval chamber and two predatory juvenile Atlantic cod (*Gadus morhua*; total length 12.5 ± 0.8 cm) in the predator chamber (which were fed a maintenance amount of commercially prepared dry feed daily during the study). The other two tanks were assigned to be the control treatment, and were identical to the experimental treatment but contained but no predators (the 'predator' chamber was vacant). To analyze larval behavior in relation to proximity to the predators, and to ensure that equal numbers of behavioral observations were made for all tank locations, the larval chamber of each tank was further divided into three grid sections using lines etched into the exterior dark paint on each tank (Bishop and Brown 1992; Killen and Brown 2006). The

Table 4.1. Summary of Acute and Chronic Exposure experiments in which the foraging behaviour of shorthorn sculpin larvae was observed with and without visual exposure to a predatory threat.

	Acute Exposure	Chronic Exposure
Duration of Experiment	hatching – 7 weeks post-hatch	2 weeks post-hatch – 7 weeks post-hatch
Frequency of Exposure	only on observation days	daily
Total Exposure Time / Day with Exposure	10-12 min	6 h
Replicate Tanks / Treatment	2	3
Observation Days / Week	2	2
Observations / Tank	9	9
Observations / Tank Section	3	3
Behaviours Recorded	orients, lunges	orients, lunges, capture success
Density (larvae l^{-1})	20	20
Water Temperature (^{o}C)	3	3

'Near ', 'Mid' and 'Far' sections were 0-15, 15-30 and 30-45 cm, respectively, away from the predator chamber.

For the Acute Exposure, behavioural observations were performed three times a week, and began two days after larvae were transferred to the experimental tanks. Following the removal of the opaque partition, *Artemia* were added to the larval chamber of the tanks (the control tanks also had the opaque partition removed during this time), and after a two-minute acclimation period, the focal animal technique (Altmann 1974) was used to observe the behavior of larvae. Using this technique, the behaviors of one individual are recorded over a specific length of time (one minute in the present study). The foraging behavior of the shorthorn sculpin larvae included two easily observable modal action patterns (MAPs; Barlow 1968). These were: 1) Orient, which was a turning of the head or body towards an *Artemia*; and 2) Lunge, which was a rapid forward movement in an attempt to capture an *Artemia*. Behaviours were recorded and tabulated using a hand-held Psion event recorder and associated software (The Observer 3.0, Noldus Information Technology Inc., Netherlands). During each period, behavioral observations were initiated for three larvae in each grid section of the larval chamber (for a total of nine observations per tank). After the behavioural observations were completed for a given tank, the predatory exposure was terminated by replacing the opaque partition. In total, predator exposures during the Acute Exposure lasted approximately 12 min. Because larvae were free to swim throughout the larval chamber, it was common for individuals to move between grid sections over the course of an observation. In

these cases, observations were divided into time intervals representing the time spent in each section. The frequencies of the observed behaviors were then converted to counts minute^{-1}. Intervals were not included in the final analysis if the focal larva spent less than 5 seconds in that section.

In the Chronic Exposure (March- April, 2005), the foraging behaviour of individual larvae was observed as described above, however: 1) the larvae were then left exposed to the predator for an additional 6 h during the regular feeding period; 2) the 6 h predatory exposure was performed daily, regardless of whether or not behavioural observations were made on that day; 3) treatments (control and predator) were conducted in triplicate tanks; 4) behavioural observations were initiated two-weeks after the larvae were transferred to the experimental tanks (although the daily predator exposures started one day after transfer); and 5) in addition to recording the number of orients and lunges performed by each focal larvae, it was also documented whether or not a lunge was successful at capturing the targeted *Artemia*. The purpose of the Chronic Exposure was to examine the long-term effects of frequent reduced foraging under predation threat. However, the previously described Acute Exposure measurements were also necessary so that observations of foraging behaviour could be related directly to changes in metabolic rate during ontogeny while not having to account for the potential effects of habituation to the predator, or the effects that reduced feeding could have on feeding motivation (both of which could potentially occur during the Chronic Exposure).

Larvae began to settle on the bottom of the tanks at approximately eight weeks post-hatch. This behavioral shift was expected as part of metamorphosis in this species, but because settlement is accompanied by a change in the behavioral repertoire, observations of foraging behavior concluded at this time.

4.2.3 Growth Measurements

Each week (in both the Acute and Chronic Exposures), eight larvae were randomly selected from each tank, anaesthetized using MS-222, and measured for standard length. Measurements were made using Matrox Inspector 3.0 image analysis software (Matrox Imaging), on images captured using a digital camera (Pixera PVC 100C). Following image capture, larvae were quickly dried with a lint-free paper towel, and then placed on dry, tared weigh-foils. Their wet mass was then measured using a microbalance (APX-60, Denver Instrument Co).

4.2.4 Measurements of Oxygen Consumption

Measurements of larval oxygen consumption were performed using a glass Blazka-type respirometer (total volume of the respirometer and the external circuit to oxygen sensor = 57 ml), and a fibre-optic flow-through oxygen sensor (this type of sensor does not consume oxygen; Presens, Germany). Data obtained using this sensor were recorded directly to a computer using the accompanying software (OXYVIEW v. 4.16). Water was moved from the respirometer and past the sensor via a short external circuit using a peristaltic

pump (Masterflex; Cole-Parmer Instrument Co.) and tubing with extremely low oxygen permeability (Masterflex Tygon Food for the majority of the circuit and a small section of Masterflex Tygon LFL at the pump-head). Preliminary experiments using hypoxic seawater (30–40% oxygen saturation) confirmed that this system did not gain oxygen after being closed for 1.5 h of monitoring. To maintain the temperature of the water in the respirometer and oxygen concentrations in the water following transfer of the larvae to the respirometer, a second external circuit supplied aerated seawater from a reservoir in a water bath set at 3°C. This circuit was closed when measurements of oxygen consumption were made, and therefore to maintain water temperature within the respirometer, the entire set-up was located in a cold-room set at 3°C. To reduce background bacterial contamination, seawater used for the 57 ml respirometer was sterilized with ultraviolet radiation and the system was cleansed daily with absolute ethanol. In addition, blank measurements were performed after each trial to quantify any possible bacterial oxygen consumption. In the vast majority of cases, there was negligible background oxygen consumption. However, in cases where a background rate of oxygen consumption was observed, this value was subtracted from the experimental data.

Larvae used for measures of oxygen consumption were reared in two 60-L holding aquaria that were separate from the experimental tanks described above. Note that the measures of metabolism reported in this Chapter are separate from those reported in Chapter Two. Most importantly, the study in Chapter Two dealt with measures of standard metabolic rate (metabolic rate under conditions of

zero activity), while this chapter contains measures of routine metabolic rate (measures of metabolism during spontaneous activity). To prepare for each measurement period (measurements of oxygen consumption were performed approximately every second day throughout development until larvae reached around 120 mg wet mass), larvae were carefully placed into the respirometer with a transfer pipette and were allowed to acclimate for 4 hours; this time period was chosen because preliminary experiments showed that the routine oxygen consumption of larval sculpin peaks immediately following transfer to the respirometer, but then stabilizes by approximately 4 hours. The number of individuals used per trial depended on the size of the larvae, but varied between 1 and 25 individuals (larvae ranged in size from 5-120 mg over the course of the study, and the number of the larvae in the respirometer was enough to make up at least 70 mg of biomass per trial). After the acclimation period, the external reservoir circuit was closed and measurements of routine oxygen consumption were made over a 20-minute period as the fish were allowed to perform spontaneous activity (larval sculpin swim almost constantly in the holding tanks and in respirometers). During the acclimation and measurement periods, the water-current speed in the respirometer was set to 1 cm s^{-1}. Larvae would occasionally orient against this current, but would also swim freely about the inside of the respirometer in various directions. Thus, I believe that most of the routine swimming of the larvae was at speeds slower than 1 cm s^{-1}. After each trial, I obtained the total wet weight of all larvae in the respirometer using a

microbalance. This value was then divided by the number of individuals to produce the average wet mass of the larvae used in the trial.

To better understand the changes in routine metabolic rate during early ontogeny in larval sculpin, I estimated the metabolic scaling exponents for three separate developmental stages. Larvae were considered 'pre-metamorphic' between the time that they completely absorbed their yolk-sac and they first began to show signs of entry into metamorphosis; as indicated by slight notochord flexion and fusing of the pigment bands on the dorsal surface and along the tail (Temple et al. 2004; ranged from 2-5 weeks in age and 6-12 mg wet mass). Larvae were considered 'metamorphic' once they began to show the morphology described above, and until they had completed metamorphosis, as indicated by the presence of full flexion, differentiation of the median finfold, uniform pigmentation, and a behavioural shift towards settling on the bottom of their holding tanks (ranged from five to eight weeks in age and 12-30 mg wet mass). Individuals were considered 'post-metamorphic' juveniles after this point (> eight weeks of age, 30-120 mg wet mass). Measurements were also performed on yolk-sac larvae, and while this data is presented, it was not used in the estimation of scaling exponents.

4.2.5 Lipid and Fatty Acid Analysis

At the beginning and end of the chronic exposure experiments, five larval samples were taken from each tank (with each sample consisting of five pooled individuals), quickly measured for wet mass using lipid-clean instruments, and

then placed into lipid-clean glass vials containing chloroform. The samples were then frozen at -80°C until the lipid and fatty acid analyses could be performed. Lipid extraction was then performed using a variation of the Folch procedure (Folch et al. 1957) as described by Parrish (1998). Briefly, each sample was homogenized with 2:1 chloroform-methanol and washed with water to remove non-lipid contaminants. The entire lower organic phase was then collected and washed four times using 2:1 chloroform-methanol.

Quantitative determination of lipid classes was performed on these extracts using rod thin-layer chromatography with flame ionisation detection on a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan). The extracts were spotted on silica gel coated Chromarods and a three-stage development system was used to separate lipid classes (described in detail by Copeman et al. 2002). After each separation, the rods were scanned and the three resulting chromatograms were combined using T-scan data scan software (RSS, Bemis, TN, USA). The signal detected in millivolts was quantified using lipid standards (Sigma). Fatty acids methyl esters (FAME) were prepared by transesterfication with 10% BF_3 in methanol at 85°C for 1 h (Copeman et al. 2002). Fatty acids were then analyzed using gas chromatography (model 6890 gas chromatograph; Hewlett-Packard, Palo Alto, California), as described by Alkanani et al. (2005). Based on whole-body lipid concentrations, the qualitative (proportional) measures of fatty acid content were then used to calculate the concentrations of each identified fatty acid (mg g^{-1} wet mass).

4.2.6 Data and Statistical Analyses

All statistical analyses were performed using Minitab version 13.1. The level of significance for all tests was $\alpha = 0.05$. Data are presented as means ± SEM (except for estimates of scaling exponents, which are also given 95% confidence limits).

We recognize the potentially confounding factor of tank effects on our treatments (differences in behaviour may be observed between tanks within a treatment due to subtle variations in factors such as temperature, light levels, or water flow rates), and thus for behavioral observations, we performed a test for tank effects by performing an ANCOVA (within each treatment) with the factors of tank and age (with the latter used as covariate). In all cases, the effects of 'tank' and the tank*age interactions were insignificant, and so the data were pooled within each treatment for all subsequent analyses. Due to the high number of observations performed for each tank during this study, this method is a highly sensitive test for tank effects. Similarly, there were no significant differences observed for larval wet mass and standard length between the experimental tanks or between treatments in the Acute Exposure.

Differences in the frequency of orients and lunges between the control and predator treatments were compared within each tank section using a General Linear Model with a normal error structure, and the suitability of the model (in terms of normality, homogeneity, and independence of residuals; Sokal and Rohlf 1995) was verified using residual-fit plots. The models were constructed with least-squares regression using the means for each observational period

(weighted by the inverse-variance for each dataset). To allow for ease of comparison to the metabolic data, larval body mass was used as a covariate for the analysis of the behavioral data (instead of larval age), with the mean expected body mass for each age obtained from the growth equations shown in Figure 4.5. The data could not be fitted using a straight line, so a quadratic term was introduced into the model ($mass^2$). This resulted in the model containing two interaction terms: predator*mass (representing the slope of the model), and predator*$mass^2$ (representing the curvature of the model). We then combined the sequential sums of squares and degrees of freedom for the linear and curvilinear aspects of the data (both interaction terms), and used this value to compute an F-ratio for the overall effect of predatory exposure on the behavior of the larvae (Sokal and Rohlf 1995).

Rates of oxygen consumption (mg O_2 h^{-1}) were calculated for each trial from the decrease in water oxygen content per unit time data using linear regression. Measures of oxygen consumption (absolute and mass-specific) were plotted on log plots against wet mass, and power curves were then fitted to the data to obtain estimates of scaling exponents.

Daily mortality (% per day) in the Chronic Exposure was estimated by calculating the mean mortality within each treatment per day (mean for all three tanks per treatment). These daily means were than used to calculate the average daily mortalities during each week of the study, which were compared between treatments using unpaired, two-tailed t-tests. Differences in wet body mass and standard length at each week, and lipid and fatty acid content at the

end of the Chronic Exposure, were also examined using unpaired, two-tailed t-tests.

4.3 RESULTS

4.3.1 Behavioural Observations

Larval sculpin did not show the 'stop-start' swimming or 'saltatory' search pattern that is displayed by some altricial fish larvae (Temple et al. 2004), and instead swam almost constantly, only occasionally stopping to perform an orient or to attempt to capture prey (lunge). They began to orient towards *Artemia*. at 1-2 days post hatch, and while the external yolk-sac of the larvae was greatly reduced by one-week post-hatch, it wasn't completely absorbed until two-weeks. The Atlantic cod displayed moderate levels of activity within the predator chamber, often approaching the transparent partition and occasionally appearing to 'attack' shorthorn sculpin larvae swimming on the opposite side.

In both the Acute and Chronic Exposures, predatory exposure decreased the frequency of both orients and lunges by the larval sculpin (Figures 4.1, 4.2; Tables 4.2, 4.3). In both experiments, the effect of the predator was most pronounced in the 'Near' section. Here, the frequency of orients and lunges differentiated from the control treatment when the larvae were around 6-8 mg wet mass (or around 2-3 weeks post-hatch), and the difference increased until the larvae reached approximately 10-15 mg wet mass. There was then a gradual and slight increase in foraging activity after this minimum point (as indicated by the concave shape of the quadratic models for orients and lunges), although

Figure 4.1 Frequencies of behavioural modal action patterns (Orients -- left panels; Lunges – right panels) for larval shorthorn sculpin *Myoxocephalus scorpius* in the Acute Exposure (see Materials and Methods for description). Filled circles represent individuals that were observed during a visual exposure to predators (juvenile Atlantic cod *Gadus morhua*), whereas open circles represent the control treatments that were not exposed to predators. The top panels represent larvae observed in the tank grid section furthest from the predator chamber (the 'Far; section; 30-45 cm from the predator chamber), the middle panels represent larvae in the middle tank grid section (the 'Mid' section; 15 - 30 cm from the predator chamber), and the bottom panels are for larvae in the closest tank grid section from the predator chamber (the 'Near' section; 0 - 15 cm from the predators). Data points denote mean ± SEM. Solid lines represent quadratic models for the changes in each MAP with increasing wet body mass (model equations and statistical comparisons are given in Table 4.1).

FIGURE 4.1

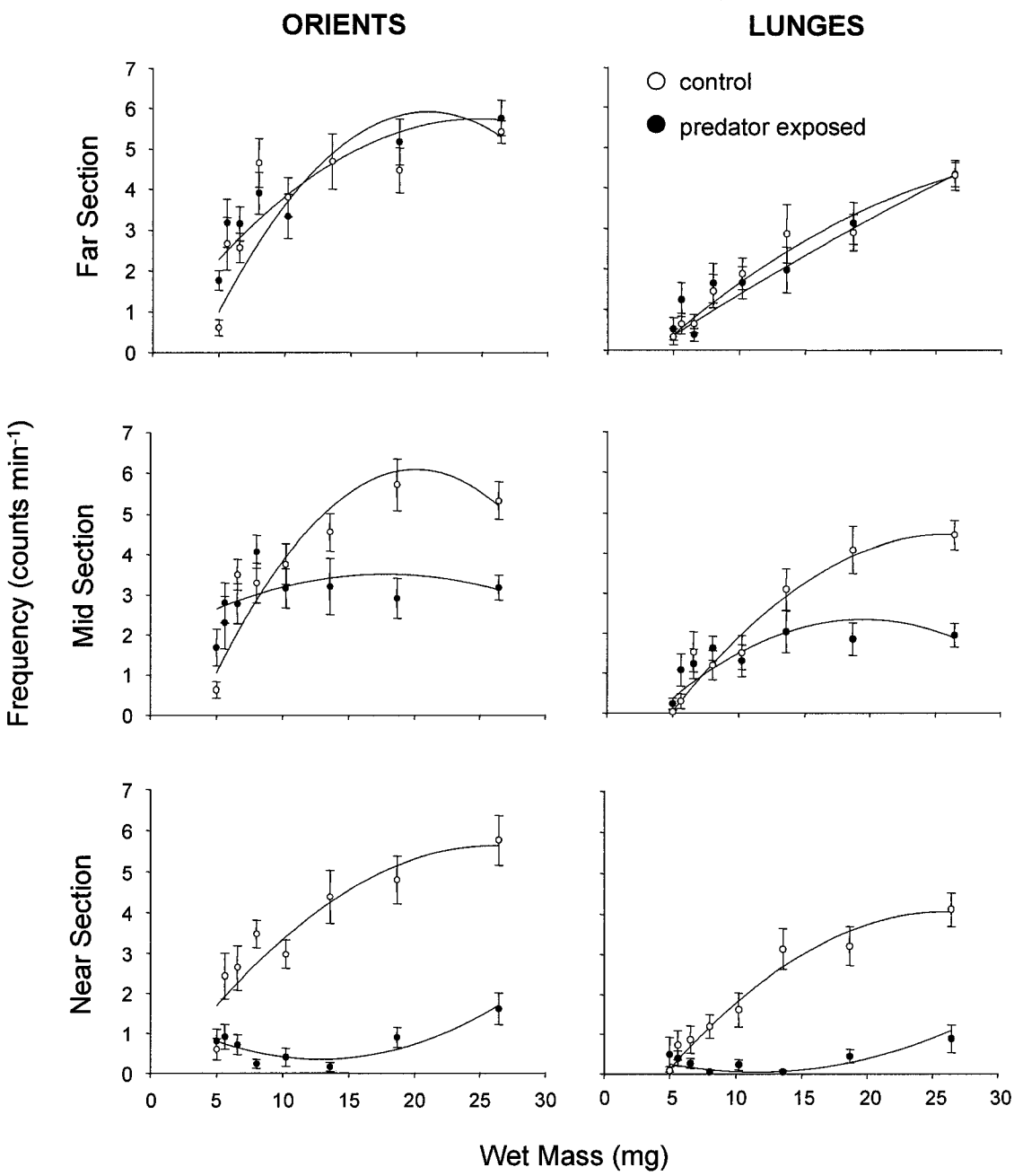

ORIENTS
LUNGES
control
predator exposed
Far Section
Mid Section
Near Section
Frequency (counts min-1)
Wet Mass (mg)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
30

Table 4.2. Model results for modal action patterns (MAPS) of larval shorthorn sculpin during the Acute Exposure. Graphical representations of regressions are shown in Figure 4.1. * indicates a significant difference between the predator treatment and the control ($p < 0.05$). ^ indicates lower order terms that cannot be interpreted independently because of a significant interaction term.

MAP	section	treatment	regression	r^2	term	*F*	*p*
Orients	Far	control	$y = -0.020x^2 + 0.81x - 2.61$	0.89	predator	4.56	0.249^
		predator	$y = -0.009x^2 + 0.44x + 0.31$	0.91	mass	103.39	<0.0001*^
					predator x mass	0.78	0.397
					predator x mass^2	1.51	0.248
					overall	1.14	0.381
	Mid	control	$y = -0.022x^2 + 0.89x - 2.82$	0.88	predator	2.28	0.162^
		predator	$y = -0.005x^2 + 0.189x + 1.83$	0.19	mass	30.11	<0.0001*^
					predator x mass	14.44	0.003*
					predator x mass^2	4.05	0.072
					overall	5.41	0.045
	Near	control	$y = -0.009x^2 + 0.46x - 0.40$	0.86	predator	134.41	<0.0001*^
		predator	$y = 0.007x^2 - 0.19x + 1.56$	0.84	mass	28.31	<0.0001*^
					predator x mass	27.23	<0.0001*
					predator x mass^2	20.47	0.001*
					overall	23.84	0.0014*
Lunge	Far	control	$y = -0.004x^2 + 0.32x - 1.10$	0.99	predator	0.43	0.526
		predator	$y = -0.001x^2 + 0.22x - 0.70$	0.94	mass	193.70	<0.0001*
					predator x mass	0.02	0.891
					predator x mass^2	0.33	0.580
					overall	0.17	0.848
	Mid	control	$y = -0.010x^2 + 0.52x - 2.30$	0.99	predator	47.71	<0.0001*^
		predator	$y = -0.009x^2 + 0.37x - 1.21$	0.86	mass	263.65	<0.0001*^
					predator x mass	54.99	<0.0001*
					predator x mass^2	<0.001	0.957
					overall	27.51	0.001*
	Near	control	$y = -0.009x^2 + 0.47x - 1.99$	0.98	predator	39.76	<0.0001*^
		predator	$y = 0.005x^2 - 0.11x + 0.68$	0.63	mass	71.82	<0.0001*^
					predator x mass	104.23	<0.0001*
					predator x mass^2	20.37	0.001*
					overall	62.27	<0.0001*

Figure 4.2 Frequencies of behavioural modal action patterns (Orients – left panels; Lunges – right panels) for larval shorthorn sculpin *Myoxocephalus scorpius* in the Chronic Exposure (see Materials and Methods for description). Filled circles represent individuals that were observed during a visual exposure to predators (juvenile Atlantic cod *Gadus morhua*), whereas the open circles represent the control treatments that were not exposed to predators. See Figure 4.1 for additional details. Data points denote mean ± SEM. Solid lines represent quadratic models for the changes in each MAP with increasing wet body mass (model equations and statistical comparisons are given in Table 4.2).

FIGURE 4.2

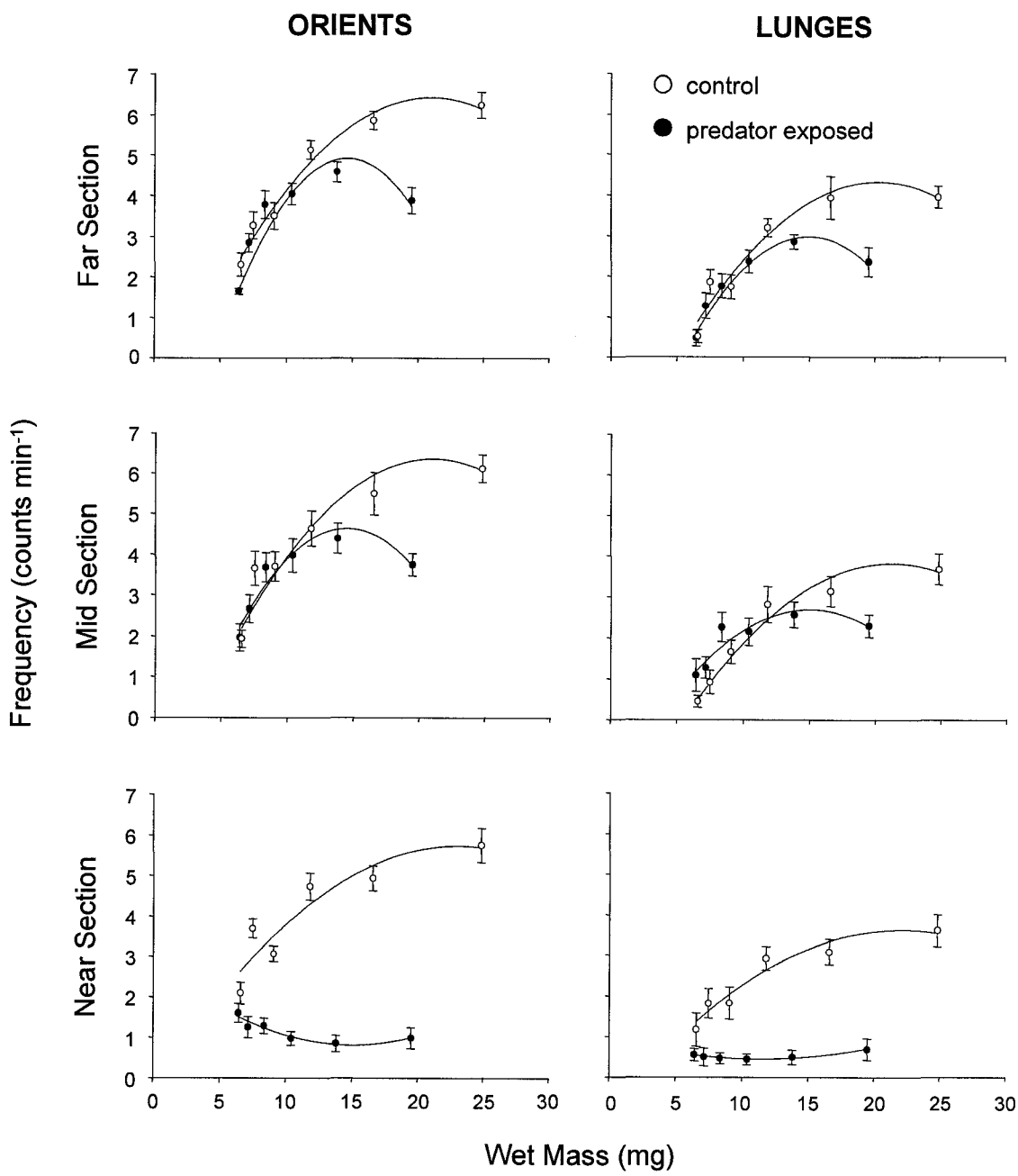

ORIENTS
LUNGES
control
predator exposed
Frequency (counts min-1)
Far Section
Mid Section
Near Section
Wet Mass (mg)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
30

Table 4.3. Model results for modal action patterns (MAPS) of larval shorthorn sculpin during the Chronic Exposure. Graphical representations of regressions are shown in Figure 4.2. * indicates a significant difference between the predator treatment and the control ($p < 0.05$). ^ indicates lower order terms that cannot be interpreted independently because of a significant interaction term.

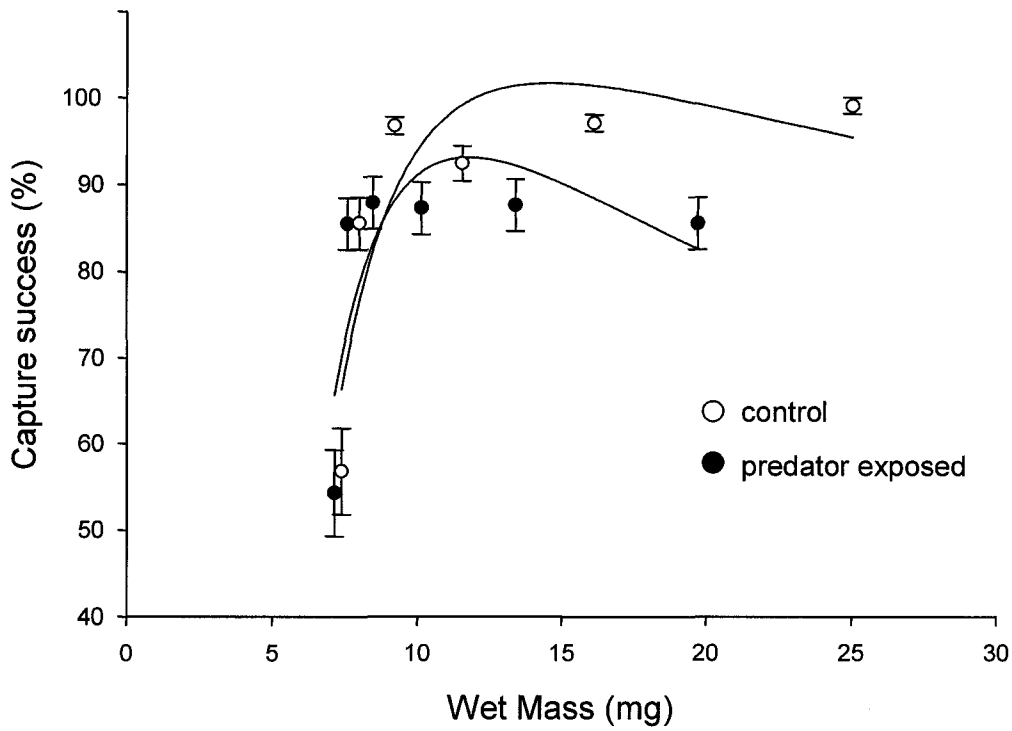
MAP	section	treatment	regression	r^2	term	*F*	*p*
Orients	Far	control	$y = -0.019x^2 + 0.80x - 1.97$	0.98	predator	91.25	<0.0001*
		predator	$y = -0.049x^2 + 1.42x - 5.43$	0.95	mass	60.12	<0.0001*
					predator x mass	3.42	0.144
					predator x mass^2	4.67	0.074
					overall	4.04	0.077
	Mid	control	$y = -0.020x^2 + 0.84x - 2.50$	0.94	predator	0.39	0.553^
		predator	$y = -0.037x^2 + 1.08x - 3.20$	0.93	mass	42.77	0.001*^
					predator x mass	16.40	0.007*
					predator x mass^2	0.94	0.371
					overall	8.66	0.017*
	Near	control	$y = -0.012x^2 + 0.53x - 0.36$	0.86	predator	101.27	<0.0001*^
		predator	$y = 0.009x^2 - 0.28x + 2.92$	0.91	mass	13.97	0.101
					predator x mass	15.17	0.008*
					predator x mass^2	2.68	0.153
					overall	8.92	0.0159*
Lunges	Far	control	$y = -0.018x^2 + 0.74x - 3.22$	0.94	predator	0.52	0.498^
		predator	$y = -o.033x^2 + 0.98x - 4.37$	0.98	mass	88.13	<0.0001*^
					predator x mass	12.56	0.012*
					predator x mass^2	0.78	0.412
					overall	6.67	0.0299*
	Mid	control	$y = -0.016x^2 + 0.66x - 3.17$	0.98	predator	18.52	0.005*^
		predator	$y = -0.021x^2 + 0.62x - 1.96$	0.87	mass	82.42	<0.0001*^
					predator x mass	26.35	0.002*
					predator x mass^2	0.16	0.700
					overall	13.24	0.0063*
	Near	control	$y = -0.009x^2 + 0.41x - 0.95$	0.93	predator	266.87	<0.0001*^
		predator	$y = 0.004x^2 - 0.09x + 0.94$	0.94	mass	21.55	0.004*^
					predator x mass	24.61	0.003*
					predator x mass^2	8.66	0.026*
					overall	16.62	0.0036*

foraging activity was always decreased as compared to the control. Both experiments also showed a significant overall decrease in both lunges and orients in the 'Mid' section (p for the 'overall' term was < 0.05; Tables 4.2, 4.3). However, the foraging activity of the predator-exposed larvae in this section did not begin to differentiate from the control until 10-15 mg wet mass (or around 5-6 week post-hatch). In the 'Far' section, there was a difference between the Acute and Chronic Exposures. In the Acute Exposure, there was no significant difference between treatments in the 'Far' section. In contrast, in the Chronic Exposure, there was a significant decrease in lunges among predator-exposed larvae, which appeared to differentiate from the controls at around 10-15 mg wet mass (although there was no significant difference between treatments for orients). One concern prior to this study was that the fish in the predator treatments would habituate to the presence of the predators (in both the Acute and Chronic Exposures). However, we found no evidence of habituation over the course of the experiments; the response to predators in the 'Near' section persisted, and indeed seemed to become stronger throughout the study in the 'Mid' and 'Far' sections.

We also examined changes in the capture success of larvae during development in the Chronic Exposure (the proportion of lunges that were successful at capturing *Artemia*). In this analysis, there were no significant differences between tank sections during each observational period, so the data from all sections was combined within each treatment (Figure 4.3). In both the predator and control treatments, the percent of lunges that were successful was

Figure 4.3 Capture success (s) of larval shorthorn sculpin *Myoxocephalus scorpius* feeding on *Artemia* with increases in wet mass (m) during early ontogeny in the Chronic Exposure. Filled circles represent individuals that were observed during a visual exposure to predators (juvenile Atlantic cod *Gadus morhua*), whereas open circles represent the control treatments that were not exposed to predators. Solid lines represent second-order inverse polynomial models that have been fitted to the data. The model equation for the control treatment is $s_c = 0.648 + (10.81 / m_c) - (79.04/m_c^2)$ ($F = 4.69$, $p = 0.119$, $r^2 = 0.76$). The model equation for the predator treatment is $s_p = 0.280 + (15.35/m_p) - (90.59/m_p^2)$ ($F = 2.32$, $p = 0.246$, $r^2 = 0.61$). Data points are mean ± SEM.

FIGURE 4.3


Capture success (%)
100
90
80
70
60
50
40
0
5
10
15
20
25
30
Wet Mass (mg)
control
predator exposed

initially low (e.g. 50-60% at ~ 7 mg wet mass). However, success rate increased rapidly with development, and was 80-90% during the second set of observations (at ~8-9 mg wet mass). During the final four sets of observations, control larvae had mean capture success rates between 92-99%, while those of the predator-exposed larvae ranged from 85-88%.

4.3.2 Metabolic Rate

A single regression throughout the entire larval period gave a scaling exponent for routine metabolism (b_r) of 1.10±0.01 (95% CL: 1.08, 1.13; Figure 4.4A). However, separate regressions for the pre-metamorphic and metamorphic developmental stages gave estimates of b_r = 1.32±0.06 (95% CL: 1.22, 1.42) and 0.98±0.05 (95% CL: 0.89, 1.06), respectively. Therefore, when expressed on a mass-specific basis, routine metabolism increased with body mass during the pre-metamorphic stage, and then reached a plateau during the metamorphic phase (Figure 4.4B). Post-metamorphosis, b_r was equal to 0.76±0.08 (95% CL: 0.61, 0.91), and thus mass-specific metabolism decreased with increases in body mass.

4.3.3 Growth and Mortality

In the Acute Exposure, there were no significant differences between treatments for either wet mass or standard length, and so the data from both treatments were pooled (Figure 4.5). For the Chronic Exposure, control larvae had wet masses that were about 27% greater than larvae exposed

Figure 4.4 Routine oxygen consumption versus body mass during the early ontogeny of shorthorn sculpin *Myoxocephalus scorpius* (top panel: absolute oxygen consumption; bottom panel: mass-specific oxygen consumption, i.e. metabolic intensity). See text for a description of the three developmental periods (pre-metamorphic, metamorphic, and post-metamorphic). Dotted line on top panel represents single regression throughout the entire larval period (without distinguishing between pre-metamorphic and metamorphic larvae). Estimate of b_r for over the entire larval period = 1.11±0.02 (95% Confidence Limits = 1.07, 1.13). Data obtained from yolksac larvae are also shown (X), but were not used in the calculation of regressions.

FIGURE 4.4

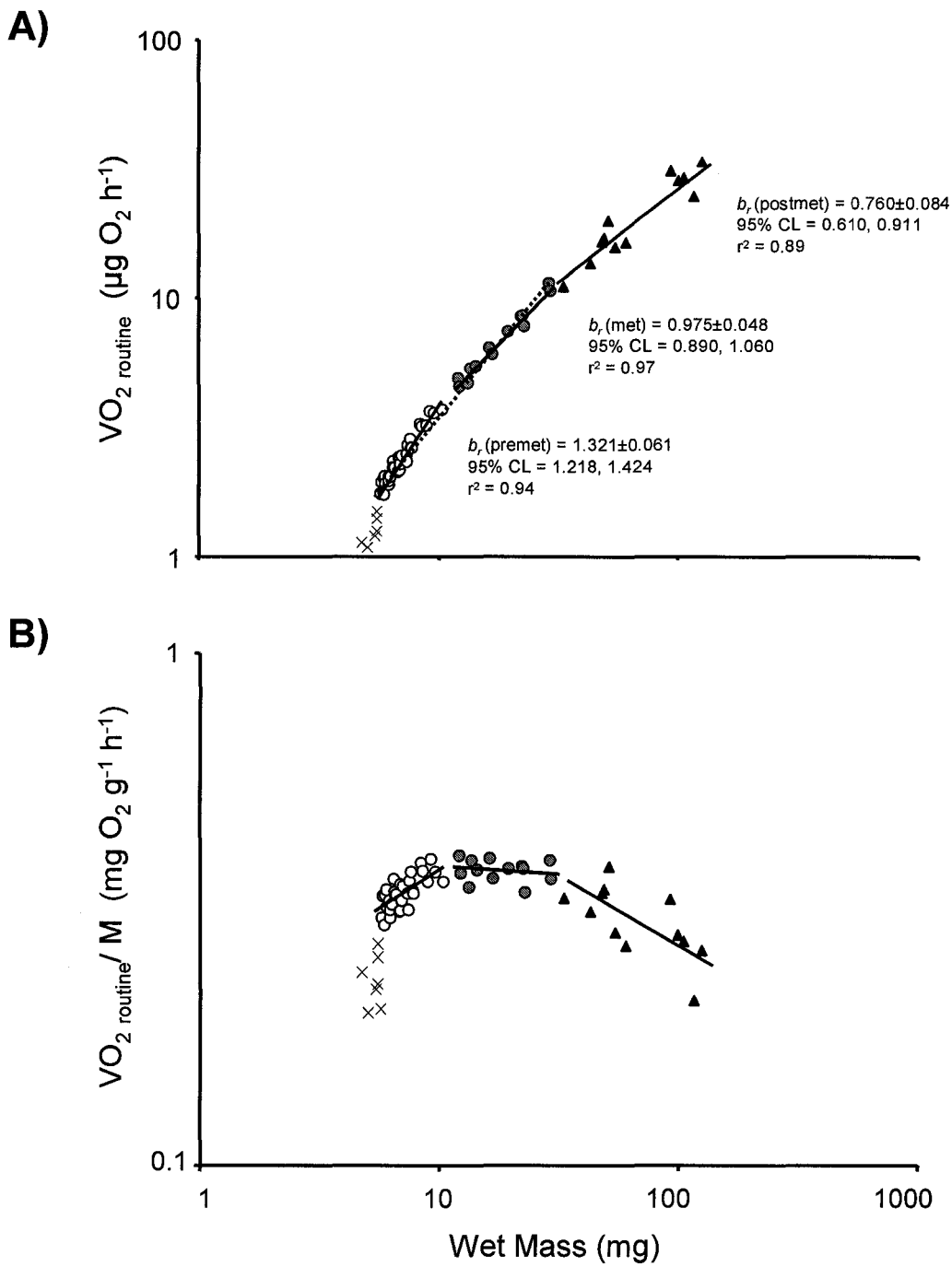
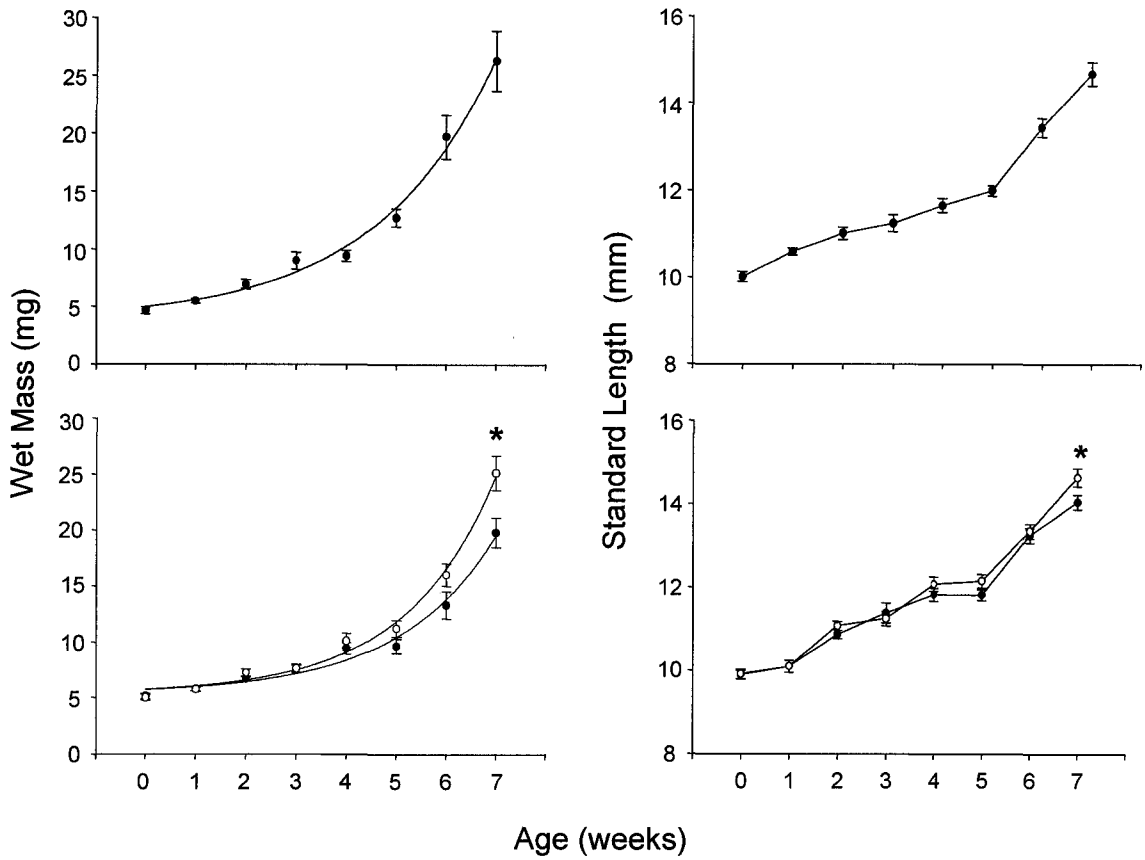

A)
100
10
1
VO_2 routine (µg O_2 h^{-1})
b_r(postmet) = 0.760±0.084
95% CL = 0.610, 0.911
r^2 = 0.89
b_r(met) = 0.975±0.048
95% CL = 0.890, 1.060
r^2 = 0.97
b_r(premet) = 1.321±0.061
95% CL = 1.218, 1.424
r^2 = 0.94
B)
1
0.1
VO_2 routine/ M (mg O_2 g^{-1} h^{-1})
1
10
100
1000
Wet Mass (mg)

Figure 4.5 Changes in wet mass and standard length of larval shorthorn sculpin *Myoxocephalus scorpius* for Acute (top panels), and Chronic Exposures (bottom panels). For the Acute Exposure, there were no significant differences between treatments for either wet mass or standard length at any age, so the data from both treatments was combined. For wet mass, this combined data set was used to generate the growth equation: $m_a = 3.79 + 1.20e^{0.42w}$. For the Chronic Exposure, filled circles represent individuals that were observed during a visual exposure to predators (juvenile Atlantic cod *Gadus morhua*), whereas the open circles represent the control treatments that were not exposed to predators. An * indicates a significant difference between treatments for a given week (unpaired t-test, $p < 0.05$, n = 24 for each data point). For wet mass in the Chronic Exposure the growth equation for the control treatment was: $m_{cc} = 5.33 + 0.41e^{0.55w}$; and for the predator treatment was: $m_{cp} = 5.36 + 0.38e^{0.51w}$. Data points denote mean ± SEM.

FIGURE 4.5



to predators by the end of week seven of the study. The control larvae were also significantly larger in terms of standard length, although this difference was only about 5% higher than the larvae exposed to predators (Figure 4.5).

Daily mortality in the Chronic Exposure was similar between treatments during weeks 2-4, but was significantly higher among the predator-exposed larvae thereafter (Figure 4.6). Overall, the cumulative mortality was 75.2±0.04% for the control larvae, and 80.7±0.04% for the predator-exposed larvae.

4.3.4 Lipid Classes and Fatty Acids

By the end of the study, individuals reared in the presence of predators (in the Chronic Exposure) had significantly (~21%) less whole-body total lipid compared to those in the control treatment (Figure 4.7). The most abundant lipid classes found in my analysis were phospholipids, sterols, and triacylglycerols. Larvae exposed to predators had significantly (~ 28%) less phospholipid at the end of the study compared to the control group (Figure 4.7). However, there were no significant differences between the treatments for either sterols or triacylglycerols, although both showed a slight decrease in concentration for the predator treatment (Figure 4.7).

Larvae exposed to predators had significantly lower levels of most identified fatty acids as compared with larvae that were not exposed to predators (Figure 4.8). However, there were no significant differences between treatments for any of the essential fatty acids (arachidonic acid (AA), eicosapentaenoic acid

Figure 4.6 Mortality (% per day) for larval shorthorn sculpin *Myoxocephalus scorpius* in the Chronic Exposure. Filled circles represent individuals that were observed during a visual exposure to predators (juvenile Atlantic cod *Gadus morhua*), whereas open circles represent the control treatments that were not exposed to predators. * indicates a significant differences between treatments (unpaired t-test, $p < 0.05$). Data points denote mean ± SEM.

FIGURE 4.6

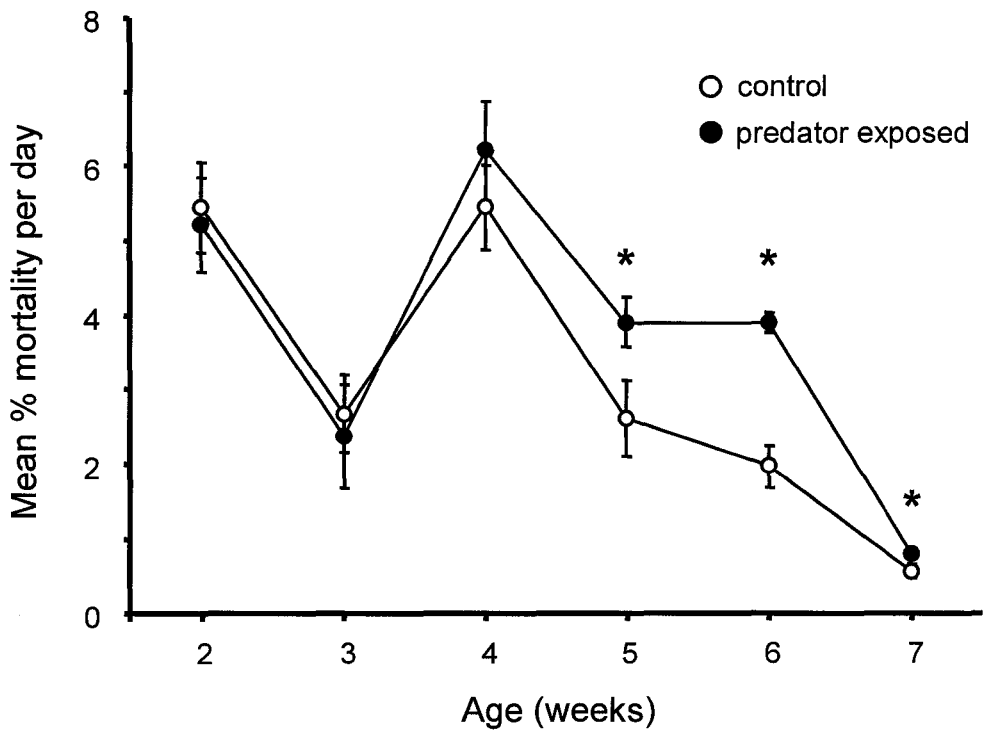

8
6
4
2
0
Mean % mortality per day
control
predator exposed
*
*
*
2
3
4
5
6
7
Age (weeks)

Figure 4.7 Final whole-body concentrations (mean ± SEM) of phospholipids (PL), sterols (ST), triacylglcerols (TAG), and total lipid for larval shorthorn sculpin *Myoxocephalus scorpius*, reared with and without exposure to predators (juvenile Atlantic cod, *Gadus morhua*) in the Chronic Exposure. Whole-body lipid concentrations immediately post-hatch were as follows; TAG, 5.35±0.78 mg g^{-1}; ST, 0.37±0.36; PL, 13.89±0.70; total lipid, 25.53±2.03. * indicates a significant differences between treatments (unpaired t-test, $p < 0.05$, n = 15 for each bar).

FIGURE 4.7

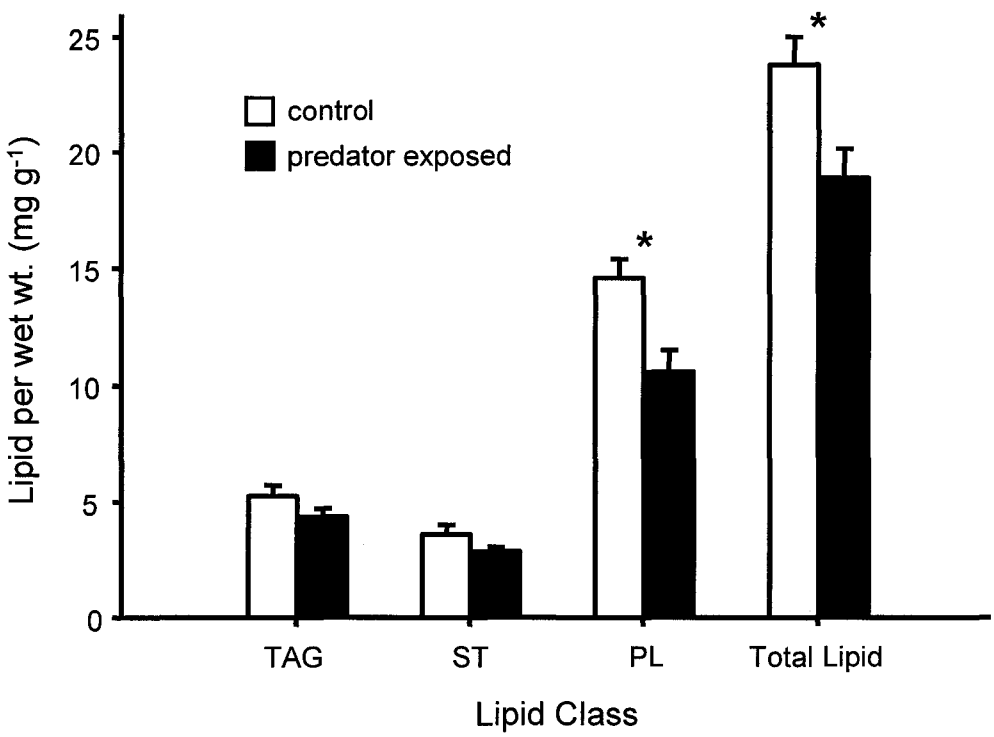
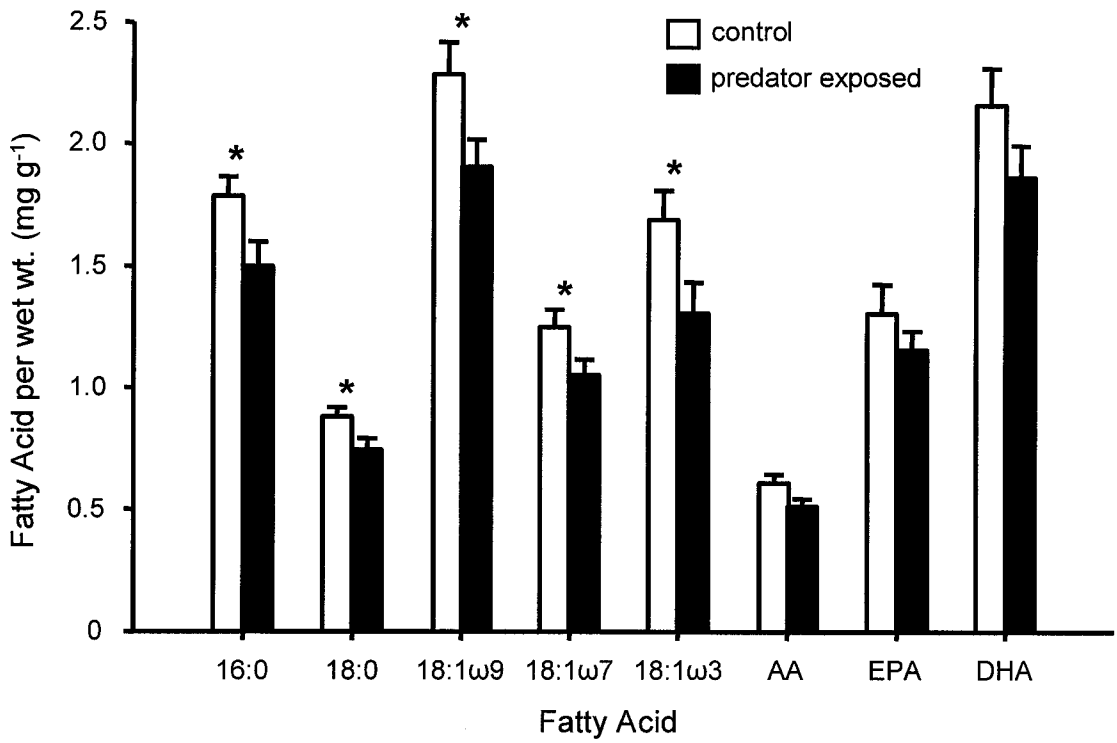

25
20
15
10
5
0
Lipid per wet wt. (mg g-1)
control
predator exposed
*
*
TAG
ST
PL
Total Lipid
Lipid Class

Figure 4.8 Final fatty acid profiles (means ± SEM) for larval shorthorn sculpin M*yoxocephalus scorpius*, reared with and without exposure to predators (juvenile Atlantic cod *Gadus morhua*) in the Chronic Exposure. Only essential fatty acids (arachidonic acid, AA; eicosapentaenoic acid, EPA; and docosahexaenoic acid, DHA), and fatty acids which were present in concentrations >5% of the total identified fatty acids are shown. Fatty acid concentrations immediately post-hatch were as follows: 16:0, 2.61±0.19 mg g^{-1}; 18:0, 0.55±0.14; 18:1ω9, 1.76±0.14; 18:1ω7, 0.89±0.07; 18:3ω3, 0.14±0.01; AA, 0.63±0.05; EPA, 2.69±0.21; DHA, 5.09±0.39. * indicates a significant differences between treatments (unpaired t-test, $p < 0.05$, n = 15 for each bar).

FIGURE 4.8


2.5
2.0
1.5
1.0
0.5
0
Fatty Acid per wet wt. (mg g-1)
control
predator exposed
*
*
*
*
*
16:0
18:0
18:1ω9
18:1ω7
18:1ω3
AA
EPA
DHA
Fatty Acid

(EPA), and docosahexaenoic acid (DHA)), although all three showed a general decrease in concentration among the predator-exposed larvae.

4.4 DISCUSSION

4.4.1 Behavioural Observations

My results support previous reports and suggest that larvae are able to sense predatory risk and adjust their foraging behaviour accordingly. In the Acute Exposure, larvae in the 'Near' section began to show reduced foraging activity between one and two weeks post-hatch (or about 5.5 mg wet mass; 10 mm standard length). This finding is in agreement with Bishop and Brown (1992), who observed that larval three-spine sticklebacks begin responding to the presence of predators after about one-week post-hatch, but in contrast to larval Atlantic cod which will first reduce foraging in the presence of predators at 33 days post-hatch (Skajaa et al. 2003). This inter-specific variation is probably due to differences in developmental trajectory between species. Atlantic cod are considerably smaller than shorthorn sculpin at hatch (Atlantic cod larvae = 3-6 mm; shorthorn sculpin = 7-9 mm; Scott and Scott 1988), and it is possible that cod larvae lack the sensory capabilities required to detect predators until they are older. Alternatively, the larvae of many marine species (including Atlantic cod) may not need to respond to predators during their first few weeks because they are almost completely transparent and it may be difficult for predators to detect their presence (i.e. it would not be advantageous to reduce foraging if there is a low probability of being detected). However, unlike larval Atlantic cod, larval

shorthorn sculpin possess significant pigmentation and an opaque yolk-sac at hatch, and both of these features should make sculpin larvae more obvious to visual predators. In spite of their apparent conspicuousness, however, sculpin larvae did not respond to predators during their first week post-hatch, although it would presumably be advantageous for them to do so. This lack of response suggests that sculpin larvae have a limited ability to detect a predatory threat immediately post-hatch.

The possibility that sculpin have reduced sensory capabilities post-hatch is supported by the observation that, in the 'Mid' section, predator-exposed larvae displayed similar foraging activity as control treatments until larvae were around 15 mg wet mass (for both the Acute and Chronic Exposures), but declined thereafter. At this point in ontogeny, the larvae may acquire the ability to detect shapes and movements from a greater distance. The predators in the present study were maintained behind a transparent partition, and so the larvae were exposed to only visual stimuli. At hatching, the larvae of most fish species have pure-cone retinas, while it is believed that rods and cones are both necessary for more advanced detection of movement and contrast (Evans and Browman 2004). Rod cells generally begin to develop in the retina during metamorphosis, and are thought to help prepare larvae for settlement and the accompanying transition to benthic habitats with reduced light levels (Noakes and Godin 1988). In my study, it was during metamorphosis that larvae in the 'Mid' section also began responding to the predators. It should also be noted that in the Acute Exposure, larvae in the tank section furthest from the predator chamber did not respond to

the presence of predators at any time during the study. Based on the above reasoning, it is likely that larvae did not possess the visual acuity required to detect the predatory threat while in the 'Far' section. Interestingly, larvae exposed to predators in the Chronic Exposure did show reduced foraging while in the 'Far' section. Possible reasons for this are discussed later, but are likely related to the long-term effects of reduced feeding and nutrient acquisition on the ability to detect and capture prey.

It was somewhat surprising that although larval sculpin decreased their foraging behaviour while exposed to the predatory threat, they did not exhibit any of the other anti-predator behaviours that are often observed in teleost fishes. Individuals of many fish species will freeze in the presence of a predator (Fuiman and Magurran 1994), but this behaviour was never observed in the present study. Another common antipredator strategy among young fish is to flee using the C-type fast-start escape response that consists of rapid, burst-type swimming (Eaton and DiDomenico 1986). No fleeing behaviour was observed in the present study, but considering that larval sculpin normally live in the water column, fleeing may not be advantageous during the larval stage of this species. First, there is little (if any) structure available for cover in open water, and so rapidly retreating to a hiding location would not be an option. Furthermore, as noted by Fuiman and Magurran (1994), if a predator is capable of more rapid movements than their prey, it may be advantageous for the prey to retreat by using subtle movements that allow the individual to remain inconspicuous. It is likely that shorthorn sculpin larvae exhibit this 'gradual retreat' upon perceiving a

teleost predator from a distance, and perhaps only perform burst-type fleeing behaviour in response to direct attacks or tactile stimulation (Bailey and Batty 1984).

4.4.2 The Relationship Between Foraging and Routine Metabolism

To examine the possible influence of metabolic demand on foraging decisions, I measured routine metabolic rate of the shorthorn sculpin larvae throughout early ontogeny. The relationship between metabolic rate and body mass is generally described by the allometric equation $MO_2 = aM^b$, where MO_2 is the metabolic rate of the organism, M is the body mass, *a* is a species-specific constant, and *b* is the scaling exponent that describes the slope of the relationship. When $b = 1$, absolute metabolic rate increases in proportion to body mass (i.e. the relationship is isometric), whereas the mass-specific metabolic rate (MO_2 / M; for which the slope of the relationship = $b - 1$) remains constant with body mass. Alternatively, a *b*-value less than 1.0 indicates that absolute metabolism increases more slowly than body mass (i.e. the relationship is allometric), and results in a mass-specific metabolic rate that decreases with body mass. Studies that have examined the routine metabolism of larval fishes have yielded substantial variation with regard to the scaling exponent for routine metabolism (b_r). Giguere et al. (1988) compiled data from 12 studies, and found that the while the mean value for b_r in larval fish was approximately 1.0, values varied between 0.65-1.69. Similarly, Oikawa et al. (1991) presented estimates of b_r from 14 studies that ranged between 0.42-1.09 during the early life stages of

teleost fishes. Despite this variation, it is often assumed that routine metabolism scales isometrically during the larval stage, and with negative allometry during the juvenile and adult lifestages (Post and Lee 1996; Glazier 2005; Killen et al. 2007). Overall, my results support this assumption, with a value for b_r over the entire larval period that is close to isometric at 1.10, and a value of 0.76 post-metamorphosis. However, I also estimated b_r during two different developmental phases within the larval stage (i.e. before and during metamorphosis), using external morphology as an indicator of development. This analysis revealed that before entry into metamorphosis, the scaling of routine metabolism shows a phase of strong positive allometry (b_r = 1.32), which is followed by a phase of isometric scaling during metamorphosis (b_r = 0.97). Thus, when the phase of negative allometry that begins after metamorphosis is complete is considered, shorthorn sculpin appear to possess a triphasic metabolic ontogeny during the early life stages. Most other studies use only a single regression for the entire larval phase (e.g. Houde and Schekter 1983; Finn et al. 2002; Peck et al. 2003). However, if it is common for larvae to have multiple scaling phases before they switch to the negative allometry that characterizes the juvenile stage, a failure to differentiate between these phases may explain some of the variation present in previous estimates of b_r for larval fish. Teleost species show great variation in the duration of their metamorphic period – some display an abrupt metamorphosis while others gradually transition to the juvenile stage (Kjorsvik et al. 2004). Variation in the type of development exhibited during the larval stage

may bias estimates of larval b_r if differences in scaling between separate developmental phases are not taken into account.

Several hypotheses have been put forth to explain the shift in metabolic scaling between the larval and juvenile stages of teleosts, that could also explain the triphasic scaling pattern observed for routine metabolism in young shorthorn sculpin. These include increased growth and protein turnover during the larval stage (Weiser 1991, 1995; Glazier 2005), the differential development of metabolically active tissues and organs (Oikawa et al. 1991), and increases in respiratory surface area as larvae switch from cutaneous respiration to the use of gills (Kamler 1992; Post and Lee 1996). In addition, changes in the amount of energy consumed while swimming during ontogeny could affect metabolic scaling, since locomotory activity is an important component of routine metabolism (Weiser 1991). Wuenschel et al. (2004) observed that in larval spotted sea trout *Cynoscion nebulosus*, the standard length at which b_r changed from isometric to negatively allometric coincided with the development of notochord flexion, and suggested that the change in scaling patterns was attributable to increased swimming efficiency at this stage. In the present study, I used notochord flexion as an indicator of metamorphosis, and partial flexion was first observed at around 12 mg wet mass (five weeks post-hatch). This was accompanied by a gradual differentiation of the median fold during metamorphosis, and development of fin rays that was largely completed by the time larvae began to settle to the bottom of the experimental aquaria. These changes may have increased the swimming efficiency, and thus caused the

switch from positive allometry during the first scaling phase to the isometric scaling observed during metamorphosis. Increased muscle thickness as larvae metamorphose into juveniles may also require branching networks of blood vessels for oxygen delivery as opposed to a reliance on epithelial diffusion. This type of change in the oxygen delivery system could alter the scaling of metabolic rate after this point of ontogeny (West et al. 1997). The evidence for these hypotheses is indirect, however, and future studies should examine whether there are significant changes in the swim performance or muscle morphology of shorthorn sculpin larvae around metamorphosis.

Whatever the specific cause of the observed scaling patterns, our data would suggest that the metabolic intensity of fishes is higher during the larval stage than at any other point during their lifetime. For this reason, it was somewhat surprising that the larval shorthorn sculpin displayed such a strong tendency to reduce foraging activity in favour of predator avoidance behaviour (in both the Acute and Chronic Exposures) since animals are generally more likely to forage under the threat of predation when they have high energetic demand (Godin and Sproul 1988; Pettersson and Bronmark 1993; Godin and Crossman 1994). In this regard, they are similar to the ocean pout observed in Chapter Three of this thesis, which also consistently reduced foraging behaviour in the presence of a predatory threat. However, the fact that foraging reductions among larval shorthorn sculpin were less severe in the 'Mid' section compared to the 'Near' section suggests that shorthorn sculpin larvae may follow a risk-adjusting strategy (where foraging is reduced in proportion to the perceived risk)

instead of an all-out risk avoiding strategy (Fraser and Huntingford 1986). Further, the mass-specific metabolic rate of the larvae peaked during metamorphosis (~ 12-30 mg wet body mass), and the foraging rate in the 'Near' section increased slightly at this stage of development (as indicated by the parabolic rise of the quadratic models for the predator-exposed larvae in the acute exposure experiment). This could indicate that the larvae had an increased motivation to forage at this time (although foraging activity was still much lower than the control larvae at this point). Still, the observation that shorthorn sculpin larvae reduce foraging activity, even while their mass-specific metabolic demands are extremely high, suggests that each instance of reduced foraging could be especially costly for larval fish.

4.4.3 Long-Term Costs of Reduced Foraging Under Predation Threat

On the one hand, frequent foraging interruptions could have important consequences for fish larvae because rapid growth is important for avoiding size-dependent predation during the early lifestages (Folkvord and Hunter 1986; Fuiman 1994). On the other hand, recent experimental evidence also indicates that extremely high growth rates are likely to be selected against, since fast-growing individuals may assume a high level of predation risk via increased foraging activity (Biro et al. 2004; Stoks et al. 2005). Overall, it appears that sculpin larvae have been selected to prioritize the immediate threat of being consumed over the energetic costs of reduced foraging. By causing an overall reduction in foraging activity, it seems that frequent predatory encounters could

influence the growth of larval shorthorn sculpin in environments with an abundance of predators.

Indeed, the results of the chronic exposure experiments suggest that the costs of reduced foraging under predation threat can accumulate over time to affect growth. By the end of the study, larvae not exposed to predators were about 27% larger in terms of wet mass. These results are in agreement with Killen and Brown (2006; see Chapter Three), who found that young ocean pout frequently exposed to predation threat show decreased rates of foraging and growth. Despite the fact that extremely high growth rates may be selected against (Biro et al. 2004; Stoks et al. 2005), growth remains important for larval fishes because size-dependent predation is a main source of mortality during this life stage (Folkvord and Hunter 1986; Bailey and Houde 1989). As young fish grow, the number of potential predators decreases, while the diversity of prey that the larvae can themselves feed upon increases (Fuiman 1994; Fuiman and Magurran 1994). For these reasons, frequent or prolonged interruptions to regular foraging behaviour could have serious consequences for the survival of larval fishes.

The predator-exposed larvae in the chronic exposure experiment showed increased rates of mortality during the final three weeks of the study, and greater cumulative mortality overall. The specific mechanisms that contributed to this mortality are not known, but the larvae of most marine fishes are extremely delicate, and normal development can be disrupted if sufficient nutrition is not obtained. For example, lipids are a primary metabolic fuel throughout larval

development for most marine fishes, and also play an important functional role in many cells and tissues (Tocher 2003). Predator-exposed larvae had reduced concentrations of whole-body lipid by the end of the seven week study, but interestingly, displayed no significant difference from the control larvae in terms of triacylglycerols. Triacylglycerols are primarily used for energy storage, and so it is surprising that the predator treatment did not show reduced triacylglycerol concentrations, especially considering their reduced foraging activity. These results contrast with those of Killen and Brown (2006), who found that prolonged exposure to predatory threat lead to decreased triacylglycerol levels in young ocean pout. However, it is possible that larval shorthorn sculpin direct most of their ingested energy towards growth, and very little to storage, thus making any decreases in triacylglycerol levels difficult to distinguish. It is also noteworthy that the samples used for lipid analysis were collected at the end of the study, whereas mortality rates for the predator treatment differentiated from the control most strongly during weeks five and six. Thus, it is likely that those individuals with the lowest energy reserves would have been among those that perished prior to the sampling period, and so in effect, my analysis only examined those predator-exposed larvae with the highest concentrations of triacylglycerols. Even so, the predator-exposed larvae did display decreased levels of phospholipids. Phospholipids are the primary component of cellular membranes and are of great importance for larval growth and tissue differentiation (Tocher 2003). Decreased levels of phospholipids could therefore reflect a decreased capacity for biosynthesis in the predator-exposed larvae. Furthermore, many marine fish

larvae can catabolize phosphoglycerides (the most predominant type of phospholipid) as an energy source (Tocher 2003) – a factor which may have contributed to the reduced phospholipid concentrations in predator-exposed larvae.

Certain fatty acids (which are components of lipid molecules) also have important functional roles in the tissues of marine fish larvae. Saturated and monounsaturated fatty acids (e.g. 16:0, 18:1ω9, etc.) are preferentially catabolized as a source of energy, and in particular, 18:1ω9 is thought to be a preferred fuel for muscles involved in swimming (McKenzie et al. 1998). All of these fatty acids showed decreased concentrations among the predator-exposed larvae, suggesting that the reduced foraging activity of these larvae forced them to utilize these fatty acids as a metabolic fuel. There are also several highly unsaturated fatty acids (HUFAs) that are known to be critical for normal development during the larval stage of marine fishes. These fatty acids – arachidonic acid (AA; 20:4ω-6), eicosapentaenoic acid (EPA; 20:5ω-3), and docosahexaenoic acid (DHA; 22:6ω-3) – are considered 'essential', in that they cannot be synthesized by marine fish and must be obtained in the diet (Sargent et al. 1999, Bell et al. 2003). They also play vital roles in numerous physiological functions including the immune response, and the development of neural tissue and visual systems (Sargent et al. 1999; Bell et al. 2003). Although the differences between treatments for all essential HUFAs were not statistically significant, there were consistently higher levels for the control treatment, suggesting that the frequent episodes of reduced foraging activity may have

affected the ability of the larvae to acquire these essential fatty acids. Killen and Brown (2006; see Chapter Three) also found that young ocean pout *Zoarces americanus* frequently exposed to predation threat exhibited decreased levels of numerous fatty acids, including AA and EPA. Deficiencies of essential HUFAs have been found to cause reductions in growth, survival, and resistance to stress in larval fishes (Bell et al. 2003). Furthermore, larval herring that receive low levels of dietary DHA have an impaired ability to capture prey (Bell et al. 1995). A similar effect may have occurred in the present study, with predator-exposed larvae of the chronic exposure experiments showing a decreased prey capture success rate. Moreover, unlike larvae in the Acute Exposure, predator-exposed larvae in the Chronic Exposure showed decreased foraging activity, even while in the 'Far' section (after around 10-15 mg wet mass). These effects could be a result of the extreme importance of the essential HUFAs for proper development of the visual system, and in the case of the reduced foraging observed in the far tank section, probably related to the long-term effects of reduced nutritional intake on the ability to detect and capture prey (as opposed to representing a predator avoidance strategy). Reduced visual capabilities could also increase the likelihood that larvae would be captured during encounters with predators, especially given that food-deprived larvae are also known to experience tissue and organ deterioration (Ehrlich et al. 1976), and to possess reduced locomotor and escape abilities (Folkvord and Hunter 1986; Yin and Blaxter 1987).

In summary, as shorthorn sculpin larvae approach and undergo metamorphosis, they consistently reduce foraging while exposed to a predatory

threat. Considering their high metabolic energy expenditure during this stage of development, each foraging interruption is especially costly at this time. Indeed, my results also indicate that these costs can accumulate over time to affect the growth and survival of these larval fish. Future studies should examine how these effects carry over to larger spatial and temporal scales.

Chapter Five

The effect of prey density on foraging mode selection in juvenile lumpfish: Balancing food intake with the metabolic cost of foraging

5.1 INTRODUCTION

In many foraging models, the energetic cost of locomotion is either ignored or treated as constant. However, it is now becoming clear that animals change their prey search tactics in response to certain environmental variables, thus altering the amount of energy spent on activity while foraging (O'Brien et al. 1989; Bautista et al. 2001). In particular, individuals of some species are capable of switching between foraging modes, usually alternating between "active" and "ambush" search strategies (McLaughlin 1989; Helfman 1990). Active foragers (also referred to as "pursuit" or "wide-ranging" foragers) move about their environment in search of prey, whereas ambush foragers (also referred to as "sit-and-wait" foragers) remain relatively immobile, and only attack prey that move into their field of view. It is generally assumed that active foragers spend more energy on movement, but capture increased numbers of prey, whereas ambush foragers capture less prey, but spend less energy in doing so.

The foraging method that organisms utilize is an important consideration when evaluating foraging models because the energy required for locomotory activity can be substantial (Bishop 1999; Wiebel and Hoppeler 2005). Moreover, total aerobic scope (i.e., the difference between minimal and maximal metabolic

rates) often constrains the simultaneous usage of oxygen-consuming physiological functions which, in addition to activity, include growth, maintenance, and digestion (Jobling 1983; Priede 1985; Bishop 1999). Assuming that the budgeting of metabolic costs is additive, the energetic demands of activity are in direct competition with these other physiological functions for allocation within an animal's energy budget. Indeed, empirical evidence suggests a tradeoff between activity and other physiological functions such as growth, because individuals that display elevated levels of activity also have decreased growth rates (Huey and Pianka 1981; Koch and Weiser 1983; Nagy et al. 1984; Secor and Nagy 1994; Rennie et al. 2005).

One of the most important factors that affects foraging mode is prey density. Norberg (1977) originally considered foraging mode in birds and mammals, and theorized that individuals should switch to the more energetically costly mode as prey density increases. Subsequent tests of this hypothesis using endothermic species have generally supported Norberg's predictions (e.g. Rudolph 1982), but experiments performed with ectotherms have instead showed that individuals use low-cost strategies at high prey densities and switch to active foraging at low densities (Formanowicz 1982, Grant and Noakes 1987). Findings such as these prompted Helfman (1990) to speculate that the relatively low metabolic demands of ectotherms may allow them to switch foraging modes in a manner opposite to that of endotherms. Specifically, he hypothesized that while endotherms must choose the foraging mode that maximizes the ratio of energy intake to energy spent (to support their increased metabolic demands),

ectotherms need only maintain a minimal prey encounter rate. More recent work has generally supported Helfman's predictions (Fausch et al. 1996). However, it has been difficult to confirm the influence of metabolism on foraging mode choice in ectotherms because most studies on the effects of prey density have not quantified the energetic demands of standard and active metabolism in the species examined.

This study examines the choice of foraging mode in juvenile lumpfish, *Cyclopterus lumpus*. Members of the family Cyclopteridae possess a ventral adhesive disc with which they to adhere to rocks, vegetation, and other available substrates (Brown 1986; Moring 1989). Previous observations have shown that for young lumpfish, this disc is important in the expression of one of two alternate foraging modes (Brown 1986): (1) an ambush strategy, where individuals remain fixed to the substrate and display relatively little movement except to attack passing prey items; and (2) an active foraging strategy, in which individuals swim through the water in search of prey. These radically different foraging modes make juvenile lumpfish well suited for studying the energetics of foraging mode selection. Specifically, my goals were to examine the effects of prey density on foraging mode choice in juvenile lumpfish, and to quantify the metabolic cost of active foraging. This data allowed me to develop a preliminary model for predicting the prey density at which lumpfish should switch between the two foraging modes. Also, by combining behavioural observations of foraging activity with measures of metabolic rate, I hoped to gain a better understanding of the

mechanisms that govern foraging mode choice in juvenile lumpfish, and ectotherms in general.

5.2 MATERIALS AND METHODS

5.2.1 Animals

Masses of lumpfish eggs (*Cyclopterus lumpus*) were collected by SCUBA divers in June of 2005 and transported to the Ocean Sciences Centre (OSC) in Logy Bay, Newfoundland. Once at the OSC, eggs were placed in aerated incubators supplied with fresh seawater and maintained at 11°C. Fish used in experiments were a mixture of individuals from four separate families. Immediately following hatching, larvae were carefully transferred to holding tanks that were maintained at the same temperature, and fed enriched *Artemia* nauplii three times daily (750 *Artemia* L^{-1}) until they were 11 weeks of age. At this time the lumpfish had fully developed fins and cutaneous pigmentation consistent with the juvenile stage of development.

5.2.2 Behavioural observations

At 11 weeks of age, individuals were transferred to experimental tanks (10 lumpfish per tank), which were flow-through 40 L glass aquaria with darkened sides (50 X 26 X 30 cm). After a one-week acclimation period, tanks (three tanks per treatment) were randomly designated to receive prey densities of either 75 or 750 *Artemia* L^{-1} (added three times daily). *Artemia* were added to each tank by mixing the appropriate number of *Artemia* (the total amount to be added to a

particular tank during that feeding period) in one litre of seawater, and then dispersing this mixture evenly across the surface of the tank. The *Artemia* were then quickly mixed homogeneously through the water column by the current created by an airstone placed in each tank. *Artemia* were observed to be swimming in the water column at all times during the day. Any excess prey and fecal matter at the bottom of the tanks were removed by siphoning at the end of each day. All experimental tanks were exposed to a 16 h:8 h light:dark photoperiod.

Observations of foraging behaviour were performed twice a week for a six-week period, beginning one week after individuals were transferred to the experimental tanks. After a two-minute acclimation period following the addition of the prey *Artemia*, I observed lumpfish behaviour using the focal animal technique (Altmann 1974). With this technique, the behaviours of one individual are recorded over a specific length of time (one minute in the present study). As described by Brown (1986), the foraging behaviour of lumpfish includes two easily distinguishable foraging modes: *Cling* and *Swim*, each of which has associated modal action patterns (MAPs; Barlow 1968). Of additional interest in the present study were: 1) *Bite*, in which the lumpfish made a rapid forward movement in an attempt to capture an *Artemia* in its mouth (could be performed while clinging or swimming); and 2) *Hop*, in which the lumpfish would temporarily leave the surface of the substrate to bite at a passing *Artemia* (could only be performed while clinging). Using these measures, I created a category called *Capture* for use in the analysis (equal to *Bite* for the swim mode, and *Hop* + *Bite*

for the cling mode). Behaviours were recorded and tabulated using a hand-held Psion event recorder and associated software (The Observer 3.0, Noldus Information Technology Inc., Netherlands).

During each data collection period, behavioural observations were performed for six individuals per tank. Furthermore, an effort was made to initiate observations with three fish that were swimming and three fish that were clinging. These observations were not always possible, particularly in the high-density treatments where it was rare to see individuals use the swim foraging mode. However, fish were free to move throughout the experimental tanks, and over the course of a given observation, it was common for individual lumpfish to switch between cling and swim modes. In these cases, observations were divided into time intervals to represent the time spent either clinging or swimming. The frequencies of the event MAPs (bites, hops, and captures) were then converted to counts minute^{-1} spent in each mode. To avoid biases caused by disproportionate counts of behaviours while in a given mode for a short duration, intervals were not included in the final analysis if the focal lumpfish spent less than 5 s performing that mode. In addition, the proportion of individuals clinging in each tank was estimated by performing a scan count of the number of fish that were in the clinging posture. This proportion was estimated twice – once before the addition of *Artemia* (prior to the observations of foraging behaviour for that tank), and once after observations for that tank had concluded (approximately 8 min after the addition of *Artemia*).

5.2.3 Growth measurements

At the beginning and end of the experiment, five lumpfish were arbitrarily selected from each tank (n = 15 per treatment), anaesthetized using MS-222, and measured for total length and wet mass. Lengths were measured using Matrox Inspector 3.0 image analysis software on images captured using a digital camera (Pixera PVC 100C). Following image capture, fish were quickly dried with a lint-free paper towel, and placed on dry, tared weigh-foils. Their wet mass was then measured using a microbalance (APX-60, Denver Instrument Co).

5.2.4 Respirometry

Lumpfish used for measures of oxygen consumption were reared in two 60-L holding aquaria that were separate from the experimental tanks described above. To help determine the effects of body size on energy expenditure during foraging in young lumpfish, two size classes were used for measurements of metabolic rate: a 'small' class (207.9±0.01 mg wet mass, 2.14±0.08 cm total length; n = 9), and a 'large' size class (594.0±0.04 mg wet mass, 2.96±0.06 cm total length; n = 9).

To prevent individuals from adhering to the inside of respirometers (see below), fish were lightly anaesthetised using MS-222 and a thin film of adhesive (VetBond® 3M Tissue Adhesive) was applied to their ventral disk with the aide of a dissecting microscope 12-15 h prior to the initiation of measurements. After revival in aerated 11°C seawater, fish were carefully placed in the respirometer (one individual per trial), and subjected to a brief 'training' trial (approximately 5

min) where they were exposed to low current velocities (0.5-1.5 body lengths (BL) s^{-1}) so they could orient against the current and acclimate to changes in water velocity. Individuals were allowed to acclimate overnight, and no mortalities resulted from the procedure. To limit disturbance to the fish during the acclimation and measurement periods, the top portion of the swim tunnel was covered with a sheet of dark plastic and each fish's swimming behaviour was observed using a mirror placed below the swim tunnel.

Lumpfish oxygen consumption at various swimming speeds was measured in a glass Blazka-type respirometer (total volume of the respirometer and external circuit that contained the oxygen sensor = 57 ml; Killen et al. 2007). Oxygen concentrations within the respirometer were measured using a flow through fibre-optic oxygen sensor (Presens, Germany), according to the methods of Killen et al. (2007). To maintain the temperature of the water in the respirometer, and to maintain oxygen concentrations in the water supplying the respirometer, an external circuit delivered aerated seawater to the respirometer from a reservoir located in a water bath set at 11°C. However, because measurements of oxygen consumption could only be made when the circuit from this external reservoir was closed, the entire system was located in a cold-room set to 11°C. To minimize the amount of background bacterial oxygen consumption, all seawater was sterilized with ultraviolet radiation, and the system was cleansed daily with absolute ethanol. In addition, blank measurements of background oxygen consumption were conducted immediately following each

trial, and when necessary, this oxygen consumption was subtracted from the experimental measurements.

Measures of active metabolic rate (and metabolic scope) were obtained for individual fish by performing a modified U_{crit} test as described by Brett and Glass (1963). Using this protocol, measurements of oxygen consumption were initially performed at zero swimming velocity, and then performed at increasing water current velocity increments of 0.5 body lengths per sec (BL s^{-1}). For the small size-class, measurements of active metabolism began at 1.0 BL s^{-1}, and for the large size-class, measurements began at 0.5 BL s^{-1} (below these speeds, the current was too slow to cause fish to consistently leave the bottom of the swim-tunnel and orient against the current). The time spent at each experimental current speed was 15 minutes, where oxygen was measured for 8-12 min commencing 3 min after each speed increase. The trial was stopped when the fish was exhausted, as indicated by an inability to avoid contact with the rear grid of the swim tunnel for at least 20 consecutive seconds. It is often difficult to obtain measures of oxygen consumption while larval and juvenile fish are swimming because they are generally averse to continuous swimming in respirometers for extended time periods (even when swimming at low velocities; Kaufmann 1990). For this reason, individuals were given a one-minute 'rest' period between each stepwise increase in current speed, during which the current in the respirometer was decreased to less than 0.5 cm s^{-1} (fish were not forced to swim at this low speed and could rest on the bottom of the respirometer).

5.2.5 Videography

To estimate the regular swim speed of lumpfish during active foraging, groups of lumpfish were transferred to plastic containers located in a cold-room at 11°C (ten lumpfish per trial). The bottom of each container was marked with 1 cm gridlines, and as fish foraged they were videotaped using a video camera (JVC GR-D250V Digital Video Camera) suspended above the container. To stimulate foraging activity, prey were added to the container (at concentrations of either 75 or 750 *Artemia* L^{-1}), and recordings were initiated after a 5-min acclimation period. Trials were performed for the same two size-classes of lumpfish ('small' 1.99± 0.04 cm total length, and 'large' 2.87±0.07cm total length) used in measurements of active metabolism. Three trials were performed for each size class-prey density combination. Recordings were reviewed using frame-by-frame analysis (Adobe Premier Version 6.0, Adobe Systems Inc.). Swim speeds of individual fish were obtained at four separate times during the recording (the swim paths analyzed were about 5 seconds in duration and appeared to be near-linear when viewed from above) and combined to calculate a mean swim speed for that fish.

5.2.6 Data and Statistical analyses

All statistical analyses were performed with Minitab version 13.1 (Minitab Inc.) with a significance level for all tests of $\alpha = 0.05$. Data are presented as means ± SEM.

5.2.6.1 Behavioural Observations

I recognize that tank effects are a potentially confounding factor, and for behavioural observations and growth parameters I performed statistical analyses to examine these effects. These analyses consisted of a two-way ANCOVA within each prey density treatment for growth data and prey capture frequencies, and a two-way ANOVA for the proportion of time spent clinging. Each test used all of the observations conducted for each week, and contained the explanatory variables of tank and study duration (in weeks; used as a covariate in each ANCOVA). In all cases, tank effects and the tank*duration interactions were non-significant ($p > 0.50$ in all cases). As a result of the large number of observations performed for each tank during this study, this test is highly sensitive for tank effects. In the absence of a significant tank*duration effect, data from individual tanks were pooled within each treatment, and the effects of rearing tanks were not considered in subsequent analyses.

Differences in capture frequency (while clinging, while swimming, and total captures minute^{-1}) between prey density treatments were tested using General Linear Models with normal error structure. Model suitability (in terms of normality, homogeneity, and independence of residuals; Sokal and Rohlf 1995) was verified using residual-fit plots. Models were constructed using the explanatory variables of prey density and study duration (used as covariate), and also included a term for the interaction between these two variables. To identify differences in the proportion of time spent clinging, and in the proportion of fish

that were clinging before and after the addition of *Artemia* for each observation, similar models were used except that the duration of the study was designated as a categorical variable instead of a covariate (because the data could not be fit using a linear regression). To identify significant differences between treatments during each week of the study, I also performed unpaired t-tests for all behaviours during each week.

5.2.6.2 Respirometry

Rates of oxygen consumption (mg O_2 h^{-1}) were calculated using linear regression for each trial from the decrease in water oxygen content per unit time. Power curves were then fitted to the relationship between oxygen consumption and swim speed, and standard metabolism ($MO_{2\ stan}$) was determined as the y-intercept of this relationship. Metabolic intensity (mass-specific metabolic rate), was determined by dividing measures of oxygen consumption for each fish by its wet mass. The absolute aerobic scope (in terms of both mg O_2 g^{-1} h^{-1} and mg h^{-1}) for each fish was calculated for each size class by subtracting $MO_{2\ stan}$ from the maximal oxygen consumption ($MO_{2\ max}$). Factorial aerobic scope was obtained by calculating the ratio of $MO_{2\ max}$: $MO_{2\ stan}$ (this ratio is identical when calculated using either metabolic rate or metabolic intensity). Total cost of transport (in kcal kg^{-1} km^{-1}) was calculated using an oxycaloric coefficient of 3.25 cal mg O_2^{-1} (Parsons and Sylvester 1992). A second-order quadratic regression was then fitted to the relationship between swim speed (BL s^{-1}) and total cost of transport. Differences between size classes for absolute aerobic scope, factorial aerobic

scope, minimum cost of transport, and swim speed at minimum cost of transport were examined using unpaired t-tests. Differences in swim speeds during foraging (as observed using videography) were detected using a two-way ANOVA with prey density and size as factors.

5.2.6.3 Behavioural Modelling

From my behavioural observations and measures of metabolic rate I calculated models to predict the prey densities at which lumpfish should switch between foraging modes (assuming decisions based on maximizing net energy gain). By examining these models from the perspective of optimality, I was able to compare my data to the original speculation of Norberg (1977), who stated that animals should switch foraging modes in a manner that maximizes net energy intake. First, the possible net energy gain was calculated for each foraging mode (swimming and clinging) at each experimental prey density (75 and 750 *Artemia* L^{-1}) using the following equation (similar to that described by Ware 1975):

$$E = I ps - A \qquad \text{(Eq. 1)}$$

where **E** is the net energy gained while either clinging or swimming; **I** is the total energy (cal h^{-1}) ingested while using either the swimming or clinging foraging mode; ***p*** is an assimilation factor representing the proportion of ingested energy that is digested (i.e. not lost as feces or urine); ***s*** is a factor that accounts for specific dynamic action (SDA; the rise in metabolic rate during the processing

and digestion of food), and is expressed as a proportion of ingested energy; and **A** is the energy spent on locomotory activity (cal h^{-1}). Values for **I** were calculated by converting the average captures min^{-1} for each foraging mode over the study to total calories ingested per hour (using 0.0068 cal *Artemia*$^{-1}$; determined for samples of *Artemia* using bomb calorimetry). I estimated ***p*** using the non-ash composition of Salt Lake strain *Artemia salina* (approximately 62% protein, 31% lipid, and 7% carbohydrates; Dhont and Van Stappen 2003), and previously-published assimilation factors for each class of nutrient (Ware 1975; Morais et al. 2004). For *Artemia*, approximately 60% of the energy from protein is recovered during digestion (***p*** for protein portion = 0.60; Morais et al. 2004). Lipid and carbohydrate assimilation factors specific to *Artemia* are unavailable, and I therefore used general estimates for each nutrient class, provided for fish by Ware (1975; ***p*** = 0.85 for lipids, and 0.40 for carbohydrates). Using this information, I calculated an overall value for ***p*** = 0.66. The exact proportion of ingested energy representing SDA in lumpfish is unknown, but SDA in juvenile coregonids fed *Artemia* has been estimated at 28% of ingested energy (Dabrowski and Kaushik 1984). Using this percentage as an estimate of SDA yields a value for ***s*** of 0.72 for the lumpfish. Values for **A** were determined from the relationships between metabolic rate and swim speed for each lumpfish size class. For swimming lumpfish, metabolic rate at the average foraging speed (as determined by videography) was used for **A.** For clinging fish, I assumed no activity, and standard metabolic rate was thus used for **A** (this is likely to slightly

underestimate **A** for the cling mode because lumpfish did display occasional movements even while clinging).

5.3 RESULTS

5.3.1 Behaviour

Overall, lumpfish at the high prey density spent significantly more time clinging than those at the low prey density (Figure 5.1; Table 5.1). However, the time spent clinging at the low density decreased from 49.08±7.39% during week one to 24.47±5.99% during week six. In contrast, the proportion of time spent clinging by lumpfish at the high density remained relatively constant over time. The proportion of individual lumpfish that were clinging in each tank generally decreased after the addition of *Artemia* (especially at the low prey density, Figure 5.1; Table 5.1). Moreover, both before and after the addition of *Artemia*, there were generally more lumpfish clinging at the high prey density (except before *Artemia* addition during week-one of the study).

While clinging, lumpfish at the high prey density were able to capture about 2.5-times more prey than individuals at the low prey density (5.90±0.22 and 2.34±0.21 captures min^{-1}, respectively, Figure 5.2; Table 2.2). However, while swimming, there was no significant difference in the amount of prey captured, with lumpfish achieving 5.29±0.15 captures min^{-1} at low density and 5.25±0.56 captures min^{-1} at high density. In total, lumpfish at the high prey density captured significantly more prey (5.85±0.18 captures min^{-1}) than those at low prey density (4.57±0.14 captures min^{-1}; 28.5% more captures overall at high

Figure 5.1 Top panel – Proportion of time spent clinging by juvenile lumpfish *Cyclopterus lumpus* exposed to low (75 *Artemia* L^{-1}; dark bars) and high (750 *Artemia* L^{-1}; light bars) prey densities. Middle and bottom panels – the proportion of juvenile lumpfish clinging before the addition of *Artemia* (at the beginning of the observational period), and after the addition of *Artemia* (count conducted at the end of the behavioural observations for a particular tank, approximately 8-10 min after the beginning of the observation). Data are means ± SEM. * represents a significant difference between the two treatments for observations conducted during a particular week (unpaired t-test, $p < 0.05$). Details for overall statistical modeling are given in Table 5.1.

FIGURE 5.1

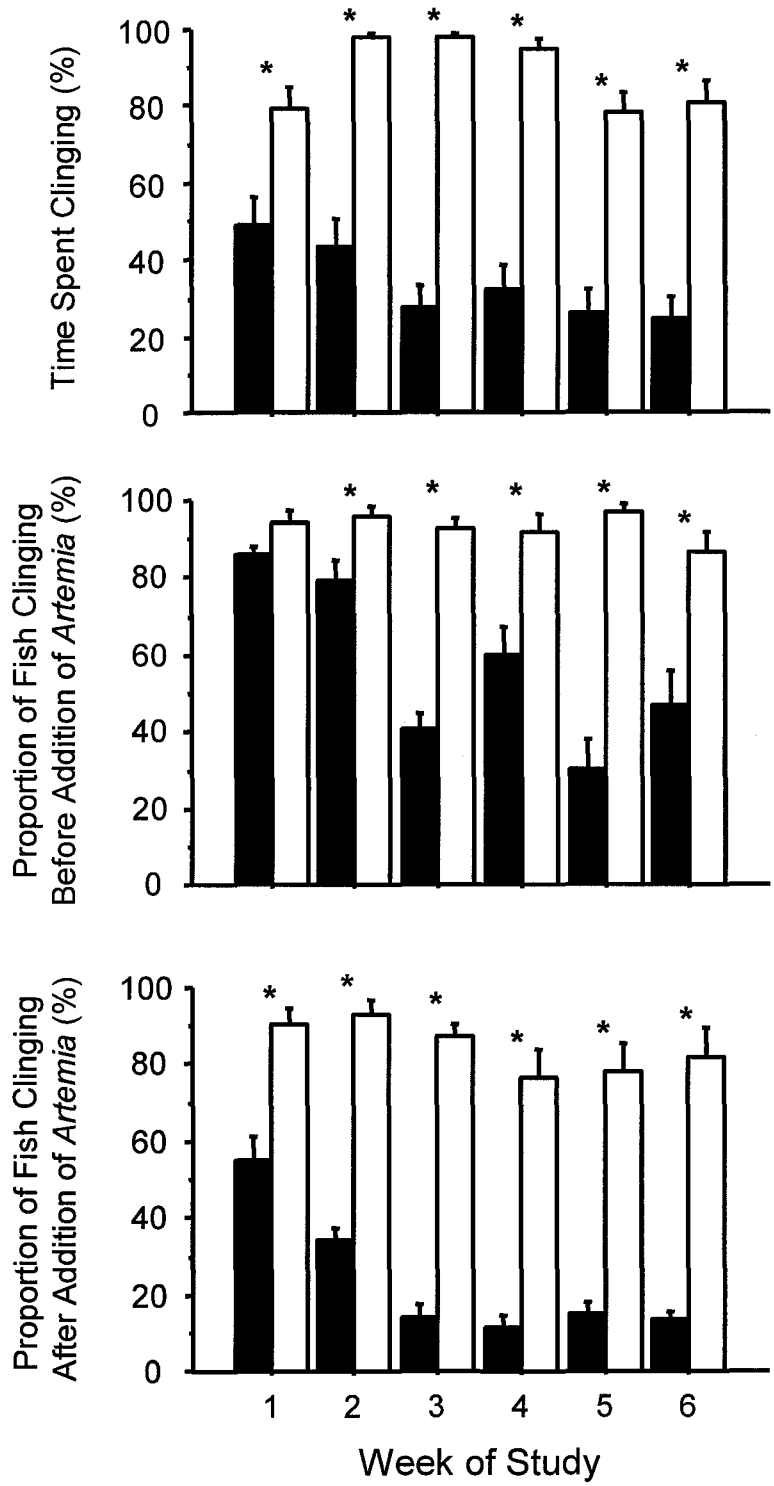

Time Spent Clinging (%)
Proportion of Fish Clinging Before Addition of *Artemia* (%)
Proportion of Fish Clinging After Addition of *Artemia* (%)
100
80
60
40
20
0
1
2
3
4
5
6
Week of Study

Table 5.1 General Linear Model results for proportion of time spent clinging for juvenile lumpfish *Cyclopterus lumpus* exposed to two different prey densities (75 and 750 *Artemia* L^{-1}), and the proportion of fish in each treatment observed to be clinging before, and 8-10 minutes after, the addition of the prey *Artemia*. For the proportion of time spent clinging, the model included the explanatory variables of prey density and week (representing time over the duration of the study which was a categorical variable). For the proportion of fish clinging before and after the addition of *Artemia*, the factor 'feeding' was also included in the model (to represent the effect of food addition). * indicates a significant effect ($p < 0.05$). ^ indicates a lower order term that cannot be evaluated independently because of a significant interaction term.

Response variable	**source**	**df**	**F**	***p***
Time spent clinging	density	1	285.57	<0.0001*^
	week	5	3.36	0.0054*^
	density x week	5	2.99	0.0116*
	residuals	414		
Proportion of fish clinging	density	1	518.83	<0.0001*^
	week	5	17.30	<0.0001*^
	feeding	1	92.41	<0.0001*^
	density x week	5	9.14	<0.0001*
	density x feeding	1	33.27	<0.0001*
	week x feeding	5	1.43	<0.0001*
	residuals	125		

Figure 5.2 Frequencies of total prey captures (top panel), and those performed while either clinging (middle panel), or swimming (bottom panel) for juvenile lumpfish *Cyclopterus lumpus*. Dark bars represent individuals in the low prey density treatment (75 *Artemia* L^{-1}), while the light bars represent those at the high prey density (750 *Artemia* L^{-1}). Data are mean ± SEM. * represents a significant difference between the two treatments for observations conducted during a particular week (unpaired t-test, $p < 0.05$). Details for overall statistical modeling are given in Table 5.2.

FIGURE 5.2

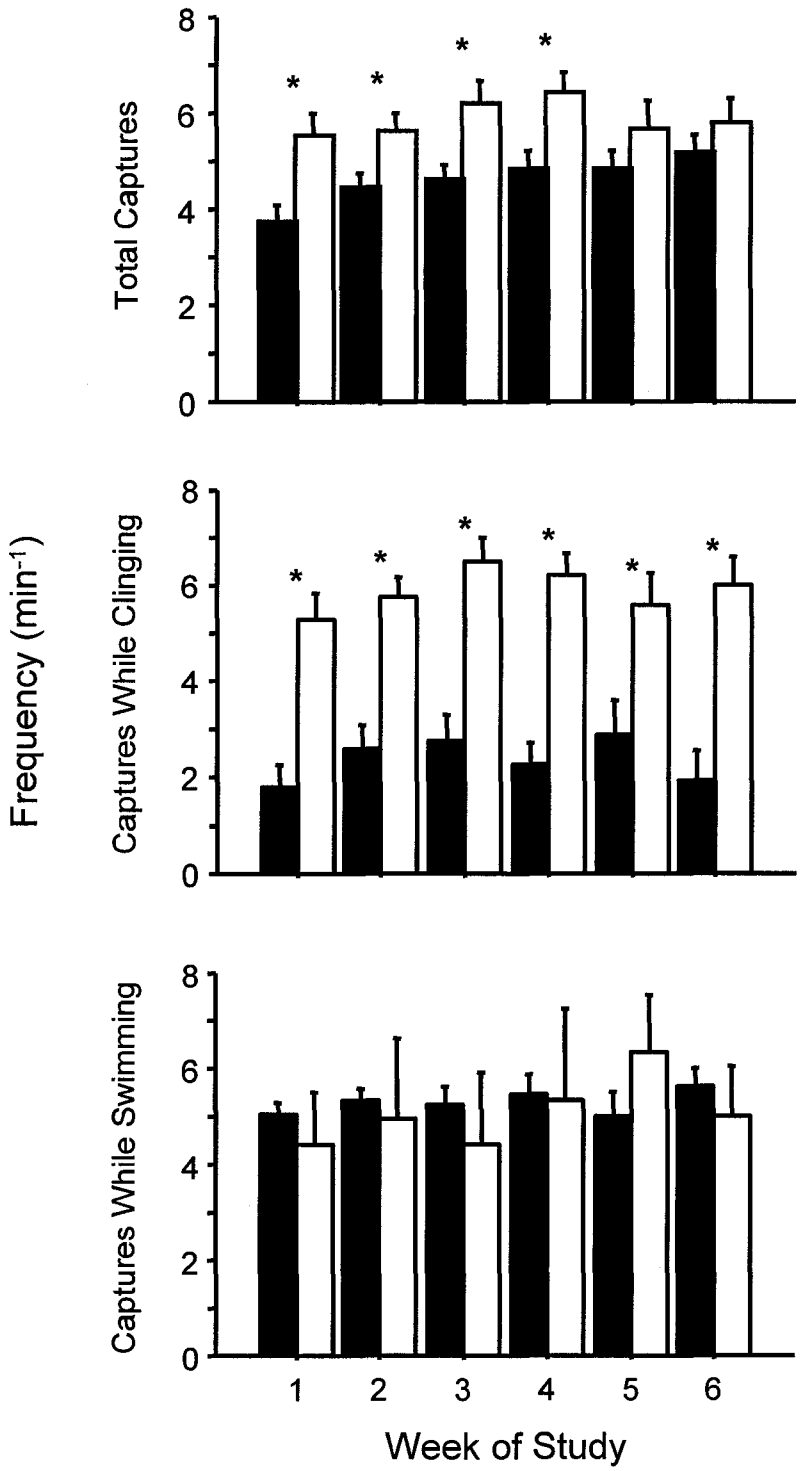

Frequency (min-1)
Total Captures
Captures While Clinging
Captures While Swimming
0
2
4
6
8
*
1
2
3
4
5
6
Week of Study

Table 5.2 General Linear Model results for total captures per minute, captures while clinging, and captures while swimming for juvenile lumpfish *Cyclopterus lumpus*. The model included the explanatory variables of prey density and week (representing time over the duration of the study; was used as a covariate). * indicates a significant effect ($p < 0.05$).

Response Variable	Source	df	F	*p*
Total captures	density	1	18.41	<0.0001*
	week	1	4.92	0.0247*
	density x week	1	3.40	0.66
	residuals	423		
Captures while clinging	density	1	26.42	<0.0001*
	week	1	1.21	0.273
	density x week	1	0.93	0.335
	residuals	320		
Captures while swimming	density	1	0.95	0.331
	week	1	2.24	0.135
	density x week	1	0.99	0.321
	residuals	239		

prey density). Total capture rates increased significantly over the course of the study, although the number of *Artemia* captured while either clinging or swimming did not change. This finding suggests that the increase in total captures over the 6-week study resulted from a shift towards more time spent swimming (particularly for lumpfish in the low density treatment; Figure 5.1).

There were no significant differences in total length (unpaired t-test, t = -0.463, p = 0.647, df = 28; Table 5.3) or wet mass (t = -0.728, p = 0.473, df = 28) between the two prey density treatments at the start of the study. However, by the end of the study, lumpfish total length and wet mass values were 29.1% and 2.5-fold greater, respectively for high prey density than for low prey density (t = -9.539, p = <0.0001, df = 28; and t = -8.375, p = <0.0001, df = 28).

5.3.2 Respirometry

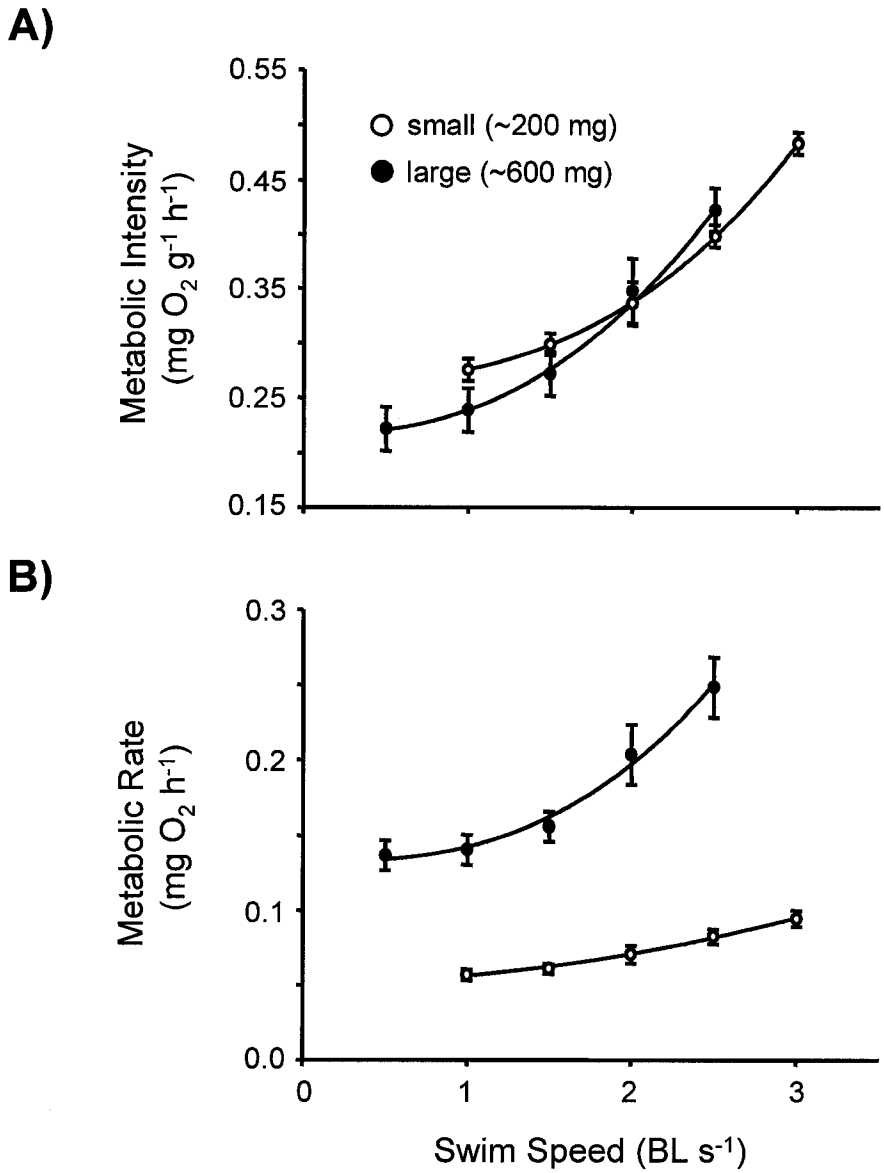
For both size classes, metabolic intensity (expressed in mg O_2 g^{-1} h^{-1}) and metabolic rate (mg h^{-1}) increased in a curvilinear manner with swim speed (Figure 5.3). Standard metabolic rate and standard metabolic intensity (i.e. the *y*-intercepts of the relationships between swim speed and oxygen consumption) for the small size class were 0.052 mg O_2 h^{-1} (0.169 cal h^{-1}) and 0.265 mg O_2 g^{-1} h^{-1}, respectively compared to values for the large size class of 0.133 mg O_2 h^{-1} (0.430 cal h^{-1}) and 0.217 mg O_2 g^{-1} h^{-1}. These values were almost identical to the actual measurements of oxygen consumption made at zero velocity (small size class: metabolic rate = 0.054 mg O_2 h^{-1}, metabolic intensity = 0.261 mg O_2 g^{-1}

Table 5.3 Measures of total length and wet mass for juvenile lumpfish *Cyclopterus lumpus* before and after the six-week study. An * indicates a significant difference between the prey density treatments ($p < 0.05$, unpaired t-test).

Prey Density (*Artemia* L^{-1})	**Total Length (mm)**	**Wet Mass (mg)**
Initial		
75	14.4 ± 0.3	120.3 ± 1.3
750	14.6 ± 0.4	119.2 ± 1.4
Final		
75	18.9 ± 0.3	201.6 ± 1.1
750	24.5 ± 0.5*	584.2 ± 1.5*

Figure 5.3 Relationship between metabolic intensity and swim speed (top panel) and metabolic rate and swim speed (bottom panel), in juvenile lumpfish *Cyclopterus lumpus* at 11°C. Open circles represent the 'small' size class (207.9±0.01 mg) and filled circles represent the 'large' size class (594.0±0.04 mg). Regression equations for metabolic intensity are: small size class: $y = 0.011x^{2.72} + 0.265$, $r^2 = 0.74$; large size class: $y = 0.022x^{2.45} + 0.217$, $r^2 = 0.76$. Regression equations for metabolic rate are: small size class: $y = 0.004x^{2.17} + 0.052$, $r^2 = 0.81$; large size class: $y = 0.0085x^{2.88} + 0.133$, $r^2 = 0.73$. Standard rates of oxygen consumption are represented by the y-intercept for each equation. Regression lines were calculated using individual data points, but for simplicity, only the mean values of the data are shown in each figure (± SEM).

FIGURE 5.3


A)
B)
Metabolic Intensity
(mg O_2 g^{-1} h^{-1})
0.55
0.45
0.35
0.25
0.15
small (~200 mg)
large (~600 mg)
Metabolic Rate
(mg O_2 h^{-1})
0.3
0.2
0.1
0.0
0
1
2
3
Swim Speed (BL s^{-1})

h^{-1}; large size class: metabolic rate = 0.125 mg O_2 h^{-1}, metabolic intensity = 0.212 mg O_2 g^{-1} h^{-1}). However, to be consistent with the literature, only extrapolated *y*-intercept values were used in this study.

Standard metabolic intensity was not significantly different between the large and small size classes, but the large lumpfish had significantly higher standard metabolic rate (ANCOVA, F = 46.48, p = <0.0001, df = 17). Furthermore, although absolute aerobic scope ($MO_{2\ max} - MO_{2\ stan}$) was not different between size classes when calculated using metabolic intensity, it was significantly greater for large lumpfish when calculated using absolute metabolic rate (Table 5.4). Factorial aerobic scope ($MO_{2\ max} / MO_{2\ stan}$) showed no difference between size classes. Minimum cost of transport was significantly greater for the small size class (6.17±0.24 kcal kg^{-1} km^{-1}, Figure 5.4) compared to the large size class (4.28±0.31 kcal kg^{-1} km^{-1}; unpaired t-test, t = -4.765, p = 0.0003, df = 17). Moreover, the swim speed at minimum cost of transport for the small size class (2.48±0.04 BL s^{-1}) was significantly greater than for the large size class (1.90±0.05 BL s^{-1}; t = -9.033, p = <0.0001, df = 17).

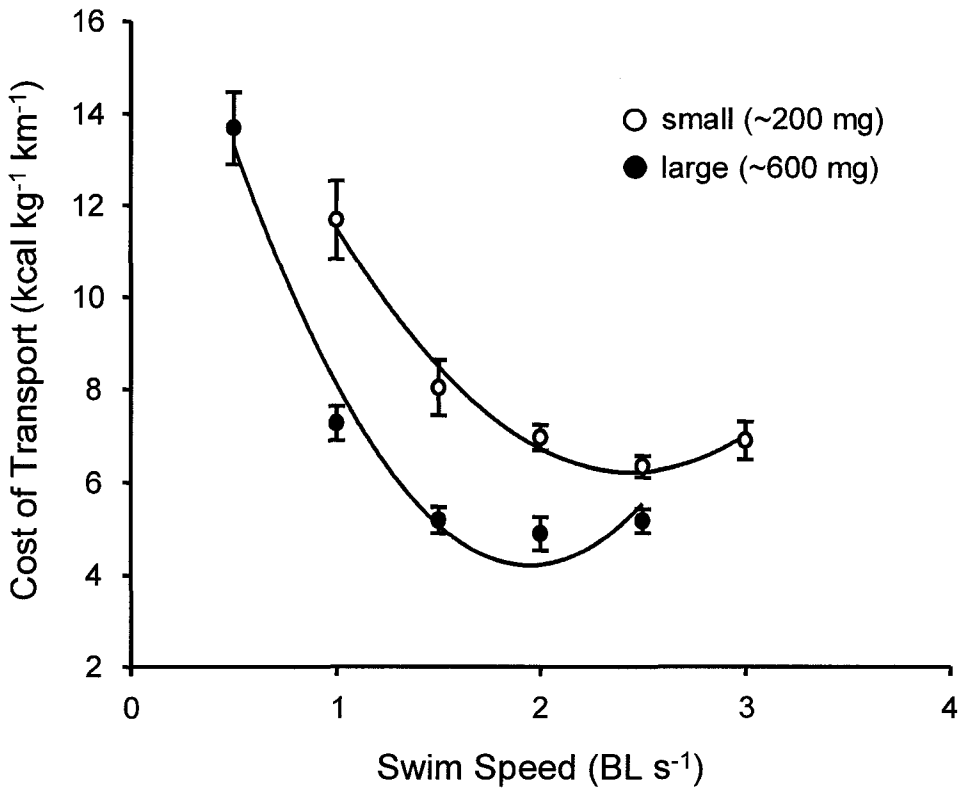
Average swim speeds while foraging that were obtained from video analyses did not differ between size classes (ANOVA, F = 0.00018, p = 0.989, df = 1, 40), but were significantly affected by prey density (ANOVA, F = 4.823, p = 0.0339, df = 1, 40). For the small size class, mean swim speeds were 0.996±0.11 BL s^{-1} at the high prey density (0.183 cal h^{-1}; 8.3% above standard), and 1.197±0.08 BL s^{-1} at the low prey density (0.189 cal h^{-1}; 11.8% above

Table 5.4 Absolute and factorial aerobic scope for juvenile lumpfish *Cyclopterus lumpus* of two size classes (small: 207.9±0.01 mg; and large: 594.0±0.04 mg). Aerobic scope is shown in terms of both metabolic intensity and metabolic rate ($MO_{2\ max} - MO_{2\ stan}$). An * indicates a significant difference between size classes.

Size Class	Absolute Scope ($mg\ O_2\ g^{-1}\ h^{-1}$)	Absolute Scope ($mg\ h^{-1}$)	Factorial Scope
Small	0.218 ± 0.01	0.042 ± 0.01*	1.82 ± 0.03
Large	0.205 ± 0.02	0.166 ± 0.02*	1.94 ± 0.09

Figure 5.4 Cost of transport for juvenile lumpfish *Cyclopterus lumpus*. Open circles represent the 'small' size class (207.9±0.01 mg) and dark circles represent the 'large' size class (594.0±0.04 mg). Regression equations are as follows: small size class: $y = 21.34 - 12.38x + 2.53x^2$, $r^2 = 0.76$; large size class: $y = 20.67 - 16.91x + 4.34x^2$, $r^2 = 0.70$. Regression lines were calculated using individual data points, but for clarity of presentation only the mean values of the data are shown in each figure (± SEM).

FIGURE 5.4

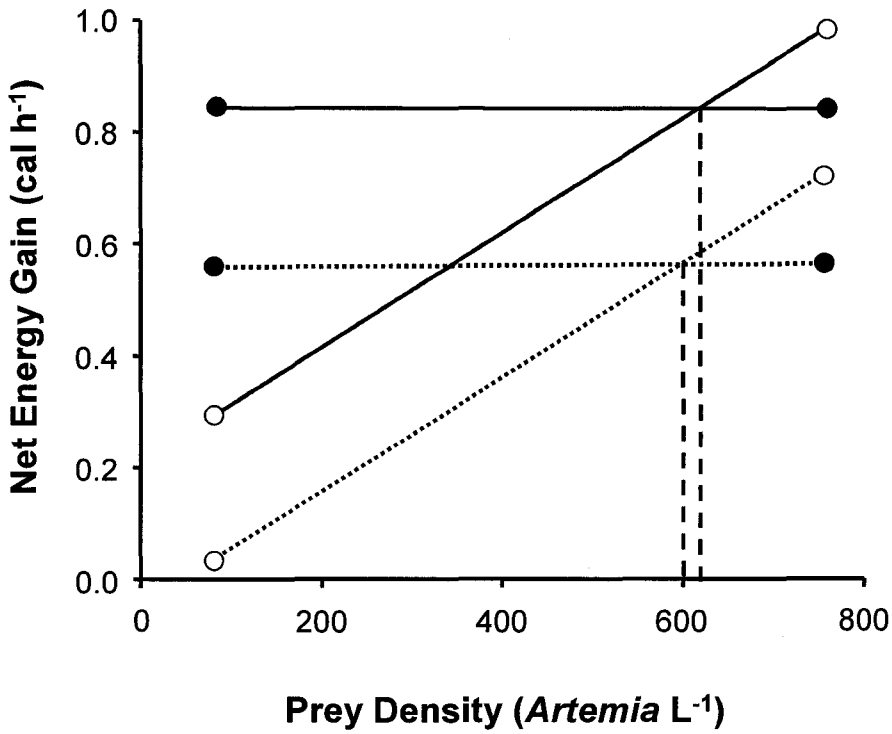

16
14
12
10
8
6
4
2
0
1
2
3
4
Cost of Transport (kcal kg-1 km-1)
Swim Speed (BL s-1)
small (~200 mg)
large (~600 mg)

standard). For the large size class, mean swim speeds were 0.989±0.04 BL s^{-1} at the high density (0.459 cal h^{-1}; 6.7% above standard), and 1.206±0.09 BL s^{-1} at the low density (0.474 cal h^{-1}; 10.2% above standard).

Values for net energy gained while either swimming or clinging were plotted against prey density (Figure 5.5). Assuming a linear increase in net energy gain with increasing prey density in the cling mode, and no increase in net energy gain with prey density in the swim mode, a foraging strategy that would maximize net energy gain would dictate that lumpfish should switch modes at the density where these linear relationships intersect. This potential switch-point occurs at approximately 620 *Artemia* L^{-1} for the small size class and at around 600 *Artemia* L^{-1} for the large size class. Although the prey consumption rates of fishes generally increase in a curvilinear manner with prey density (Type II functional response; Holling 1959), the relationship is often very near linear at low and intermediate prey densities (with levels of prey intake approaching an asymptote only at very high prey densities). In this regard, intake rates among young fishes feeding on planktonic prey continue to increase at densities as high as 4000 prey L^{-1} (which far exceeds the prey densities used in the present study; Puvanendran and Brown 1999). For this reason, I believe that the assumption that net energy gain increases linearly with prey density (in the cling mode) is valid under the conditions of the current study.

Figure 5.5 Potential net energy gain by juvenile lumpfish *Cyclopterus lumpus* foraging by either clinging (open circles) or swimming (filled circles). Lumpfish acting to maximize their net energy gain should switch foraging modes at the prey density at which the net energy gain obtained by swimming and clinging intersect. According to these assumptions, smaller juveniles (solid lines) should switch at approximately 620 prey L^{-1}, and larger juveniles should switch at approximately 600 prey L^{-1} (dotted lines).

FIGURE 5.5


1.0
0.8
0.6
0.4
0.2
0.0
Net Energy Gain (cal h-1)
0
200
400
600
800
Prey Density (Artemia L-1)

5.4 DISCUSSION

My results indicate that prey density is an important factor in determining foraging mode in juvenile lumpfish. Lumpfish exposed to high prey density spent a greater proportion of time clinging, whereas those with low prey density spent more time swimming. This pattern is contrary to early predictions regarding alternate foraging modes by Norberg (1977), but support Helfman's (1990) later hypothesis regarding foraging mode choice that was specific to ectotherms. Norberg (1977) stated that ambush foraging should be used at low prey densities because the energetic costs of activity easily outweigh food intake when prey is scarce. However, Norberg's theoretical work mainly focused on mammals and birds, for which the energetic cost of activity may be 5-50 times resting metabolic rate (Bishop 1999; Weibel and Hoppeler 2005). In contrast, Helfman (1990) suggested that the lower metabolic rate of ectotherms might allow them to actively forage at low prey densities and switch to an ambush strategy when prey are abundant. My results support this reasoning because the energetic cost of locomotion in actively foraging lumpfish is only 6-12% higher than at rest (depending on size and prey density). This relatively low cost of activity for lumpfish allows an active foraging strategy at low prey densities to remain energetically profitable.

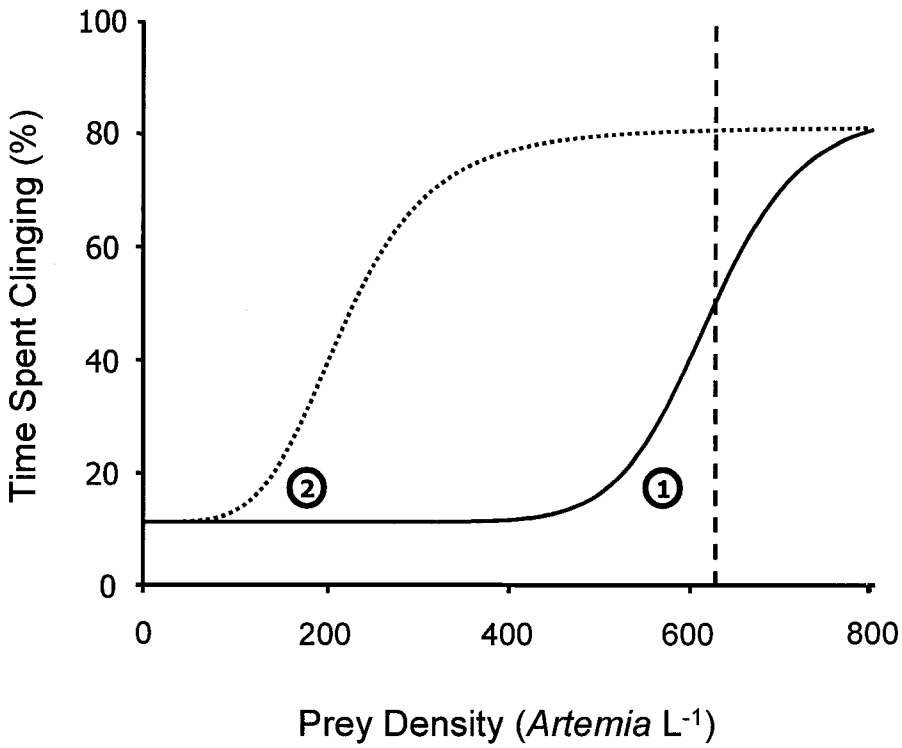
Based on the hypothesis that juvenile lumpfish switch foraging modes to maximize their net energy intake, I devised a model for predicting the prey density at which they should switch between the swim and cling foraging modes. According to this model, lumpfish that maximize their net energy gain should only

cling at relatively high prey densities, and should spend the majority of their time swimming at all lower prey densities (Figure 5.6, scenario 1). The same conclusion might be reached after simply considering the low cost of swimming activity in the lumpfish (i.e. given the low cost of activity, why don't lumpfish swim all the time?). However, the limited information available for young lumpfish suggests they do not follow this pattern of behaviour. For example, larval and juvenile lumpfish in the laboratory predominantly use the cling mode at densities as low as 250 *Artemia* L^{-1} (Williams and Brown 1991). Furthermore, in the wild, young lumpfish spend large amounts of time attached to substrates or seaweed (Moring 1989; Moring and Moring 1991), even though summer and fall zooplankton densities off the coast of Newfoundland are generally less than 100 individuals L^{-1} (Dower et al. 2002).

This apparent discrepancy between model predictions and lumpfish behaviour could be explained by the model's assumption that lumpfish maximize net energy intake. The observation that lumpfish behaviour does not fit the model suggests that this assumption should be rejected, and instead supports Helfman's (1990) speculation that ectotherms maintain a minimum prey encounter rate, and do *not* necessarily maximize net energy gain. Given that the cling position seems to be the 'default' foraging mode of young lumpfish, I propose that they reduce swimming whenever possible, but possess the behavioural flexibility to switch to the swim mode at very low prey densities (Figure 5.6, scenario 2). As indicated by my model, lumpfish still receive a net energetic gain if they use the cling mode at intermediate and perhaps even low

Figure 5.6 Potential behavioural responses (proportion of time spent clinging) of juvenile lumpfish *Cyclopterus lumpus* to changes in prey density. Scenario one (solid sigmoidal curve) indicates a response that maximizes net energy gain, and agrees with the model described in Figure 5.5 (for the small size class). According to this scenario, the cling mode would be the dominant foraging strategy (individuals would spend greater than 50% of their time clinging) at prey densities above 620 prey L^{-1} (the switch-point that is predicted by the model for the small size class; is indicated by the vertical dashed line). Scenario two indicates that juvenile lumpfish switch to the cling mode at prey densities much lower than is predicted by the model, and suggests that lumpfish maintain some minimum prey encounter rate (and do not maximize net energy gain).

FIGURE 5.6


100
80
60
40
20
0
Time Spent Clinging (%)
0
200
400
600
800
Prey Density (*Artemia* L^{-1})
2
1

densities, even though they would not maximize the gains possible by swimming at these same densities. Only the large size class in my study would receive extremely low net energy gains via clinging at the lowest prey densities, and so it is possible that larger juvenile lumpfish switch to active foraging at a higher prey density relative to smaller individuals.

The question, however, still remains: why wouldn't lumpfish maximize their net energy gains? First, it should be noted that numerous studies show that animals often behave as energy satisficers when foraging and are not optimizers (Ward 1992; Nolet et al. 2006); an important aspect of satisficing is that an animal forages only enough to fulfill some minimal requirement (Ward 1992). However, this threshold is difficult to define (Nonacs and Dill 1993), especially for juvenile fishes that not only must satisfy maintenance requirements, but also obtain surplus energy that can be used for some amount of growth. Given the extreme importance of growth during early life stages of fishes (mainly to avoid size-dependent predation; Bailey and Houde 1989; Fuiman 1994), it is somewhat surprising that juvenile lumpfish do not maximize net energy gains while foraging. It should be emphasized, however, that ectotherms have low metabolic demands compared to endotherms, and so even during the juvenile stage they may be able to support their metabolism through ambush foraging alone while exposed to high and intermediate prey densities. In addition, the reduced energy intake resulting from ambush foraging may be offset by the benefits that come with reduced exposure to predators. For lumpfish, swimming makes an individual more obvious to predators, and larval lumpfish increase the amount of time spent

clinging when in the presence of a predator (Williams and Brown 1991). The presence of predators could cause active foraging to be detrimental to overall fitness, except at very low prey densities.

From an energetics perspective, reduced activity may also be beneficial for ectotherms because it allows them to conserve their limited aerobic scope for other physiologically demanding processes such as growth and digestion (and not simply because it reduces energy expenditure *per se*). Fish face an increased risk of mortality when operating near the upper limit of their aerobic scope (Wood et al. 1983; Wood 1991), and there is powerful selection for individuals that can reduce their power requirements so that they do not approach this upper limit (especially for slow swimming fish or those with a limited aerobic capacity; Priede 1985). As a result of their small size and high metabolic intensity, young fish have a reduced aerobic scope compared to adults, and must fit the demands of growth, digestion, and activity into an extremely tight energy budget (Weiser et al. 1988; Kaufmann 1990; Weiser and Medgysey 1990; Post and Lee 1996; Killen et al. 2007). This issue is an especially important concern for juvenile lumpfish because my data show that lumpfish have a very limited aerobic scope (factorial aerobic scope = 1.80-1.95), even compared to juvenile fish of other species such as Danube bleak *Chalcalburnus chalcoides* and roach *Rutilus rutilus* (2.5-4; Kaufmann 1990); Atlantic cod *Gadus morhua* (2.5; Soofiani and Priede 1985); and pike *Esox lucius* (2.7; Weiser et al. 1992). Thus, it is likely that young lumpfish use the cling posture to reduce activity and preserve space within their narrow energy budget

for other physiological processes. In particular, they may prioritize growth, which is important during the early life stages of fish (Bailey and Houde 1989; Fuiman 1994).

Additional evidence that juvenile lumpfish do not forage in a way that maximizes net energy gains comes from the observation that, even when actively foraging, they swim at speeds different from that which minimizes their cost of transport. Although the speed that minimizes the cost of transport in fish is typically greater than that which provides the minimum cost per unit time (Priede 1985; Gamperl et al. 2002), foraging at this increased swim speed actually decreases overall energy expenditure because individuals increase prey encounter rates (Ware 1975; Pyke 1984), and therefore need to forage for a shorter duration to capture the same amount of food. However, if lumpfish were to forage at this increased swim speed, the rate of energy expenditure while swimming would comprise a significant portion of their available aerobic scope. For example, the lumpfish in my study swam at speeds of 0.99–1.21 BL s^{-1} (depending on prey density and size), which equates to 7-15% of their absolute aerobic scope. By contrast, if juvenile lumpfish swim at the speed that minimizes their cost of transport (1.90 BL s^{-1} for the large size class, and 2.48 BL s^{-1} for the small size class), this activity would comprise approximately 46-70% of their absolute aerobic scope. Therefore, although swimming at these speeds would decrease foraging times and reduce overall energy expenditure, it would greatly constrain the ability of lumpfish to simultaneously perform additional physiological functions while swimming.

Lumpfish clinging at the high prey density captured 2.5 fold more prey than those clinging at the low density. This result supports the contention that the low cost foraging mode is usually the least efficient at decreased prey densities (Norberg 1977), and highlights why lumpfish may switch to active foraging when prey is scarce. Interestingly, the number of prey captured between treatments was not significantly different among fish that were swimming. This result was unexpected because prey intake rates of young fish that forage while swimming usually increase with prey density (Houde and Schekter 1980; Munk 1995). In contrast, lumpfish seem to respond to increases in prey availability by adopting the cling posture (as opposed to increasing their prey intake while swimming). This response supports the view that the cling position is the 'default' foraging mode of young lumpfish, and that they reduce swimming whenever possible.

In summary, the foraging mode utilized by juvenile lumpfish is heavily influenced by prey density. Model predictions that assume lumpfish maximize their net energy intake do not match previous observations of lumpfish behaviour, suggesting that this species switches foraging modes to maintain some minimum level of prey intake. Although juvenile lumpfish do not appear to optimize their energy intake while foraging, they may be maximizing their overall fitness because adopting a 'cling' posture allows lumpfish to conserve space in their extremely limited aerobic scope (< 2 fold). Future experiments should confirm this hypothesis by determining the precise density at which juvenile lumpfish increase the proportion of time spent clinging, and by examining how other

factors (e.g. the presence of a predator) may further constrain foraging mode choice beyond the effects of prey density alone.

Chapter Six

Summary

The experiments detailed in this thesis combine measurements of metabolic rate and energy storage with observations of animal behaviour to study tradeoffs encountered during the early life stages of marine fishes. In this concluding chapter, the contributions of this integrated approach are summarized, and suggestions made for areas of future research.

The study described in Chapter Two was the first to quantify standard metabolism, maximal metabolism, and aerobic scope over the entire life history of any fish species. Previous studies have presented original data for either the larval, juvenile or adult life stages, but never all three (Post and Lee 1996; Bokma 2004). There are inherent difficulties in measuring the metabolism of fishes, and this obstacle may explain the paucity of studies that examine metabolic scaling in this group. First, most fish species show random movement in respirometers and routine metabolism (metabolic rate while the fish performs a specified amount of activity) is therefore often measured instead of standard metabolic rate. Second, unlike other vertebrates, the life histories of many fish species span a range in wet mass that may be 4-8 orders of magnitude. For this reason, it is technically difficult to measure standard and maximal metabolism over a size range large enough to reliably estimate scaling exponents.

The species examined in Chapter Two allowed me to circumvent these difficulties, and provide a direct comparison of metabolism and aerobic scope

among various life stages. This approach enhanced our understanding of the metabolic challenges faced by young fishes, and how they could ultimately affect behaviour and survival. For example, larval and juvenile fishes have an extremely high mass-specific metabolic demand and a greatly reduced aerobic scope compared to adult individuals, and changes in metabolism and aerobic scope throughout ontogeny vary considerably depending on the pattern of early development. As a result, species that undergo relatively abrupt developmental changes early in life (e.g. shorthorn sculpin) may have extremely reduced aerobic scope during specific points in early development (i.e. metamorphosis). Chapter Two therefore suggests that metabolic constraints faced by young fishes have important implications for behavioural ecology. This hypothesis was the underpinning of the research conducted in the remainder of this thesis.

One area in which a limited aerobic scope could affect the ecology of young fishes is the ability to respond to changes in water temperature. At all life stages, temperatures above a species-specific thermal range (defined by upper and lower "pejus" tempertures; Pörtner, 2002) cause increased maintenance costs and a reduction in aerobic scope that constrains physiological function. Pörtner (2002) argues that pejus temperatures should be more important than critical temperatures for determining population abundances and species distributions, because growth and reproductive potential can become impaired beyond pejus temperatures without direct mortality from thermal stress. In support of this hypothesis, Pörtner and Knust (2007) observed that the abundance of eelpout *Zoarces viviparous* in the North and Baltic Seas showed

reductions after years in which water temperatures reached the upper pejus temperature for this species (but not the critical temperature). Despite the potentially important role of temperature in defining the distribution and abundance of populations, most work examining thermal-tolerance in fishes has been performed on adult individuals. However, the delicate early life stages are known to be more sensitive to changes in temperature as compared to adult individuals (Rombough 1988; Johnston and Hall 2004). Furthermore, it is survival through early life that is believed to be a critical factor influencing the population size and distribution of adult fishes (Trippel and Chambers, 1997). If water temperatures approach the upper pejus limits of larval or juvenile fishes, it could cause a further decrease in their already limited aerobic scope. Unfortunately, we currently have very little knowledge of how aerobic scope is affected by temperature in larval fishes. More data is needed on the standard and maximal metabolic rates of young fishes and how these variables respond to temperature change so that we may better understand temperature sensitivity during the early life stages.

A limited aerobic scope may also affect the dispersal potential of the pelagic larvae of marine fish species. It has been speculated that larval fishes face a tradeoff during the pelagic phase in that the longer they spend swimming before settlement, the less onboard energy they have available for growth and maintenance (Leis 2006). However, because larvae have a reduced aerobic scope, an additional tradeoff may be that their ability to grow and swim simultaneously is limited. To illustrate, consider a larvae that spends all of its time

swimming at its critical swim-speed (U_{crit}). This larvae would obviously be spending a great deal of energy (which would therefore not be available for growth), but even if it did have excess energy stores to put into growth, it wouldn't be able to do so because it would operating at its maximum aerobic capacity while swimming at U_{crit}. This example is extreme in that larvae probably spend very little time swimming at U_{crit} (Leis 2006), but any time larvae spend swimming should theoretically cut into the scope that is simultaneously available for other physiological processes. As a result, larvae may "choose" to swim less (i.e. decrease their dispersal potential) so they can grow more. To date there have been no attempts to investigate the role of aerobic scope in determining the dispersal potential of larval fishes. One testable prediction is that larvae with an increased scope would be more willing to swim for extended periods or at higher speeds (which would suggest a higher dispersal potential). In addition, the larvae of many species are known to feed while swimming (Hindell et al. 2003). In larvae with full guts, swimming ability might be limited since they will need to devote a large portion of their aerobic scope toward digestion and nutrient assimilation. Species or individuals with relatively high aerobic scopes may have an increased ability to feed while "on the run". Alternatively, species with low aerobic scopes may have to "rest" for some period after feeding (or at least reduce swim speed), which would effectively reduce their dispersal potential.

Chapters Three and Four examined the effects of mass-specific changes in metabolic demand on the predator-avoidance behaviour of young fish, and are the first to document the long-term cost of the tradeoff between foraging and

predator avoidance during the early life stages of any fish species. Specifically, the results of these studies indicate that young ocean pout and larval shorthorn sculpin decrease foraging activity while exposed to a predatory threat. Ocean pout display this tendency immediately after hatching, but in the case of the shorthorn sculpin, the ontogeny of this decision-making ability is delayed, and appears to be related to developmental changes in visual acuity and the ability to detect a predatory threat. Moreover, the willingness of shorthorn sculpin larvae to forage under predation threat does not change significantly during stages of development where metabolic demand is high (i.e. during metamorphosis). This pattern suggests that reductions in foraging activity are extremely costly (especially considering that the mass-specific metabolism of young fishes is already high as compared with adults). Indeed, for young ocean pout and shorthorn sculpin, the costs of these frequent foraging interruptions accumulate over time and result in reduced growth and energy (lipid) storage during this fragile stage of development.

These studies represent an important first step towards establishing the potential affects, other than direct consumption, of predators on young fishes. For example, the mere presence of predators in the environment may reduce foraging activity and growth, with potentially serious consequences during the early life stages of fishes. Indeed, the experiments in Chapter Four show increased levels of mortality in larval shorthorn sculpin frequently exposed to predators. However, there are at least three avenues of research stemming from these results that require further study. First, although increased energy stores

are generally assumed to be associated with increased fitness during the early life stages of fishes (Fraser 1989), this concept needs more rigorous investigation. For example, future research could examine how increased body size or lipid stores affect competitive ability among fish in a common environment, or whether increased lipid stores are beneficial during bouts of reduced prey availability or increased temperature. Second, the studies described in Chapters Three and Four likely represent a "worst-case scenario" for the young fishes, and it is important to work back from this point and evaluate whether the presence of predators has important sublethal effects at larger spatial and temporal scales. Finally, although the experimental conditions used in Chapters Three and Four resulted in reduced foraging among the young fishes exposed to predators, future work should examine how this response is influenced by various environmental factors. For example, young fish may be less able to detect (and therefore respond to) predators under conditions of high turbidity (Abrahams and Kattenfeld 1997). In addition, although the experiments of Chapter Three found that temperature had no effect on foraging under the threat of predation, this area requires further study.

Perhaps the most interesting finding in Chapters Three and Four was that larval fishes prioritized anti-predator behaviour over foraging. It has traditionally been accepted that rapid growth is critical during early life stages – both to avoid size-dependent predation, and to increase the range of available food items (Bishop and Brown 1992; Pepin et al. 1992; Johnsson 1993). For this reason, it was somewhat surprising that young ocean pout and shorthorn sculpin do not

behave in a manner that maximizes growth. However, despite the apparent benefits of rapid growth, it is clear that many fish probably do not grow maximally given that hormonal and genetic manipulation can increase growth rates (i.e. fish generally grow slower than the rate that is physiologically possible, even when rations are not limiting; Devlin et al. 2002). In addition, recent research suggests that extremely rapid growth rates may not be advantageous from a fitness standpoint. Individuals with increased growth rates forage more often, and may therefore experience increased predator encounter rates (Abrahams 2006). Being physically smaller may also provide some direct benefits. For example, larger individuals may be more conspicuous or attractive to predators compared to their smaller conspecifics. Larger capelin larvae *Mallotus villosus* are preferentially consumed by predatory stickleback, *Gasterosteus aculeatus,* and jellyfish, *Staurophora mertensi* (Litvak and Leggett 1992). Smaller fish may also be able to out-maneuver predators during escape attempts (Domenici 2001; Abrahams 2006), or exploit certain refuge habitats (e.g. hypoxic areas) that cannot be as easily tolerated by predators (Robb and Abrahams 2003). Thus, it appears that although growth is important for young fishes (especially during the larval stage), the scenario is much more complicated than was once assumed according to the "get big quick" paradigm.

The study described in Chapter Five is one of only a few attempts to study foraging mode selection in juvenile fishes. Furthermore, only a handful of studies have applied the principles of optimal foraging theory to juvenile fishes, and none have incorporated measures of the energetic cost of locomotor activity into a

foraging model. The latter is a major strength of this thesis because it does not rely on assumptions of energy expenditure or inappropriate data from the literature (a common criticism of many foraging models). The predictions of the foraging model devised in Chapter Five do not agree with previous observations of lumpfish behaviour, and it appears that juvenile lumpfish do not maximize their net energetic gain. Instead, the data suggest that juvenile lumpfish switch foraging modes in a manner that reduces activity and conserves space in their limited aerobic scope. This behavioural flexibility would be of great benefit to this species because it helps young individuals operate within their extremely tight energy budget and divert energy towards growth (or other physiological functions) as opposed to activity.

An interesting question that arises from the research in Chapter Five is why lumpfish have such a low scope for aerobic activity. Although ectotherms have a greatly reduced aerobic scope compared to endotherms, and the differential effects of scaling on minimal and maximal metabolic rates cause aerobic scope to be limited during the early life stages of fishes (see Chapter Two), juvenile lumpfish appear to possess an exceptionally limited scope even compared to the juvenile stage of other fish species. One possible explanation is that because of their relatively sedentary lifestyle, they simply do not require a large aerobic capacity. A ventral adhesive disc probably provides several benefits, including reduced exposure to predators, and an increased ability to remain in a preferred microhabitat despite strong wave action in coastal areas.

In general, juvenile lumpfish appear to be equipped for a life of very little activity, and thus may not need a large aerobic scope.

To conclude, the strategy of quantifying both physiological and behavioural parameters proved to be extremely beneficial for studying the biology of young fishes, and this approach yielded many insights that would not have been possible if either discipline had been applied alone. This integrated approach enhances understanding of how physiology can affect animal behaviour (e.g. how increased metabolic demand or decreased aerobic scope may affect foraging decisions), and conversely, how behaviour can alter an individual's physiology (e.g. how foraging rates can affect lipid stores and fatty acid profiles). Further studies on the complex inter-relationship between physiology and behaviour should lead to significant advances in our understanding of the ecology of young fishes, as well as other animals.

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