DEVELOPMENT OF LARVAL FISH: A MULTI-SPECIES PERSPECTIVE

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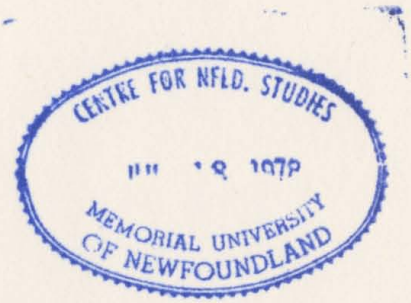
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Development of larval fish: A multi-species perspective

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Abstract

This thesis progresses from the previous examinations of finfish early life history by first examining the differences and similarities of larval fish development among species, and then building on this knowledge base to test for species similarities based on phylogeny and/or ecology. The first analysis undertaken was a corroboration of external characteristics with internal characteristics (Chapter 2). Second, a detailed analysis of lipids in larval fish across multiple species was completed (Chapter 3). The third analysis chapter (Chapter 4) examines the interplay among developmental processes within larval fish, and how these tradeoffs vary between species. The fifth chapter further extends this question and utilises variation amongst individuals to determine which of these processes are optimised to produce larvae that survive. In the final analytical chapter (Chapter 6), all of this information is incorporated into an exploratory multi-species model.

The contribution of this thesis in its entirety to the field of marine fish ecology is in three general areas: 1) the relevance of body size to larval fish; 2) the potential applicability of a multi-species model of larval fish development; and 3) sources of larval fish mortality. Research within this thesis favoured the use of mass to determine internal state of a larval fish, although to allow comparison across studies, reporting of both mass and length continues to be the most effective approach. Explicitly explaining the similarities and differences observed among species was not assisted by the use of phylogenetic analysis, but ecological patterns such as demersal or pelagic life cycle phases, and season of hatch were somewhat more successful. Additional information

about sources of larval fish mortality resulted from highlighting the importance of variables that characterise developmental events during ontogeny (i.e. development progress variables, Chapters 4 and 6) and through the analysis of patterns of intrinsic selection (Chapter 5).

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I shall refrain from thanking my cats, rather I suggest all typos are results of misplaced paws.

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List of abbreviations and symbols

%	percent
µg	microgram
µm	micrometre
C16ALC	16 carbon alcohol
C16KET	16 carbon ketone
C18FFA	18 carbon free fatty acid
C19HC	19 carbon hydrocarbon
C22FFA	22 carbon free fatty acid
C32DAG	32 acyl carbon diacylglycerol
C36WE	36 carbon wax ester
C43SE	43 carbon steryl ester
C45SE	45 carbon steryl ester
C48TAG	48 acyl carbon triacylglycerol
C54TAG	54 acyl carbon triacylglycerol
C60TAG	60 acyl carbon triacylglycerol
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATP	adenosine triphosphate
°C	degree Celsius
DNA	deoxyribonucleic acid
DF, df	degrees of freedom

F	Fisher's F-statistic
g	gram
GC-MS	gas chromatograph-mass spectrometer
h	hour
L	litre
ln	natural logarithm
mg	milligram
ml, mL	millilitre
mm	millimetre
MS-222	tricaine methane sulphonate
n, N	number
p	probability
r	correlation coefficient
RNA	ribonucleic acid
R^2	coefficient of determination
SD	standard deviation
SE	standard error
ST	cholesterol

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Co-authorship statement

The experiments contained within this thesis were designed and conceptualised by K.E. Morton, with assistance from J.A. Brown, P. Pepin and I. Fleming. K.E. Morton was responsible for all data collection and analysis.

Chapter 1 General Introduction

1.1 Why are larvae important to marine ecology?

The larval period in fish is loosely defined as the period between hatching and ossification, and occurs in many of the more than 24,000 species of fish. Although researchers vigorously debate the exact definition of "larva" (e.g. Balon 1999), the generally high rate of mortality in the larval period has broad implications to several fields of study (see review Leggett and Deblois 1994). In fisheries management, as early as 1914 Hjort identified the larval period as a key stage in the success or failure of a year class in marine fish. Aquaculturists continue to be constrained by the supply of high quality juveniles and knowledge of maintenance of immature fish (Planas 1999; Kamler 2008). Biological theorists work to understand the transition between trophic levels and environments between life stages; and to use the diverse comparative basis for understanding evolution, functional morphology (e.g. Shelbourne 1956; Higgs 1998), and heterochrony (e.g. Godfrey and Sutherland 1995) amongst other reasons. Early life history has been a focus of study for naturalists, zoologists, marine biologists, and evolutionary biologists for several centuries in ongoing attempts to understand not only the life cycles of individual fish species, but also how life cycles vary across species (Hall and Wake 1999).

This thesis progresses from the earlier research on the early life history stages of fish by first examining the differences and similarities of larval development among species, and then building on this knowledge to test for similarities based on phylogeny

and/or ecology. In this introductory chapter I will briefly review the factors that affect the survival of larval fish, summarise what understanding of larval fish development dynamics has been gained through the use of allometric relationships, and finally review multi-species syntheses of larval fish developmental research.

1.2 **What causes larval fish to die?**

In addition to the plethora of interesting research opportunities presented by larval fish that attract scientific inquiry, one ultimate question remains a central focus: why do so many larval fish die? The early life history stages of fish present a unique challenge to fisheries managers, aquaculturists, and conservationists, with many species producing millions of offspring from which far less than 0.01% survive to maturity. This represents a major bottleneck in the population growth and maintenance of many fish species (e.g. Houde 1989a). While it is beyond the scope of this introductory chapter to review in detail all of the theories surrounding larval fish mortality, it is necessary to contextualise this thesis by highlighting the breadth of factors that influence losses during the larval period. Research into larval fish mortality can be broadly classified as investigating either external pressures on the larva, hereafter referred to as extrinsic, or details of the larva itself, hereafter referred to as intrinsic.

1.2.1 *Extrinsic sources of mortality*

Marine larval fish are relatively small and mostly planktonic. Significant effort has been, and continues to be, focused on the effect of the environment on larval mortality. Larval fish were previously assumed to be entirely passive and unable to select

nursery habitats. Therefore, the currents that larvae are released or hatch into were considered to determine the nursery habitat for the entire larval period (e.g. Norcross and Shaw 1984). The temperatures of a nursery habitat, as well as the variability of the temperature experienced by the larvae, are well researched abiotic influences on larval fish survival. The mortality rates of larval fish generally increase as temperatures rise (Houde 1989b, Pepin 1991). However, concurrent with increased losses, elevated temperature also leads to more rapid development and growth, which can result in a decrease in the duration of the larval stage (Houde 1989b, Pepin 1991). When the influences of increased mortality and growth are combined, the net effect of increasing temperature is that there may be little or no effect on net survival (Pepin 1991), although there is likely to be significant variation on net survival.

Larval fish were also assumed to be subject to all of the variations of the nursery habitat. The amount of environmental variability (e.g. in temperature) of the nursery environment has also been explored as a source of mortality in larvae, but has not resulted in consistent predictions (Pepin 1991). More recently, it has been demonstrated that the larvae of many fish species can move quite significantly within the water column relative to their size. This allows larvae to effectively select currents and nursery environments (e.g. Bradbury et al. 2003, Leis 2006) that reduce the potential negative effects of abiotic factors on their health and survival.

How much food and when it is available is critical. Increased prey density should logically decrease mortality, but prey availability has generally been found to be patchy. The incumbent spatial effects of a patchy distribution of prey on foraging likely have stronger influences on the mortality of larval fish than actual prey abundance (Ware 1975,

Bailey 1984, McGurk 1986, Rice et al. 1993). Hjort (1914) proposed the 'critical period' hypothesis, which postulates that larval fish are highly sensitive to temporal variability in prey availability. This was further refined by Cushing and Harris (1973) who linked the timing of spawning to the timing of plankton blooms. This work, as well as others, proposes that starvation is a major source of larval mortality.

1.2.2 *Intrinsic sources of mortality*

Apart from the external influences on larval fish mortality, evidence suggests that larvae are also subject to intrinsic influences on mortality. Faster growing, bigger larvae within a cohort have been shown repeatedly to succeed while other members of the cohort fail (e.g. Balon 1979, Houde 1989a, Pepin and Myers 1991, Meekan and Fortier 1996). This effect has been recorded within a cohort and verified through nucleic acid (Clemmesen 1993, Pepin et al. 1999) and otolith increment analyses (Meekan and Fortier 1996), suggesting that two larvae of the same cohort that experience the same extrinsic influences are not necessarily equally likely to succeed. Fluctuations in selective pressures between cohorts have long been known to maintain genetic diversity in addition to mutational variation (for review see Philipi and Seger 1989), but understanding the intrinsic functional tradeoffs preventing animals from achieving optimal growth rates remains elusive (see review by Dmitriew 2011). This thesis builds on and improves the understanding of intrinsic differences by comparing individual larvae within standardised rearing conditions.

1.3 What does body size tell us about larval fish?

Larval fish are generally very small compared to their final adult stages, often growing many orders of magnitude in size before reaching adulthood or even the juvenile stage. The importance of size to survival is well documented in ecology, and examples of the positive influences of increased size on larval fish are many (Miller et al.1988). Bigger larvae can swim faster (Bainbridge 1958), forage more efficiently (Dower and Kim 2009), better avoid predators (Bailey 1984), process food more efficiently (Ware 1978, Houde and Schekter 1980), and are therefore more likely to survive and eventually produce more offspring.

Ecologists often group larvae as a function of their size. For example, measures such as total length, body mass, size at hatch, size at metamorphosis, and combinations of these measures are pervasive in larval fish research. Body size has been used as a measure of growth potential (e.g. Houde 1989a), condition (well-being, or robustness; e.g. Suneetha et al. 1999), and in many studies as a method of assessing developmental state (e.g. Ben Khemis et al. 2003). For example, Gozlan et al. (1999) used the rate of change in body size (as measured by 23 characteristics including standard length) to define different developmental stages (*sensu* Balon 1986) and the endpoint of the larval period. Houde (1989a) inferred growth rates based on body size, and in conjunction with otolith data, predicted the survival likelihood of an entire stock. Researchers have also used size to classify animals into developmental states (e.g. Herzka et al. 2001, Ben Khemis et al. 2003). For example, the proxy of length has been used to indicate the onset of stomach differentiation (Ben Khemis et al. 2003) and that animals are ontogenetically capable of

settling (Herzka et al. 2001). The use of body size in larval fish ecology is widespread and therefore any inaccurate use would subsequently affect many fields including fisheries management (e.g. Houde 1989a, Froese and Binohlan 2000, Bailey et al. 2003, Valle and Ramos 2003), conservation (e.g. Herzka et al. 2001, Govoni et al. 2003), predator-prey interactions (e.g. Huuskonen et al. 1998, Ojanguren and Brana 2003, Pepin et al. 2003), phylogeny (e.g. Smith 2001), ontogeny (e.g. Post and Lee 1996, Fuiman et al. 1998), husbandry (e.g. Cunha et al. 2003), and ecological theory (e.g. Post and Lee 1996, Fuiman et al.1998, Dower et al. 2002, Jaworski and Kamler 2002).

Despite the widespread use of body size as a proxy for developmental state in fisheries ecology, its use in this manner also presents difficulties. Larval fish have historically been underrepresented in the examination of body size as a proxy for internal measures. Much of the research based on the use of body size has focused primarily on juvenile and adult fish owing to the difficulties incumbent in working with the larval stage (e.g., challenges in rearing and manipulation, logistic challenges of working with small sized animals), and because the economic value is principally in the adult fish. As size is biologically advantageous for predator avoidance, foraging, competition, reproductive success, and reducing mortality (Erzini 1994), an increase in size is often considered the primary goal of immature fish. Fish are therefore considered to allocate the bulk of ingested energy to growth until a sufficient size is reached, and subsequently to shift focus to reproduction (Weatherley et al. 1987). Sexually immature fish, such as larvae, are by definition non-reproductive, thus there is an underlying belief that the bulk of available energy is devoted to basic size enhancement. As a result, sexually immature

fish are often modelled on the same principles as adults, but with the reproduction term dropped (Weatherley et al. 1987).

Condition indices also use body size, and are simple measures used to assess animals in such studies as population health, effects of nutritional supplements in aquaculture, meat quality, and parasite load as well as to observe population reactions to pollution (for reviews see Ferron and Leggett 1994 and Shulman and Love 1999). When the indices were first introduced (circa 1950), technology was limited so that the biochemical evaluations of fish were expensive, time consuming, and not widely available (Craig 2005). The most commonly applied morphometric condition index for fish is Fulton's condition factor (K), derived from the simple allometric relationship between weight and length, and calculated as $100W/L^3$ (e.g. Hoar et al. 1979). More complicated methods of assessing condition from morphometrics utilise image analysis software, but these tend to be more species-specific and less widely used (e.g. Smith et al. 2005). More recently, alternative non-lethal methods such as bioelectric impedance (Cox and Hartman 2005) and microwave (Crossin and Hinch 2005) have been developed, although bioelectric impedance may not provide significantly more information than body mass alone (Pothoven et al. 2008), and microwave requires species specific calibration (Whiterod 2010).

1.4 Can variations in larval fish composition be understood through a general model?

A few examples of successful multi-species models have demonstrated relationships between development and body size (Miller et al. 1988), temperature and growth rate (Pepin 1991), and environment and egg size (e.g. Johnston and Leggett 2002). However, although there are many models that find general relationships governing larval fish, there are perhaps as many or more that find significant differences among species.

It is likely, therefore, that the reality lies somewhere in between – that there is an underlying framework that provides a good descriptor of larval fish development. Two organisational schemes are already being used informally to explain larval fish groupings: ecological designations and phylogenetic relatedness. These frameworks have not yet been explicitly tested as guiding principles of larval fish development, although both have been proposed as explanations for the variation observed in larval fish development (e.g. Pepin 1991, Leis 2006). Ecological designations such as spawning season, and pelagic or demersal development have been mentioned as potential explanations of observations (e.g. egg size, Sargent and Gross 1987, larval size, Winemiller and Rose 1993) and the field of ecomorphology, which explicitly attempts to explain functional morphology through ecology supports these types of inferences (e.g. Wainright 1996). Phylogeny, or the relatedness of species under study, has also been suggested to affect larval development (Pepin 1991) and the effect of phylogeny on similarities between adult fish has been explicitly tested (e.g. Rochet 2000, Mank and Avise 2006). Ecology and

phylogeny are pre-existing frameworks of organisation that may help group larval fish development strategies.

1.5 Summary of thesis objectives

The ultimate goal of this thesis was to examine the potential for a comprehensive multi-species model for development of larval fish. With this objective in mind, the project attempted to incorporate all species that were readily available at the Ocean Sciences Centre of Memorial University of Newfoundland, Canada. Ten species were reared in controlled laboratory conditions: *Gadus morhua* (Atlantic cod), *Mallotus villosus* (capelin), *Ulvaria subbifurcata* (radiated shanny), *Cyclopterus lumpus* (Atlantic lumpfish), *Pseudopleuronectes americanus* (winter flounder), *Myoxocephalus scorpius* (shorthorn sculpin), *Myoxocephalus aenaeus* (grubby sculpin), *Limanda ferruginea* (yellowtail flounder), *Liparis atlanticus* (Atlantic snailfish), and *Hippoglossus platessoides* (American plaice). In addition, wild *Ulvaria subbifurcata* larvae were collected to corroborate some of the laboratory derived data.

The first analysis undertaken (Chapter 2) was a corroboration of external characteristics with internal characteristics, *sensu* Fuiman et al. (1998). The aim was to determine whether there was a simple external characteristic that could be used to examine the developmental progress across multiple species of larval fish, in hopes of providing a useful metric for further multi-species investigations.

Second, a detailed analysis of the size-dependent patterns of variation in neutral lipids in larval fish across multiple species was undertaken (Chapter 3). Lipids are high-energy compounds and the focus of significant research in larval fish development (e.g.

Sargent 1995, Wiegand 1996, Rainuzzo et al. 1997). Within this large proportion of the overall energetic budget of larval fish, I was able to test the hypothesis that larvae sharing common ecological designations share similar developmental trajectories. Phylogeny was also examined as a potential delineator of developmental trajectories.

After these investigations, the investigation moved to a broader examination of larval fish development, and in the third analytical chapter (Chapter 4) I examined the interplay between developmental processes within larval fish, and how these possible tradeoffs varied between species. The fourth analytical chapter (Chapter 5) further extended this question and utilised variation amongst individuals to determine if there is intrinsic selection in the absence of known selective pressures and which descriptors of physiological state are optimised to produce larvae that survive.

In the final analytical chapter (Chapter 6), all of this information was incorporated into an exploratory multi-species model. Through path analysis, the relative influences of different developmental processes on increasing mass, and how these varied across species, were explored.

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Chapter 2 Metrics comparing ontogenetic and biochemical measures of developmental state in larval fish

2.1 Introduction

Consistently and conveniently assessing the developmental state of fish larvae is necessary for many aspects of marine ecology. Measures of body size (e.g. length, mass) have been used extensively as indices of the internal state of a larva, both as measures of condition and development (e.g. Houde 1989, Suneetha et al. 1999, Herzka et al. 2001, Ben Khemis et al. 2003). For example, Ben Khemis et al. (2003) used a body length of 5.5 mm to estimate the point of stomach differentiation in winter flounder. Houde (1989) used body mass not only to estimate growth rates, but to predict stock production. Using body size to estimate developmental state and condition is convenient, fast, and inexpensive. In addition, inferring internal state from larval morphometrics allows researchers to proceed without destroying the animal, thereby reducing the total mortalities while allowing single individuals to be followed throughout development. Finally, some analyses require the entire animal and therefore preclude other, simultaneous whole-animal measurements (e.g. either carbon or neutral lipids can be measured, but not both). While research in this area, led by studies on condition factors, is extensive (for details see Chapter 1), little has been done to investigate the suitability of the morphometric-internal state relationship across species. This study tests the ability of a single morphometric to predict the internal state of a larva, both within and among species.

As research strives towards understanding the general principles that govern the early life history of fishes, it is desirable to have convenient metrics that allow inter-

specific comparison. While both mass and length can be informative, they have traditionally failed when applied in inter-specific comparisons (Fuiman et al. 1998). Fuiman et al. (1998) were relatively successful in developing a multi-species gauge of ontogeny through the inter-calibration of age, total length, mass, and eye length from hatch until metamorphosis. These standardised measurements were compared to precise ontogenetic events (e.g. the development of gill arches). They found that natural logarithmic transformation of total length provided the morphometric variable with the least inter-specific variance. However, the applicability of this ontogenetic index is limited to species where the entire development is known and metamorphosis occurs at a defined and agreed upon state of development. It is not applicable to animals before organogenesis or after metamorphosis (Jaworski and Kamler 2002), making a more reliable and versatile inter-species morphometric desirable.

With the ability to digitally analyse photographs of larvae, a greater variety of alternate morphometric measurements (e.g. eye diameter, body depth, head height and head length) can be used to estimate developmental state and nutritional status of larvae (Portt and Balon 1984, Cottrill et al. 2002, Gisbert and Williot 2002, Lochmann and Ludwig 2003). However, such estimates of state and/or status are often chosen because of accepted disciplinary standards rather than a demonstrated effectiveness. For example, biochemical studies usually report mass (e.g. Jaworski and Kamler 2002), while field studies usually report length (e.g. Voss et al. 2006). Length is the more traditional measure of larval fish body size because it is easily measured and highly repeatable, whereas accurate measurements of mass (both wet and dry) can be difficult to obtain (Fuiman et al. 1998). Recently, an increasing proportion of studies have begun reporting

combinations of metrics, such as length and mass (e.g. Conover and Ehtisham 1997, Pepin and Penney 1997, Fuiman et al. 1998, Otterlei et al. 1999). The present study focuses on the most commonly used morphometrics, mass and length, as well as other simple external morphometrics to determine their value in estimating internal state variables of larvae, both within and among species in the style of Fuiman et al. (1998).

To comprehensively document the internal state of a larva, measurements of both biochemical elements (carbon, nitrogen, nucleic acids and lipids) and developmental features (ossification and organogenesis), hereafter referred to as state variables, are compared to external morphometrics and mass. Each larva was individually processed and analysed, allowing the variability among larvae to be be considered in the analysis using a novel lipid analysis method. To address the lack of a widely applicable larval development metric, I first evaluated whether a single metric can be used as a general indicator of all internal state variables, irrespective of species. Second, I investigated which metric performs best for each state variable across all species. Next, each species is examined independently for these same questions: a species-specific metric to assess all internal state variables simultaneously, and finally a metric which performs best for each state variable of each species. To improve the separation of intrinsic effects from environmental effects, all species were reared in similar experimental settings, temperatures, and feeding regimes as; differences in rearing temperature (Sickland et al. 1988, Weigand 1996, Green 2004, Johnston 2006) and diet (Sargent et al. 1999, Cahu et al. 2003) can affect larval development.

2.2 **Materials and methods**

2.2.1 Rearing and sampling

Nine species of larval fish were reared under controlled laboratory conditions: *Gadus morhua* (Atlantic cod), *Mallotus villosus* (capelin), *Ulvaria subbifurcata* (radiated shanny), *Cyclopterus lumpus* (Atlantic lumpfish), *Pseudopleuronectes americanus* (winter flounder), *Myoxocephalus scorpius* (shorthorn sculpin), *Limanda ferruginea* (yellowtail flounder), *Liparis atlanticus* (Atlantic snailfish), and *Hippoglossus platessoides* (American plaice) (For rearing details, please see Appendix 2.1). Atlantic cod were reared in collaboration with the staff of the Dr. Joe Brown Aquatic Research Building (JBARB). Feeding protocols, feed enrichment, feed preparation, stocking densities and light regimes for all other species were matched to those used at the JBARB. Larvae were stocked at densities of 50 larvae L^{-1}. Initially, all species were fed rotifers (*Brachionus plicatilus)* enriched with protein selco (INVE, Belgium) and microalgae (*Isochrysis* sp.) at a density of 4000 prey L^{-1} three times daily (02:00, 10:00, 18:00) until the larvae were observed to feed upon enriched *Artemia* sp. nauplii (determined by gut examination). *Artemia* sp. nauplii were then provided three times daily (02:00, 10:00, 18:00) at a density of 4000 prey L^{-1} until metamorphosis. Larvae were maintained under continuous lighting because many species perform better in larviculture under such conditions (see Villamizar et al. 2011 for a review). All species were incubated and reared at an average temperature of 6°C, with the exception of Atlantic cod, which were incubated at 6°C and reared at a constant temperature of 8°C. Short term (<24 hr) inconsistent departures of rearing temperatures (range 4-8°C) in all other species were unavoidable because of limitations in the seawater facilities at the

Ocean Sciences Centre. Although the species were spawned at different seasons, the common temperature in the laboratory setting varied from those experienced in the wild but was within the known range for all species sampled (for details see Appendix 2.2).

Randomly selected larvae of each species were sampled once a week. Sampling began at hatching and continued beyond metamorphosis (defined for the purposes of this study as complete ossification). Fifty Atlantic cod, shorthorn sculpin, Atlantic lumpfish, radiated shanny and winter flounder larvae were sampled during each sampling event. As fewer eggs were obtained for yellowtail flounder, Atlantic snailfish, American plaice and capelin, only 25 larvae were sampled each week until the supply of larvae was exhausted. Larvae were euthanised with an overdose of tricaine methane sulphonate (MS-222) or phenoxyethanol.

All larvae were photographed laterally with a digital camera (Pixera PVC 100C) immediately after euthanasia. Measurements of head length, head height, body depth at pectoral fin, body depth at anal fin insertion, eye diameter, yolk sac length, yolk sac height, and body length were obtained from the images using Matrox Inspector 3.0 image analysis software (Matrox Imaging; for details see Appendix 2.3). After photography, larvae from each species were divided equally among five analytical procedures to provide measurements of (1) dry mass, carbon, and nitrogen, (2) organogenesis, (3) ossification, (4) lipids, and (5) DNA and RNA.

2.2.2 Carbon and nitrogen processing

Samples for carbon and nitrogen processing were dried at 24°C on pre-massed foil until a constant mass was achieved. This mass was recorded and the samples stored in a

dessicator until processing, when they were encapsulated in aluminum. Total carbon and nitrogen of individual fish was determined using a Perkin-Elmer CHNS/O 2400 analyser, which uses thermal conductivity through combustion and reduction to estimate the mass of each element.

2.2.3 Neutral lipid extraction

Individual larvae for lipid analysis were placed in chloroform and stored under nitrogen gas at -20°C. Tricaprin was added at this point as a surrogate spike, which is an amount of quantified standard added to the sample to account for any variability in laboratory processing. By comparing the amount of tricaprin recovered to that from the other lipids in the sample, the amount of each lipid can be accurately quantified using previously developed correction factors. Tricaprin was chosen after preliminary analysis showed no similar compounds in the samples. Amounts of neutral lipids and the surrogate spike were extracted using the modified Folch method (Folch et al. 1957) described by Parrish et al. (1999). Single larvae were homogenised in cold chloroform:methanol (2:1, v/v) using a clean metal rod. The sample was then sonicated in chloroform:methanol:water (8:4:3, v/v/v), centrifuged and the organic layer removed. This was repeated three additional times and the organic layers pooled. The sample was then mixed with two drops of N,O-bis(trimethylsilyl)-acetamide and two drops of N,O-bis(trimethylsilyl)-trifluoroacetamide and heated at 70°C for 15 minutes. The sample was then passed through a Pasteur pipette containing either 2 g Florisil (Fisher Scientific, Springfield, NJ) or silica. Neutral lipids were eluted with 8 mL of chloroform-methanol-formic acid (99:1:1, v/v/v).

Neutral lipids were quantified using a Hewlett-Packard 6890/7863 gas chromatograph-flame ionization detection (GC-FID) (see Yang et al. 1996 and Hooper and Parrish 2009 for operational details). A standard containing 13 lipid components (16 carbon ketone (C_{16} KET), 19 carbon hydrocarbon (C_{19}HC), 16 carbon alcohol (C_{16}ALC), 18 carbon free fatty acid (C_{18}FFA), 22 carbon free fatty acid (C_{22}FFA), cholesterol (C_{27}ST) 36 carbon wax ester (C_{36}WE), 32 acyl carbon diacylglycerol (C_{32}DAG), 43 carbon steryl ester(C_{43}SE), 45 carbon steryl ester (C_{45}SE), and three triacylglycerols (48 acyl carbon triacylglycerol C_{48}TAG, 54 acyl carbon triacylglycerol C_{54}TAG, 60 acyl carbon triacylglycerol C_{60}TAG)) was run each time a series of samples was analyzed. Each larva generated a single chromatogram. The resultant chromatogram was analysed using HP ChemStation Chromatography Software (Version B00.00) to integrate the area under each peak. Peaks were cut in two approaches: first, peaks which corresponded to the concurrently run standard were cut, and then all other peaks were cut.

Lipid quantities reported as a single component (e.g. 16 carbon ketone) indicate the peak of the chromatogram most likely representing that component. When reported as 'total neutral lipids', this value includes all peaks cut except the surrogate spike. Lipids reported as percentages are of all peaks. Lipids were quantified by comparison with the surrogate spike tricaprin. Lipids were grouped into categories of use: long term storage (triacylglycerols), structural components (steryl esters, sterols), and intermediate metabolites (free fatty acids, DAG) (Kattner et al. 2007). Values were compared to those created by T. Hooper (pers. communication, Hooper and Parrish 2009) to ensure reliability, as using the chromatograph-flame ionization detection for larval fish is a novel method.

2.2.4 Nucleic acid processing

Animals to be used for RNA/DNA analysis were individually flash frozen in liquid nitrogen and stored at -80°C. Nucleic acids were extracted using a 1% sarkosyl Tris-EDTA buffer (Trizma Base, HCl, EDTA) and then quantified using a 1-dye/1 enzyme protocol. Ethidium bromide (ISC Bioexpress) was added and the fluorescence measured for total nucleic acids, RNase (Sigma Chemical Company) was added and the fluorescence measured for deoxyribonucleic acids (DNA), and finally DNase (Sigma Chemical Company) was added and the fluorescence measured to verify that the resulting fluorescence equaled that of daily blank samples. Serial dilutions of rRNA from calf liver (18S and 28S) (Sigma Chemical Company) and DNA from calf thymus (Sigma Chemical Company) were used to create a calibration curve from which nucleic acid concentrations were estimated. Technique repeatability was quantified using a sample of homogenate divided amongst all sampling events to a coefficient of variaiblty of 1.85% (DNA) and 1.00% (RNA), well within the expected accuracy (7.62% and 4.21%, respectively, reported by Clemmesen 1993).

2.2.5 Histology

Larvae for histological analysis were preserved in Bouin's solution, dehydrated in an ethanol series of 30%, 50%, 70%, 80%, 95% and 100% for 60 minutes at each concentration, with the final concentration of 100% repeated. Samples were cleared using three 1-h washes of Hemo-de® (Scientific Safety Solvents, TX, USA) and infiltrated in two 1-h baths of hard paraffin (melt point of 60-68 °C) before being embedded.

Specimens were longitudinally-sectioned in a 5 μm series and stained with haematoxylin and eosin by AML laboratories (Rosedale MD, USA). Three slides for each larva were analysed using Matrox Inspector 3.0 image analysis software on images captured using an Olympus Q-Color-5 camera. The number of gill arches, the thickness of the intestinal epithelium, and the area of the liver were measured 3 times on 3 different slides and an average of these values established for each larva.

2.2.6 Ossification

Processing of samples for ossification analysis followed Campbell (1986). Larvae were initially preserved in a 10% formalin solution. The samples were dehydrated in 95% ethanol for two 24-h periods, stained with Alcian blue for 8 h and then neutralised in borax. Samples were bleached using a 3% hydrogen peroxide solution and subsequently neutralised again in borax. A trypsin enzyme-buffered solution was used to clear the specimens, which were then processed through an alcohol hydroxide solution series followed by Alizarin Red S staining. Final preparation of the samples was accomplished using a series of glycerin solutions (40%, 70%) and finally storage in 100% glycerin. The resulting structures were then colourimetrically quantified for red and blue using the histogram features of Matrox Inspector 3.0 image analysis software.

2.2.7 Data analysis

A dry mass-length relationship was developed for each species to allow comparison for researchers who prefer to use dry mass (for details see Appendix 2.4).

Using these relationships, dry mass was estimated from body length for samples for which processing precluded its estimation.

Species-independent model

The first question addressed was the potential for a morphometric variable to predict all state variables across all species. The best morphometric was defined as possessing the least variation among species and state variables in the style of Fuiman et al. (1998). To allow for this comparison, variation associated with each state variable was standardised by regressing each morphometric (e.g. total length) on each state variable (e.g. carbon). The variance of the residuals for each morphometric and state variable combination was calculated. Then all residuals pertaining to a given state variable were pooled and the total variance for that state variable (e.g. carbon total residual variance) estimated. Using these standardised variances for each morphometric/state variable combination, the coefficient of variation (CV = s.d./mean x 100) was calculated.

The second species-independent investigation aimed to determine which morphometric variable predicts each state variable with the least variation across species. Using the unstandardised residuals derived in the first analysis, the best predictor morphometric was selected as the morphometric that showed the least variance of residuals. Pairwise contrasts adjusted for multiple comparisons (*F*-tests) were applied to determine significant differences among variances.

To verify the validity of a species-independent model, an ANOVA (analysis of variance) was performed for each combination of morphometric and state variable on the unstandardised residuals.

Species-specific models

Once species-independent models were examined, species-specific models were explored. The first two analyses outlined above were repeated for each species for which there was sufficient information. A species-specific regression for each combination of morphometric and state variables was calculated and the subsequent residuals used to perform the same analyses to determine the best universal morphometric within each species and the best morphometric to predict each state variable.

Natural logarithmic transformation resulted in an approximate error-normal distribution of residuals when a linear relationship was estimated between the various morphometric and state variables, except in the case of gill arch count, carbon, and nitrogen. All analyses were repeated with and without gill arch count, which did not alter the conclusions. Carbon and nitrogen were ln-transformed for the analyses across state variables (analyses one and three) to allow for consistent comparisons, but for analysis within carbon and nitrogen (analyses two and four) the data were not transformed. Morphmetric values overlapped among species and five species (*G. morhua*, *M. scorpius*, *C. lumpus*, *P. americanus*, *U. subbifurcata*) were sampled successfully from hatch to beyond metamorphosis and provided sufficient information for species-specific analysis in addition to species-independent analysis. All species were included in the species-independent models.

2.3 **Results**

2.3.1 *General data description*

The morphometric variables of total length, dry mass, head length, head height, eye diameter, body depth at pectoral fin, and body depth at anal fin were all highly significantly correlated in a species-independent analysis ($r \geq 0.769$, $p < 0.005$; detailed in Appendix 2.5). Within species, all morphometrics were highly significantly correlated ($r \geq 0.577$, $p < 0.001$; detailed in Appendix 2.6- Appendix 2.10). Although the variables displaying the highest correlations differed among species, the lowest correlation between morphometrics was always for the relationship of body depth at the pectoral fin with another variable: with head height in *G. morhua* (0.863) and *M. scorpius* (0.577), and with head length in *C. lumpus* (0.879), *P. americanus* (0.782) and *U. subbifurcata* (0.868), although all of these values were still highly significant. Despite the high level of correlation among all morphometrics, it appears that body depth at pectoral fin changes in a slightly different manner relative to other variables.

2.3.2 *Species-independent patterns*

The first question addressed was which morphometric variable showed the lowest coefficient of variation across all state variables in a species-independent analysis. Using all the data, functional regressions of each state variable in relation to each morphometric, irrespective of species, were performed and the residuals retained for further analysis. All relationships were significant at ≤ 0.05, except for those between total length and both liver area and ossification, where the p-values were 0.107 and 0.472, respectively (Table 2.1). The highest r^2 value of 0.888 was observed between carbon and dry mass, even

though the log-transformation was not ideal for this relationship. The lowest explained variances were observed between ossification and total length, with a r^2 of 0.002. Based on this criterion, body depth at the pectoral fin showed the least variation among all state variables with a coefficient of variation of 7.44% (Fig. 2.1). The standardised variance of the residuals of each state variable/morphometric combination illustrates the wide distribution of variances for each morphometric (Fig. 2.2).

The second investigation aimed to determine which morphometric variable predicts each state variable with the least variation across species. While most morphometrics provide a significant relationship with state variables, the quality of fit and the variable explaining the most variance was different depending on the state variable investigated (Fig. 2.3). Body depth measures at the pectoral and anal fin performed best for six of the state variables (intestinal epithelial thickness, ossification and intermediate metabolite lipids; RNA, storage lipids and gill arches), but overall the best morphometric is dependent on the state variable under investigation.

There was a significant species effect on the relationship between morphometrics and most state variables, with a few exceptions (carbon in relation to dry mass, liver area in relation to both eye diameter and total length, gill arches in relation to all morphometric variables, and intestinal epithelial thickness in relation to all morphometrics except head length and total length) (Table 2.2, Fig. 2.4). The residuals of the species-independent model were not normally distributed, further suggesting that a species-independent model is not ideal.

2.3.3 *Species-specific patterns*

Gadus morhua

In the case of *G. morhua*, eye diameter showed the lowest coefficient of variation across all state variables (7.51%; Fig. 2.1). For the state variables gill arch count, intestinal epithelial thickness, liver area, metabolic intermediate lipids, ossification, RNA and storage lipids, there were no significant differences between the variances associated with each morphometric (Fig. 2.1, detailed in Appendix 2.11). Although eye diameter was the best predictor across all state variables, no single morphometric consistently predicted better when each state variable was examined individually.

Myoxocephalus scorpius

For *M. scorpius*, dry mass (5.44%) and total length (5.44%) showed the lowest coefficient of variation across all state variables (Fig. 2.1). Body depth at the anal fin insertion showed the lowest variance of residuals for intermediate metabolite lipids, storage lipids, structural lipids, ossification and RNA, although body depth at anal fin was not significantly better than any other morphometric except for ossication (detailed in Appendix 2.12).

Cyclopterus lumpus

Head height was the morphometric with the lowest coefficient of variation across all state variables for *C. lumpus* (8.61%; Fig. 2.1). This pattern was repeated frequently when state variables were examined individually (detailed in Appendix 2.13). For

example, storage lipids and RNA had the lowest variance of residuals with body depth at anal fin, and ossification and structural lipids with body depth at pectoral fin. Body depth measures (i.e. head height, body depth at anal fin or body depth at the pectoral fin) showed the least variance of residuals with all state variables except intestinal epithelial thickness.

Pseudopleuronectes americanus

Across all state variables, body depth at pectoral fin showed the lowest coefficient of variation (18.7%) for *P. americanus* (Fig. 2.1, detailed in Appendix 2.14). There were no significantly lower variances observed in *P. americanus* with the exception that eye diameter performed better than body depth at pectoral fin and head length when related to structural lipids. Despite this, there was considerable variability in the morphometric that best explained each state variable, with head length, gill arches, eye diameter, body depth at anal fin, dry mass, total length and body depth pectoral also representing the lowest coefficient of variation for specific state variables.

Ulvaria subbifurcata

As with *P. americanus*, body depth at pectoral fin was the morphometric which showed the lowest coefficient of variation across all state variables in *U. subbifurcata* (12.1%; Fig. 2.1, detailed in Appendix 2.15). There were no significant differences in residual variances among morphometric variables detected for DNA, RNA, gill arch count, intestinal epithelial thickness, liver area, ossification, metabolic intermediate lipids, storage lipids, or structural lipids ($p > 0.05$; detailed in Appendix 2.15). Although body

depth at pectoral fin had the lowest coefficient of variation across state variables, at the level of individual state variables differing morphometrics performed best.

2.4 **Discussion**

2.4.1 *Multispecies*

From a utilitarian perspective, body depth at the pectoral fin is the best multi-species developmental morphometric as it shows the greatest explanatory power across species and state variables. This morphometric was included in this study because of its historical relevance to condition indices (for reviews, see Ferron and Leggett 1994, Shulman and Love 1999). A condition index is defined by Shulman and Love (1999) as a measure that effectively represents the state of an organism; has a similar range as the process examined; is representative of the population and can be easily determined under field conditions. Similarly, Lochmann and Ludwig (2003) found that while using 19 measures of body size together provides the most accurate measure of the condition of young fish, using body depth at pectoral fin alone provides nearly as good an approximation. Moreover, where the relationship between body depth and ontogeny has been examined, body depth was the least variable morphometric across differing temperatures (Koumoundourous et al. 2001).

When each state variable was examined in the multi-species analysis, no single morphometric best explained all state variables. For example, measures of condition, such as nucleic acids, metabolic intermediate lipids and storage lipids, were frequently best explained by measures of body depth (i.e. depth at the pectoral fin, head depth and depth

at anal fin) while carbon and nitrogen were best explained by dry mass in most cases. The latter is consistent with other studies and reflective of the elemental nature of carbon and nitrogen and their link to body mass (e.g. Gnaiger and Bitterlich 1984). Less clear were the histological and ossification measures, which showed no significant pattern as to which morphometric performed better than others. Histological and ossification studies of development are normally qualitative rather than quantitative (Baglole et al. 1997, Hernandez et al. 2001), so this quantitative assessment is novel and difficult to compare to previous results. Histological and ossification measures were much better explained when species were examined individually (see species-specific). For all state variables, except carbon and intestinal epithelial thickness, there was a significant effect of species on the morphometric relationships. These different relationships between morphometrics and state variables among species handicap attempts to develop a single, good measure of internal state for fish larvae, and reinforce the need for multi-species studies to understand species-specific differences.

2.4.2 *Species-specific*

When species were examined individually, a measure of body depth was the best morphometric for using across multiple state variables in two of the five species which were sampled from hatch to metamorphosis: *P. americanus* and *U. subbifurcata*. Body depth at pectoral fin also performed well in *C. lumpus*. With more detailed examination of each state variable within each species, *G. morhua*, *C. lumpus*, and *M. scorpius* shared similarities in the relationships between morphometrics and carbon, nitrogen, storage lipids, RNA, and intestinal epithelial thickness. These same three species showed carbon

and nitrogen were best estimated by dry mass and storage lipids, RNA and DNA were all best described in these species by a measure of body depth. Indices of organ development were most strongly related to a measure of head size in species-specific investigations, potentially linking organ development to increased complexity rather than simply increased energy reserves, or alternatively representing a link between gape size (which often limits prey size ingestion, Pepin and Penney 1997) and digestive tract functioning. In four out of five species (excluding *P. americanus*) DNA was represented best by body depth at either the pectoral or anal fin. *Pseudopleuronectes americanus* showed the most distinct relationships between morphometrics and state. Apart from being the only flatfish that was sampled from hatch to metamorphosis, *P. americanus* was also the only species documented to feed on bivalve larvae (Pepin and Penney 1997). As flatfish experience a unique developmental transformation, whereby the head rotates and body orientation shifts 90 degrees, this may be why it shares so few morphometric similarities with the other species studied here.

2.4.3 *Caveats*

The species included in this study have different natural diets (Pepin and Penney 1997), temperature ranges (Buckley 1989, Walsh 1992), and some were collected from the wild (natural spawning) while others were from artificial crosses. However, these confounding factors do not align with the groupings observed. Rather, these similarities in relationships between state and morphometry likely represent a fundamental link between morphometry and state. This is consistent with the literature, in which lipids and nucleic acids are considered indicators of condition, which can also be estimated as a ratio of

body depth or weight to length (for reviews, see Ferron and Leggett 1994, Shulman and Love 1999). Comparison with literature suggests that the trait values reported here and in following chapters are reliable, and that the fish were growing well although survival was not recorded. RNA/DNA values were consistent with those reported for *U. subbifurcata* and *P. americanus* (Pepin et al. 1999), and comparison of data from *U. subbifurcata* reared in the laboratory with wild-collected individuals showed no significant differences in the slopes or intercepts of any functional groups of lipids, with the exception of the intercept for the intermediate metabolites (a highly variable group). The major components of structural and storage lipids, sterols and mid-length triacylglycerols generally increased as expected (Desvilettes et al. 1997), indicating that the animals' nutritional needs were being met (Fraser 1989) and further supporting the conclusion that the values reported here are representative of normally developing larvae.

Some limitations and potential sources of error were unavoidable in this study. The non-linearity of the gill arch count is a result of the nature of the metric, but when the analyses were repeated without gill arch count the results were unchanged. Body depth at pectoral may have performed the best as a common metric among species because it was least responsive to differences in individual species' temperature-dependent development, as the species in the present study occur naturally across a range of temperatures, but were reared at a common temperature. Temperature, among other external factors, is known to affect phenotype (Fuiman et al. 1998, Jaworski and Kamler 2002, Georgakopoulou et al. 2007). By rearing the larvae in the laboratory the goal was to minimise the effect of uncontrolled environmental variables to allow a clear assessment of multi-species similarities in the relationships of morphometric and state variables,

independent of environmental stochasticity. Environmental effects on wild larvae would likely serve to increase the differences among species.

2.4.4 *Conclusions*

Morphometrics were highly correlated with each other within a species, though body depth at pectoral fin was consistently the least related to the other morphometrics when each of the five species sampled from hatch to metamorphosis were examined independently. The strong correlation among the morphometrics in each species makes it tempting to disregard the importance of selecting the correct morphometric. However, I was looking specifically for the morphometric which accounts for the most variation in the data and has the least variation between species. Subtle differences in correlation can be meaningful as small errors at this early stage in data collection decrease confidence in the results (e.g. Portt and Balon 1984, Pepin et al. 2001). Studies of morphometric allometry document the discord among different measures of body shape, and the change in body shape during the larval period is well documented (e.g. Gisbert and Williot 2002, Peck et al. 2005, Kouttouki et al. 2006). The finding that all morphometrics are not equal is consistent with previous work and emphasizes the importance of morphometric selection for accurately describing the state of the larvae, even when different morphometric measures are highly correlated (see Ferron and Leggett 1994 for review).

In contrast with Fuiman et al. (1998), total length was consistently the poorest predictor of state variables relative to other morphometrics. This is particularly relevant to the ongoing debate between using mass and using length in the study of larval fish. Measuring dry mass is problematic on small specimens and can preclude the

measurement of many other variables. For example, total lipid and dry mass cannot be measured for the same larvae. Here I used simultaneously sampled larvae to create a species and study specific length-mass relationship, which was then applied to estimate dry mass based on the images taken of each larvae used for each analytical procedure. The results suggest that the transformation of length measurements to dry mass improves the estimation of the internal state of larval fish across species, even though direct measurements were not available for all state variables measured.

Body depth at the pectoral fin performed better for "among species" analysis, and "within species" measures of body depth or mass always outperformed longitudinal measures. However, the most precise morphometric predictor was not the same among species or state variables, suggesting that the desired internal state variable and the species should guide the selection of the most appropriate morphometric proxy. Because of the species- and variable-specific nature of the morphometric required to characterize larval fish, the best path for increasing efficiency, decreasing costs, and decreasing mortalities in species-specific studies might be the suggestion of Ferron and Leggett (1994) to subsample the larvae for the desired internal state variable. This creates an environment, study and species-specific relationship for the proxy. However, for inter-specific comparisons this is not practical. As Fuiman et al. (1998) stated, "A clear recommendation for inter-specific comparisons is more problematic." In this study, I removed the confusion surrounding the terms "development" and "ontogeny" by limiting the analysis to practical state measures and expanded the analysis to include different morphometrics than previously considered, yet retained the objective of simple, convenient and repeatable measures. This study suggests that using measures of body

depth to estimate the internal state of larvae may allow better inter-specific comparisons than other morphometrics.

2.5 Tables and figures

Table 2.1 r^2 and p values of species-independent relationships between morphometrics and state variables. All variables were ln-transformed. For each state variable the highest r^2 value is shaded and the lowest r^2 value is underlined.

	Dry mass	Total length	Head length	Head height	Eye diameter	Body depth (pectoral fin)	Body depth (anal fin)
Carbon	0.888	0.569	0.815	0.863	0.830	0.845	0.879
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Nitrogen	0.438	0.572	0.527	0.471	0.567	0.503	0.396
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Structural lipids	0.747	0.488	0.766	0.775	0.724	0.748	0.773
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Storage lipids	0.546	0.272	0.543	0.567	0.460	0.539	0.629
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Intermediate metabolite lipids	0.317	0.232	0.315	0.316	0.222	0.339	0.280
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
DNA	0.798	0.633	0.848	0.835	0.843	0.815	0.844
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RNA	0.217	0.103	0.269	0.256	0.237	0.288	0.193
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Gill arches	0.857	0.548	0.734	0.813	0.767	0.799	0.780
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Intestinal epithelial thickness	0.831	0.687	0.832	0.847	0.859	0.830	0.877
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Liver area	0.061	0.032	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	0.024	0.107	0.007	0.018	0.017	0.006	0.003
Ossification	0.082	[illegible]	0.046	0.092	0.049	0.148	0.064
	<0.001	0.472	<0.001	<0.001	<0.001	<0.001	<0.001

Table 2.2 Summary of ANOVA of residuals generated by the general model analysed for species effect. The degrees of freedom listed below each state variable are for the species effect. Each cell lists the sum of squares, Fisher's *F*-statistic, p-value (in bold), error degrees of freedom, and error sum of squares.

	Dry mass	Total Length	Head length	Head height	Eye diameter	Body depth (pectoral fin)	Body depth (anal fin)
Carbon	10.57	587.41	121.31	27.85	93.47	38.45	88.22
(DF = 8)	1.19	67.75	11.85	2.71	9.77	3.78	9.43
	0.30	**<0.001**	**<0.001**	**0.01**	**<0.001**	**<0.001**	**<0.001**
	367	362	362	362	367	362	362
	406.54	392.33	463.09	464.23	438.93	460.10	423.44
Nitrogen	17.66	692.51	131.71	18.88	148.05	58.98	46.33
(DF = 8)	4.61	210.15	26.71	3.78	41.26	13.43	12.52
	<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	377	372	372	372	377	372	372
	180.68	153.23	229.29	232.02	169.09	204.22	172.03
Structural	269.96	1055.75	189.89	176.11	377.42	222.86	183.61
lipids	14.28	57.35	9.74	9.09	23.86	10.96	9.53
(DF = 8)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	239	235	235	235	232	235	235
	564.85	540.75	572.62	569.04	458.79	597.24	565.96
Storage	633.34	1612.98	603.26	00.10	904.94	614.08	377.88
Lipids	17.05	43.21	15.49	12.64	25.43	15.75	10.55
(DF = 8)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	213	210	210	210	208	210	210
	988.74	979.83	1022.15	1038.75	925.11	1023.51	939.84
Metabolic	842.06	1085.30	629.26	691.62	736.87	612.18	835.27
intermediate	20.14	25.84	14.24	16.04	16.98	14.15	19.45
lipids	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
(DF = 8)	212	210	210	210	209	210	210
	1108.05	1102.60	1160.07	1132.00	1133.59	1136.05	1127.24
DNA	81.05	346.51	52.11	73.87	55.33	93.07	77.66
(DF = 8)	11.95	59.68	9.37	13.69	10.58	16.08	16.05
	<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	246	243	244	243	241	244	242
	208.61	176.36	169.63	163.88	157.55	176.48	146.33
RNA	49.21	292.24	72.12	67.12	29.94	76.46	63.34
(DF = 8)	6.33	43.18	10.54	10.15	4.60	11.47	12.59
	<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	249	246	247	246	244	247	245
	242.09	208.09	211.31	203.27	198.62	205.80	154.03

Table 2.2 (cont.)

	Dry mass	Total Length	Head length	Head height	Eye diameter	Body depth (pectoral fin)	Body depth (anal fin)
Gill arch	0.16	0.23	0.10	0.10	0.24	0.15	0.10
count	0.91	1.21	0.55	0.51	1.34	0.84	0.55
(DF = 4)	**0.46**	**0.31**	**0.70**	**0.73**	**0.26**	**0.50**	**0.70**
	81	79	79	79	81	79	79
	3.66	3.70	3.60	3.69	3.58	3.59	3.58
Intestinal	1.41	6.68	.68	2.81	3.31	1.64	3.26
epithelial	1.06	5.23	3.85	2.17	2.30	1.26	2.40
thickness	**0.38**	**<0.001**	**0.01**	**0.08**	**0.06**	**0.29**	**0.06**
(DF = 4)	90	88	88	88	90	88	88
	29.42	28.10	26.74	28.56	32.37	28.69	29.86
Liver area	27.72	6.25	14.60	28.72	7.29	22.25	33.47
(DF = 3)	8.79	2.57	6.15	13.37	2.29	10.05	13.81
	<0.001	**0.06**	**<0.001**	**<0.001**	**0.07**	**<0.001**	**<0.001**
	74	73	73	73	74	73	73
	58.32	59.31	57.77	52.27	58.99	53.88	58.97
Ossification	0.24	0.47	0.32	0.20	0.31	0.14	0.25
(DF = 6)	13.16	28.16	17.71	10.79	17.41	7.67	13.21
	<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	345	340	340	340	345	340	340
	1.06	0.94	1.02	1.07	1.04	1.06	1.07

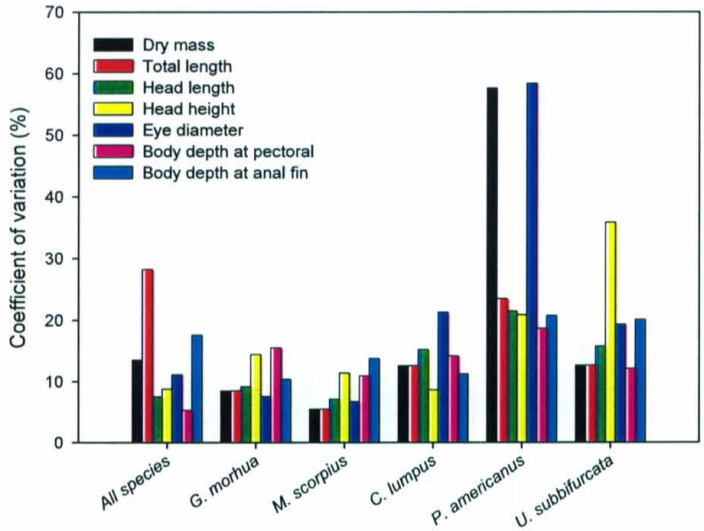

Figure 2.1 Coefficient of variation for each morphometric in the species-independent model ("All species") and individual species. The coefficient of variation is the amount of variability each morphometric shows across all state variables. High values indicate a measure which is not likely to accurately predict all the internal state variables measured in this study.

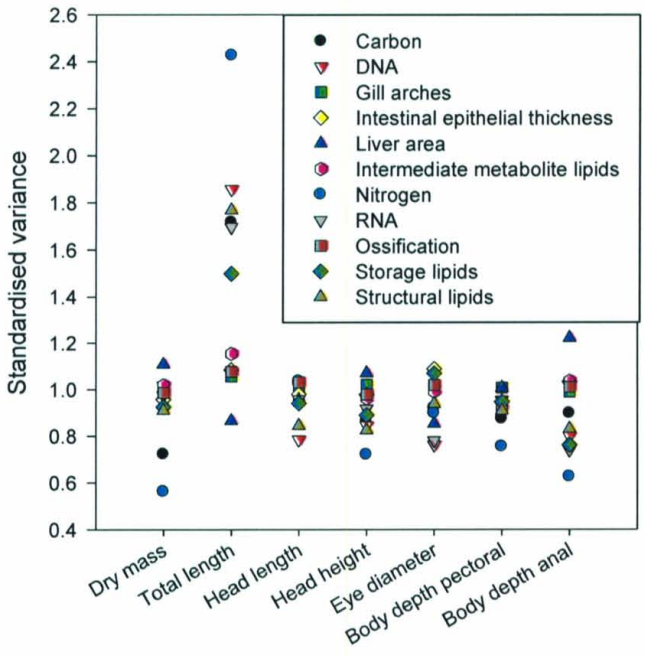

Figure 2.2 Standardised variance of residuals for each combination of state and morphometric variable across all species. Low values indicate a high proportion of variability explained between the variable indicated on the x-axis and that indicated by the symbol.

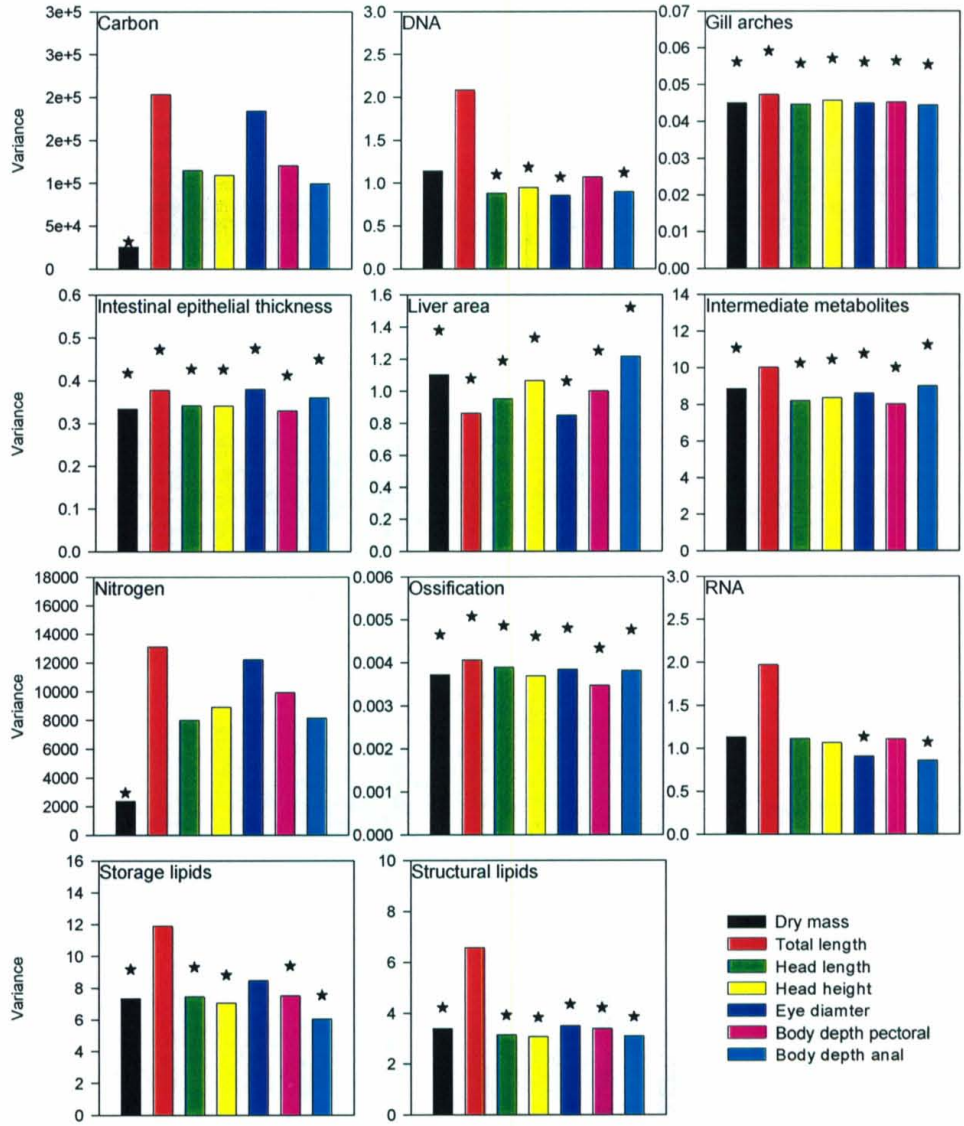

Figure 2.3 Variance in each morphometric relative to each state variable for the species independent model. The morphometrics marked with stars are not significantly different from the lowest variance based on *F*-test. Note that nitrogen and carbon were not ln-transformed (see text for explanation).

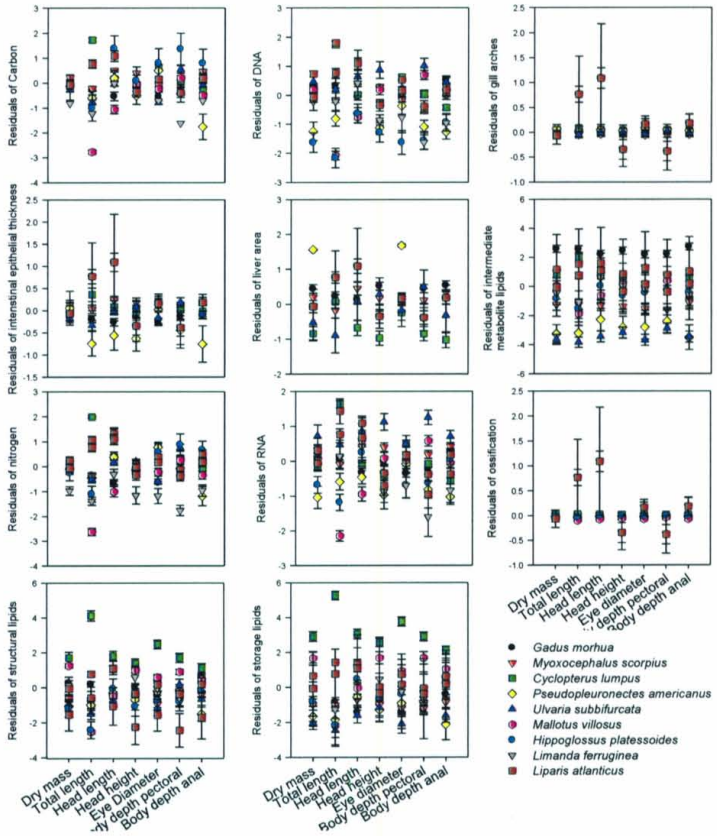

Figure 2.4 Residuals generated by the general model of the relationship between each morphometric variable and each state variable by species. Mean (symbol) and standard error (bars) shown.

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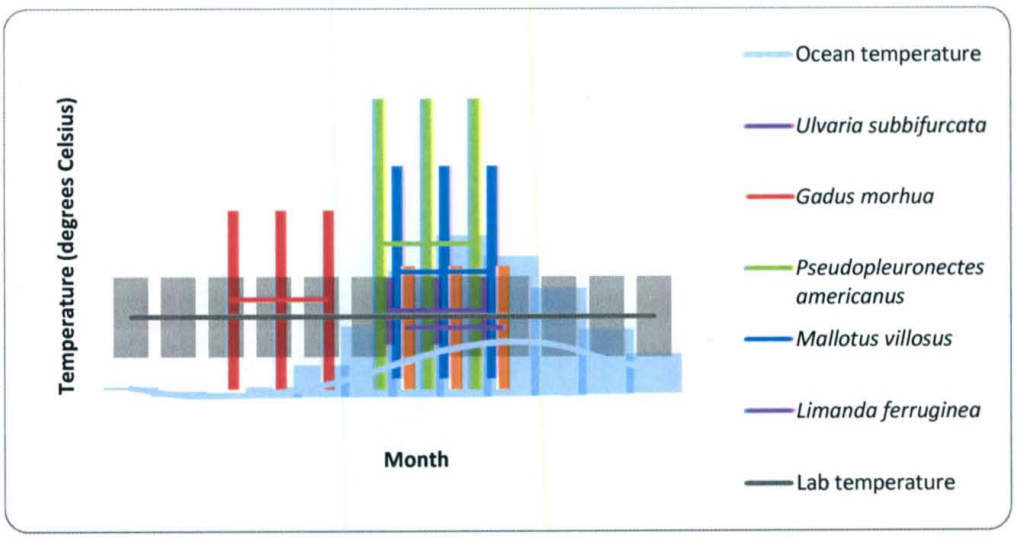
2.7 Appendices

Appendix 2.1 Rearing details. Each species was reared once.

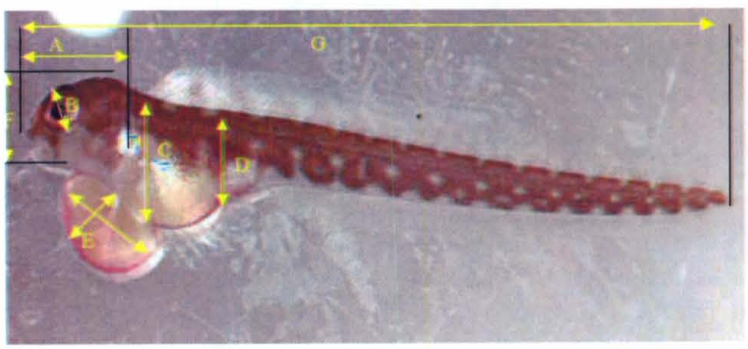
Species	Number of females	Number of males per female	Total number of males	Rearing tank details	Egg source
G. morhua	25	25(1)	25	3000-L circular tanks	Captive – natural
M. scorpius	5	n/a	n/a	130-L rectangular tanks	Wild – natural
C. lumpus	>3 (2)	n/a	n/a	130-L rectangular tanks	Wild – natural
P. americanus	7	3	9	20-L circular tanks	Captive – stripped
U. subbifurcata	6	n/a	n/a	20-L circular tanks	Wild – natural
M. villosus	n/a	n/a	n/a	20-L circular tanks	Wild – natural
H. platessoides	2	4	4	20-L circular tanks	Wild –stripped
L. ferruginea	4	2	2	20-L circular tanks	Captive – stripped
M. aenaeus	8	n/a	n/a	20-L circular tanks	Wild – natural
L. atlanticus	4	n/a	n/a	20-L circular tanks	Wild – natural

(1) Communal spawning
(2) Egg batches were broken up when received. Egg colouration indicates at least three different batches contributed, but could be more.

Appendix 2.2 Known temperature ranges of species under study in chronological order. Source: Scott and Scott 1988 (*Mallotus villosus*), Buckley 1989 (*Pseudopleuronectes americanus*), Walsh 1992 (*Limanda ferruginea*), Froese and Pauly 2007 (*Ulvaria subbifurcata, Gadus morhua*).



Appendix 2.3 Morphological measures. A. Head length B. Eye diameter C. Body height at pectoral fin D. Body height at anus E. Yolk sac volume F. Head height G. Total length



Appendix 2.4 Relationships between dry mass (mg) and length (mm) for each species of larval fish calculated using fish sampled at the same sampling event with the fish used in this study. Additional data from fish from the same population measured by Pepin, P. (unpublished) was used for species *H. plattesoides*, *L ferruginea.*, and *L. atlanticus*. Dry mass = $e^{\alpha} L^{\beta} e^{\frac{SE(\beta)^2}{2}}$ (Hayes et al. 1995, L= total length)

Species	Parameters	R^2	*p*-value	Minimum length (mm)	Maximum length (mm)
G. morhua (n= 90)	α=-6.903	0.906	<0.001	4.16	20.3
	β =2.966				
	SE(β) =0.192				
M. scorpius (n=70)	α =0906	0.669	<0.001	8.28	17.18
	β =0.906				
	SE(β) =0.3				
C. lumpus (n=66)	α =-6.11	0.840	<0.001	5.49	13.35
	β =3.151				
	SE(β) =0.169				
P. americanus (n=7)	α =-4.904	0.671	0.391	3.88	8.12
	β =1.69				
	SE(β) =0.592				
U. subbifurcata (n=10)	α =-7.379	0.791	0.030	5.91	10.36
	β =3.085				
	SE(β) =0.20				
M. villosus (n=65)	α =-8.208	0.963	0.001	3.63	6.70
	β =2.797				
	SE(β) =0.04				
H. platessoides (n=77)	α =-8.142	0.938	<0.001	2.73	24.46
	β =3.26				
	SE(β) =0.20				
L. ferruginea (n=41)	α =-7.715	0.974	<0.001	1.87	6.92
	β =3.536				
	SE(β) =0.09				
L. atlanticus (n=)	α =6.569	0.983	<0.001	1.87	13.63
	β =3.146				
	SE(β) =0.145				

Appendix 2.5 Correlation matrix of morphometric variables across all species, shown with correlation coefficient, significance, and number of samples.

	Dry mass	Total length	Head length	Head height	Eye Diameter	Body depth at pectoral fin	Body depth at anal fin
Dry mass	----	0.811	0.908	0.942	0.914	0.915	0.942
		<.001	<.001	<.001	<.001	<.001	<.001
		2211	2211	2211	2205	2211	2211
Total length	0.811	----	0.837	0.784	0.861	0.769	0.771
	<.001		<.001	<.001	<.001	<.001	<.001
	2211		2211	2211	2180	2211	2210
Head length	0.908	0.837	----	0.942	0.939	0.898	0.929
	<.001	<.001		<.001	<.001	<.001	<.001
	2211	2211		2211	2181	2212	2210
Head height	0.942	0.784	0.942	----	0.936	0.934	0.953
	<.001	<.001	<.001		<.001	<.001	<.001
	2211	2211	2211		2180	2211	2210
Eye Diameter	0.914	0.861	0.939	0.936	----	0.934	0.912
	<.001	<.001	<.001	<.001		<.001	<.001
	2205	2180	2181	2180		2181	2179
Body depth	0.915	0.769	0.898	0.934	0.934	----	0.929
at pectoral	<.001	<.001	<.001	<.001	<.001		<.001
fin	2211	2211	2212	2211	2181		2210
Body depth	0.942	0.771	0.929	0.953	0.912	0.929	----
at anal fin	<.001	<.001	<.001	<.001	<.001	<.001	
	2211	2210	2210	2210	2179	2210	

Appendix 2.6 Correlation matrix of morphometrics across *G.morhua*. (*r*, *p*, N=522)

	Dry mass	Total length	Head length	Head height	Eye Diameter	Body depth at pectoral fin	Body depth at anal fin
Dry mass	----	----	0.964	0.956	0.979	0.922	0.978
			<.0001	<.0001	<.0001	<.0001	<.0001
Total length	----	----	0.964	0.956	0.979	0.922	0.978
			<.0001	<.0001	<.0001	<.0001	<.0001
Head length	0.964	0.964	----	0.967	0.979	0.879	0.955
	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
Head height	0.956	0.956	0.967	----	0.974	0.863	0.943
	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001
Eye Diameter	0.979	0.979	0.979	0.974	----	0.911	0.972
	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
Body depth at pectoral fin	0.922	0.922	0.879	0.863	0.911	----	0.931
	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
Body depth at anal fin	0.978	0.978	0.955	0.943	0.972	0.931	----
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

Appendix 2.7 Correlation matrix of morphometrics across *M. scorpius*. (r, p, N =571)

	Dry mass	Total length	Head length	Head height	Eye Diameter	Body depth at pectoral fin	Body depth at anal fin
Dry mass			0.892	0.826	0.915	0.710	0.873
			<0.001	<0.001	<0.001	<0.001	<0.001
Total length			0.892	0.826	0.915	0.710	0.873
			<0.001	<0.001	<0.001	<0.001	<0.001
Head length	0.892	0.892		0.823	0.901	0.615	0.854
	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
Head height	0.826	0.826	0.823		0.838	0.577	0.801
	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
Eye Diameter	0.915	0.915	0.901	0.838		0.883	0.685
	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001
Body depth at pectoral fin	0.710	0.710	0.615	0.577	0.883		0.784
	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001
Body depth at anal fin	0.873	0.873	0.854	0.801	0.685	0.784	
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Appendix 2.8 Correlation matrix of morphometrics across *C. lumpus* (r, p, N =500)

	Dry mass	Total length	Head length	Head height	Eye Diameter	Body depth at pectoral fin	Body depth at anal fin
Dry mass			0.924	0.968	0.908	0.960	0.946
			<0.001	<0.001	<0.001	<0.001	<0.001
Total length			0.924	0.968	0.908	0.960	0.946
			<0.001	<0.001	<0.001	<0.001	<0.001
Head length	0.924	0.924		0.925	0.898	0.879	0.889
	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
Head height	0.968	0.968	0.925		0.918	0.964	0.940
	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
Eye Diameter	0.908	0.908	0.898	0.918		0.893	0.883
	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001
Body depth at pectoral fin	0.960	0.960	0.879	0.964	0.893		0.954
	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001
Body depth at anal fin	0.946	0.946	0.889	0.940	0.883	0.954	
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Appendix 2.9 Correlation matrix of morphometrics across *P. americanus* (r, p, N =471)

	Dry mass	Total length	Head length	Head height	Eye Diameter	Body depth at pectoral fin	Body depth at anal fin
Dry mass			0.891	0.895	0.908	0.832	0.854
			<0.001	<0.001	<0.001	<0.001	<0.001
Total length			0.891	0.895	0.854	0.832	0.854
			<0.001	<0.001	<0.001	<0.001	<0.001
Head length	0.891	0.891		0.911	0.886	0.782	0.841
	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
Head height	0.895	0.895	0.911		0.862	0.851	0.833
	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
Eye Diameter	0.908	0.854	0.886	0.862		0.818	0.846
	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001
Body depth at pectoral fin	0.832	0.832	0.782	0.851	0.818		0.837
	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001
Body depth at anal fin	0.854	0.854	0.841	0.833	0.846	0.837	
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Appendix 2.10 Correlation matrix of morphometrics across *U. subbifurcata.* (r, p, N =90)

	Dry mass	Total length	Head length	Head height	Eye Diameter	Body depth at pectoral fin	Body depth at anal fin
Dry mass			0.918	0.960	0.948	0.935	0.933
			<0.001	<0.001	<0.001	<0.001	<0.001
Total length			0.918	0.960	0.948	0.935	0.933
			<0.001	<0.001	<0.001	<0.001	<0.001
Head length	0.918	0.918		0.891	0.911	0.868	0.916
	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
Head height	0.960	0.960	0.891		0.949	0.922	0.926
	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
Eye Diameter	0.948	0.948	0.911	0.949		0.899	0.905
	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001
Body depth at pectoral fin	0.935	0.935	0.868	0.922	0.899		0.902
	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001
Body depth at anal fin	0.933	0.933	0.916	0.926	0.905	0.902	
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Appendix 2.11 Residual variance of the general models after regression of each state variable relative to each morphometric for *Gadus morhua.* All values were ln-transformed excluding those for carbon and nitrogen. Numbers in bold are not significantly different from zero when using an *F*-test. The values for dry mass and total length are the same as mass was estimated from total length, except for carbon and nitrogen where the data was not transformed (see results for explanation). Shaded blocks indicate the lowest values.

Source	Dry mass	Total length	Head length	Head height	Eye diameter	Body depth (pectoral fin)	Body depth (anal fin)
Carbon	**31309**	78384	120963	96621	94863	88489	**40319**
Nitrogen	**2600**	7877	11940	9698	9522	8894	3985
Structural lipids	**1.255**	**1.255**	1.745	2.258	**1.344**	1.783	**1.106**
Storage lipids	**3.927**	**3.927**	**4.579**	**5.058**	**4.034**	**4.195**	**3.631**
Intermediate metabolite lipids	**2.554**	**2.554**	**2.577**	**2.629**	**2.549**	**2.609**	**2.554**
DNA	**0.422**	**0.422**	**0.571**	**0.609**	**0.431**	0.811	**0.438**
RNA	**0.701**	**0.701**	**0.931**	**0.959**	**0.721**	**0.744**	**0.632**
Gill arches	**0.037**	**0.037**	**0.037**	**0.038**	**0.036**	**0.037**	**0.036**
Intestinal epithelial thickness	**0.173**	**0.173**	**0.166**	**0.172**	**0.176**	**0.180**	**0.171**
Liver area	**0.401**	**0.401**	**0.428**	**0.332**	**0.408**	**0.372**	**0.391**
Ossification	**6.24E-03**	**6.24E-03**	**6.28E-03**	**6.26E-03**	**6.27E-03**	**6.33E-03**	**5.97E-03**

Appendix 2.12 Residual variance of the general models after regression of each state variable relative to each morphometric for *Myoxocephalus scorpius*. All values were ln-transformed excluding those for carbon and nitrogen. Numbers in bold are not significantly different from zero when using an *F*-test. The values for dry mass and total length are the same as mass was estimated from total length, except for carbon and nitrogen where the data was not transformed (see results for explanation). Shaded blocks indicate the lowest values.

Source	Dry mass	Total length	Head length	Head height	Eye diameter	Body depth (pectoral fin)	Body depth (anal fin)
Carbon	**44082**	79363	66617	70889	64553	63566	**36997**
Nitrogen	**4071**	7485	5854	6524	5914	6466	**3468**
Structural lipids	**2.019**	**2.019**	**1.865**	**1.751**	**2.031**	**2.149**	**1.751**
Storage lipids	**5.294**	**5.294**	**4.925**	**5.206**	**5.098**	**4.958**	**4.567**
Intermediate metabolite lipids	**6.992**	**6.992**	**6.530**	**6.503**	**6.680**	**6.211**	**5.986**
DNA	**0.377**	**0.377**	**0.353**	**0.331**	**0.343**	0.498	**0.372**
RNA	**0.828**	**0.828**	**0.840**	**0.805**	**0.772**	**0.817**	**0.717**
Gill arches	**0.068**	**0.068**	**0.071**	**0.074**	**0.066**	**0.066**	**0.070**
Intestinal epithelial thickness	**0.270**	**0.270**	**0.234**	**0.261**	**0.276**	**0.255**	**0.253**
Liver area	**1.114**	**1.114**	**0.833**	**0.955**	**0.927**	**0.936**	**0.835**
Ossification	**1.32E-03**	**1.32E-03**	**1.30E-03**	1.61E-03	**1.20E-03**	1.45E-03	**9.82E-04**

Appendix 2.13 Residual variance of the general models after regression of each state variable relative to each morphometric for *Cyclopterus lumpus*. All values were ln-transformed excluding those for carbon and nitrogen. Numbers in bold are not significantly different from zero when using an *F*-test. The values for dry mass and total length are the same as mass was estimated from total length, except for carbon and nitrogen where the data was not transformed (see results for explanation). Shaded blocks indicate the lowest values.

Source	Dry mass	Total length	Head length	Head height	Eye diameter	Body depth (pectoral fin)	Body depth (anal fin)
Carbon	**18708**	84766	138929	94557	204294	62375	73422
Nitrogen	**826**	4001	6696	4273	10517	3221	4031
Structural lipids	**2.709**	**2.709**	**2.810**	**2.761**	**2.694**	**2.677**	**2.686**
Storage lipids	**2.727**	**2.727**	**3.183**	**2.834**	**2.960**	**2.728**	**2.531**
Intermediate metabolite lipids	**2.662**	**2.662**	**2.836**	**2.594**	**2.596**	**2.735**	**2.674**
DNA	**0.195**	**0.195**	**0.216**	**0.176**	0.323	**0.223**	**0.254**
RNA	**0.238**	**0.238**	**0.320**	**0.241**	0.468	**0.239**	**0.225**
Gill arches	**0.010**	**0.010**	**0.010**	**0.009**	**0.009**	**0.010**	**0.010**
Intestinal epithelial thickness	**0.448**	**0.448**	**0.417**	**0.436**	**0.447**	**0.437**	**0.446**
Liver area	**0.834**	**0.834**	**0.944**	**0.737**	**0.791**	**0.757**	**0.772**
Ossification	**4.53E-04**	**4.53E-04**	5.60E-04	**4.51E-04**	**5.32E-04**	**3.92E-04**	**4.24E-04**

Appendix 2.14 Residual variance of the general models after regression of each state variable relative to each morphometric for *Pseudopleuronectes americanus*. All values were ln-transformed excluding those for carbon and nitrogen. There was insufficient information to perform this analysis for liver area. Numbers in bold are not significantly different from zero when using an *F*-test. Shaded blocks indicate the lowest values.

Source	Dry mass	Total length	Head length	Head height	Eye diameter	Body depth (pectoral fin)	Body depth (anal fin)
Carbon	**1101.335**	**1202.945**	**886.467**	**774.263**	**1280.887**	**668.915**	**695.535**
Nitrogen	**79.743**	**85.009**	**61.024**	**51.843**	**82.709**	**50.04**	**49.063**
Structural lipids	**2.746**	**2.754**	**2.824**	**2.492**	**1.561**	3.047	2.845
Storage lipids	**5.718**	**6.375**	**6.578**	**6.218**	**2.867**	**6.751**	**6.634**
Intermediate metabolite lipids	**4.783**	**4.983**	**5.295**	**5.303**	**5.465**	**4.872**	**4.513**
DNA	**1.002**	**1.152**	**0.838**	**1.094**	**1.221**	**1.027**	**1.224**
RNA	**1.115**	**1.186**	**0.808**	**1.004**	**1.329**	**1.352**	**0.943**
Gill arches	**0.041**	**0.060**	**0.038**	**0.048**	**0.043**	**0.047**	**0.045**
Intestinal epithelial thickness	**0.687**	**0.133**	**0.151**	**0.150**	**0.525**	**0.151**	**0.147**
Liver area							
Ossification	**4.72E-05**	**6.13E-05**	**6.58E-05**	**6.23E-05**	**5.26E-05**	**6.24E-05**	**6.33E-05**

Appendix 2.15 Residual variance of the general models after regression of each state variable relative to each morphometric for *Ulvaria subbifurcata*. All values were ln-transformed excluding those for carbon and nitrogen. Numbers in bold are not significantly different from zero when using an *F*-test. The values for dry mass and total length are the same as mass was estimated from total length, except for carbon and nitrogen where the data was not transformed (see results for explanation). Shaded blocks indicate the lowest values.

Source	Dry mass	Total length	Head length	Head height	Eye diameter	Body depth (pectoral fin)	Body depth (anal fin)
Carbon	111.357	111.832	149.075	**30.65**	179.995	130.938	136.437
Nitrogen	**4.264**	**4.235**	**3.306**	**1.455**	**4.181**	**4.261**	**2.469**
Structural lipids	**1.541**	**1.541**	**1.744**	**1.957**	**2.217**	**1.620**	**1.697**
Storage lipids	**2.101**	**2.101**	**2.666**	**3.023**	**3.686**	**2.308**	**2.249**
Intermediate metabolite lipids	**1.646**	**1.646**	**2.014**	**1.928**	**1.967**	**1.667**	**1.989**
DNA	**0.466**	**0.466**	**0.419**	**0.469**	**0.468**	**0.439**	**0.378**
RNA	**0.248**	**0.248**	**0.213**	**0.252**	**0.261**	**0.223**	**0.159**
Gill arches	**0.041**	**0.041**	**0.041**	**0.039**	**0.035**	**0.042**	**0.042**
Intestinal epithelial thickness	**0.075**	**0.075**	**0.049**	**0.099**	**0.099**	**0.067**	**0.046**
Liver area	**1.235**	**1.235**	**1.380**	**1.114**	**1.014**	**1.107**	**1.284**
Ossification	**4.26E-04**	**4.26E-04**	**4.53E-04**	**4.43E-04**	**4.00E-04**	**4.21E-04**	**4.54E-04**

Chapter 3 Non-polar lipid class dynamics in larval fish: A comparison among species

3.1 Introduction

In many species of marine fish, a larva is unlikely to survive to metamorphosis. The larval period of marine fish presents specific challenges including transforming from an embryo to a small fish, and usually changing drastically in size. These form and size challenges require considerable energy, and lipids are important sources of energy at all life stages in fish (Sargent 1995, Wiegand 1996, Rainuzzo et al. 1997). Larval fish store energy primarily as large, high energy triacylglycerols (Cowey and Sargent 1977) ("storage lipids"), which are used either in a time of starvation or when the need for energy is in excess of intake (e.g. metamorphosis, as reviewed by Wiegand 1996). Lipids can also be used directly in the form of transitory, energetically available, small components (e.g. free fatty acids and 32 acyl carbon diacylglycerol; "intermediate metabolite lipids"; Kattner et al. 2007). Finally, some lipids, such as sterols, are used as the building material of cell membranes and other arrangements (Rainuzzo et al. 1997) ("structural lipids"). Lipid dynamics in larval fish are distinct from adult lipid dynamics, as initial reserves stored in the yolk sac reflect maternal investment (Sargent 1995). In this study, I examine whether neutral lipid dynamics in larval fish during development can be generalised among species, between closely related species, or within ecological groupings.

Generalizing among species has been proposed to predict a variety of features of larval fish (e.g. growth, mortality, metabolism, feeding) across a breadth of taxa (e.g.

Giguere and St. Pierre 1988, Miller et al. 1988, Houde 1989, Pepin 1991, Fuiman et al. 1998). While some models are successful (e.g. relationship between hatching size and development time: Miller et al. 1988), others are less successful (e.g. attempts to quantify a species independent model for the relationship between temperature and mortality in larval fish: Houde 1989, Pepin 1991). Within lipids, maternally derived energy reserves, are known to differ greatly among species (Wiegand 1996), with different utilisation rates of triacylglycerols (Rainuzzo and Jorgensen 1992), as well as of other lipids (Wiegand 1996). Extrapolation from one species to a species-independent relationship can thus be misleading.

The study of phylogeny provides an evolutionary framework for life history variation (see review by Mank and Avise 2006) and may assist to categorise larval lipid dynamics among species. For example, in a review of the effects of size and temperature on development and mortality in larval fish, Pepin (1991) noted evidence of taxonomic effects in growth and mortality rates. As larval lipid dynamics are fundamentally limited by the basic molecular properties of the compounds and the manner in which their use has evolved, fish that are closely related may have more similar lipid dynamics during development than those more distantly related.

Larval lipid dynamics may also evolve convergently among species as a response to common ecological factors, such as spawning season, egg development location, and larval development location (e.g. Mank and Avise 2006). For example, adult fish, regardless of species, accumulate more and different lipids in spring and summer than at other times of the year. This seasonal accumulation may result in differences in the amounts and forms of lipids that are maternally allocated to larvae (Conover 1992,

Sargent 1995). Additionally, larvae spawned in the late summer and fall must develop faster than larvae spawned in the spring because of a shorter growing season and higher over-wintering mortality in small animals (Conover 1992). Differences in rearing environments may also generate differing patterns of lipid dynamics. For instance, pelagic eggs and larvae are often small, with limited energy reserves and are characteristically produced in higher numbers than demersal eggs and larvae (Moyle and Cech 1988, see review Winemiller and Rose 1993). Moreover, larvae from pelagic eggs are less likely to receive parental care and more likely to have long, risky larval periods than demersal larvae (Sargent and Gross 1987, Moyle and Cech 1988, see review by Winemiller and Rose 1993). These differences could well affect maternal allocation of lipids and lipid accumulation dynamics for eggs and larvae.

In this study, a species-independent model relating dry mass to larval lipids was evaluated. Subsequently, the role of phylogeny was examined as a potential factor influencing patterns of larval lipid dynamics across fish species. Finally, I tested for an association between lipid composition of larvae and species' ecological characteristics.

3.2 **Materials and methods**

3.2.1 *Rearing and sampling*

Nine species of larval fish were reared under controlled laboratory conditions: *Gadus morhua* (Atlantic cod), *Mallotus villosus* (capelin), *Ulvaria subbifurcata* (radiated shanny), *Cyclopterus lumpus* (Atlantic lumpfish), *Pseudopleuronectes americanus* (winter flounder), *Myoxocephalus scorpius* (shorthorn sculpin), *Limanda ferruginea* (yellowtail flounder), *Liparis atlanticus* (Atlantic snailfish), and *Hippoglossus*

platessoides (American plaice). Basic ecological characteristics were compiled for each species from available literature or from direct observations (Table 3.1). For rearing, sampling and lipid analysis details see Chapter 2.

To confirm the relevance of lipid measurements from laboratory reared larvae to wild larvae, field samples of *U. subbifurcata* were collected on four occasions (July 19 and 27, and August 3 and 11, 2006) from Conception Bay, Newfoundland, Canada using a 1-m diameter plankton net towed obliquely for approximately 15 minutes. The net was rinsed and the cod-end emptied into a chilled cooler. Live larvae were immediately removed with fine forceps, placed in a Petri dish, given an overdose of phenoxyethanol and photographed using a microscope and a Canon A60 digital camera. Samples were then stored at -80°C in lipid-clean test tubes. Within 6 hours, the samples were placed in chloroform and capped under nitrogen gas for preservation until lipid analysis. Samples were then treated identically to the laboratory derived samples. Temperatures from a nearby oceanographic station indicate that water temperatures at collection ranged from 3 to 13°C across the vertical profile of the tows.

3.2.2 *Data analysis*

All variables were ln-transformed to achieve a normal error distribution. Body length was used to estimate dry mass through relationships developed with simultaneously sampled fish from each species to allow for comparison with the majority of literature available on lipids (for details see Appendix 2.4). Each larva was treated as an independent observation point to preserve variation among individuals. An analysis of covariance (ANCOVA) was performed for each lipid functional group incorporating dry

mass as a continuous variable and species as a class variable to determine whether a general model was applicable to compare lipid content and mass.

Residual means and standard errors for each species were calculated at hatching and metamorphosis (when possible) from the general model (using all species). The resultant residual patterns were then analysed for phylogenetic and ecological differences independent of body size. The measure of phylogenetic distance between each pair of species was calculated from a taxonomic tree compiled from the literature and online sources (Figure 3.1, Miya et al. 2003, Genbank). The difference between the lipid contents of pairs of species was then regressed against relatedness. To determine whether ecological factors (location of egg development in the water column, location of larval development in the water column and season of spawning) had an effect on lipid content of larval fish, the same residuals from the general species-independent regression analysis were used. Residuals of each lipid functional group (structural, storage, and intermediate metabolite lipids) were contrasted to determine if there was a significant effect of egg development location, larval development location and spawning season using a nested ANOVA. The significance level used was $p < 0.05$. To establish the efficacy of using laboratory reared larval fish as a proxy for wild larval fish, estimated dry mass was entered as a continuous explanatory variable with location (laboratory *vs* field) included as a categorical variable. The interaction term between rearing location and dry mass was also included.

For details on lipid chromatogram processing see Chapter 2. Briefly, all peaks apparent in the chromatogram were cut and the area beneath integrated. When reporting the resulting data, the following conventions were observed: when a specific compound

(e.g. 16 carbon ketone) is cited, it refers to the peak most likely to represent that compound; when percentages are reported they are of all neutral lipids detected. To determine the relationships among species, a principal components analysis was performed on the residuals of the general species-independent regression between each lipid functional group and body size, using all species. Individual lipid composition was further examined through the percentage contributions of single lipid classes (e.g. free fatty acids) to the total neutral lipid content of each larva.

3.3 **Results**

The data from the nine species covered more than 4 orders of magnitude in the amount of lipid and over 2 orders of magnitude in dry mass, representing a sizeable range for both variables (Fig. 3.2). Including the lowest (youngest) and highest (oldest) values for larvae across all species, this data set represented a change in structural lipids from 0.041 µg to 889 µg, while storage lipids and intermediate metabolites ranged from 0.002 µg to 551 µg and 0.001 µg to 57.8 µg, respectively. Dry mass ranged from 0.0274 mg to 9.36 mg based on 249 observations distributed among nine species. Data from five species exhibited sufficient overlap in their ranges of dry mass to provide a valid comparison of the lipid-dry mass slopes among species and be included in the general ANCOVA model (*G. morhua, M. scorpius, C. lumpus, P. americanus, U. subbifurcata*). For the species-independent lipid-dry mass regressions used to estimate residuals for the phylogenetic and ecological analyses, all species were included.

In the comparison of data from *U. subbifurcata* reared in the laboratory with wild-collected individuals, there were no significant differences in the slopes or intercepts of

structural or storage lipids versus dry mass (Figure 3.3, for details see Appendix 3.1). Metabolic intermediate lipids of field collected larvae were significantly higher than in the laboratory reared specimens.

3.3.1 *Species-independent patterns*

The ANCOVAs relating structural, storage and intermediate metabolite lipids to dry mass explained 85.2%, 75.0% and 65.6% of the variation, respectively, when species terms were incorporated (for details see Appendix 3.2). The effect of dry mass accounted for approximately 36% of the variance in structural lipids and 48% of the variance in storage lipids. The species intercept accounted for 5% of the variance in structural lipids and 17% of the variance in storage lipids. For metabolic intermediate lipids the partitioning of the variance was reversed, with the species term contributing 47% to the model followed by dry mass (16%). Species showed different patterns (Figure 3.2) and significant interaction terms between mass and species existed for structural lipids and intermediate metabolite lipids, as well as a significant species term for storage lipids (for details see Appendix 3.2). Visual evaluation and estimated species values (α and β, for details see Appendix 3.3) showed that there are many species which deviate from a general model in both intercept and slope.

3.3.2 *Species-specific patterns*

Each species demonstrated an increase in structural and storage lipids as mass increased ($p<0.05$), with the slope ranging from 0.472 $\mu g\ mg^{-1}$ (*C. lumpus*) to 1.76 $\mu g\ mg^{-1}$ (*P. americanus*) of lipid per mg dry mass for structural lipids, and 0.884 $\mu g\ mg^{-1}$ (*C.*

lumpus) to 1.65 μg mg^{-1} (*U. subbifurcata*) of lipid (Fig. 3.4, for details see Appendix 3.3). Both structural and storage lipids increased in similar rates relative to dry mass among G. *morhua, M. scorpius* and *P. americanus*. In *C. lumpus* and *U. subbifurcata* structural lipids increased at a greater rate than storage lipids.

Relationships for intermediate metabolite lipids versus dry mass were less consistent. The lowest slope was found in *P. americanus* (-0.860 μg intermediate metabolite lipids mg^{-1}) and the greatest slope was in *M. scorpius* (1.28 μg intermediate metabolite lipids mg^{-1}) although all species had a significant relationship between dry mass and intermediate metabolites except for *P. americanus*. Explained variances were generally low, reflecting a high degree of variability among individual larvae.

3.3.3 *Individual lipid classes*

When individual lipid classes were examined, the mean values of metabolic intermediate lipids 18 carbon free fatty acid and 22 carbon free fatty acid generally decreased from hatching towards metamorphosis, while 32 acyl carbon diacylglycerol showed very little change during development (Table 3.2). Eighteen carbon free fatty acid ranged among species from 0.6% to 48.3% of total lipids (*G. morhua* at hatching). Twenty two carbon free fatty acid was undetectable in *M. scorpius* and *C. lumpus* at metamorphosis. The highest amount of 22 carbon free fatty acid acid was observed in *L. atlanticus* at hatching, at 15.9% of total neutral lipids. A range of average contributions to total neutral lipids was observed for 32 acyl carbon diacylglycerol: from 0.1% in *P. americanus* at hatching to 22% in *L. atlanticus* at hatching, but the actual change with

increasing body mass within species was low. The largest change was a drop from 1.3% at hatching to 0.5% at metamorphosis in *U. subbifurcata.*

An examination of individual classes of structural lipids display mixed trends (Table 3.2). Sterols represented a high percentage of neutral lipids in all species, ranging from 25.7% in *L. atlanticus* at hatching to 84.8% in *P. americanus* at metamorphosis. In all five species reared to metamorphosis, sterols increased from hatching to metamorphosis, except in *C. lumpus* where the change was very small.

Storage lipids also showed a mixture of trends, with an increase in 54 acyl carbon triacylglycerol in four out of five species reared to metamorphosis, but mixed results in 48 acyl carbon triacylglycerol and 60 acyl carbon triacylglycerol (Table 3.2). Fifty-four acyl carbon triacylglycerol ranged from 2.5% in *H. platessoides* at hatching to 40.9% in *C. lumpus* at metamorphosis, and increased from hatching to metamorphosis in all species except *M. scorpius.* Forty-eight acyl carbon triacylglycerol represented a smaller fraction of neutral lipids, from a minimum 0.5% in *U. subbifurcata* at hatching to 3.4% in *M. scorpius* and *M. villosus* at hatching. Finally, 60 acyl carbon triacylglycerol ranged from being undetectable (*G. morhua*, *M. scorpius*, *U. subbifurcata* at metamorphosis) to representing 16% (*H. platessoides* at hatching).

3.3.4 *Phylogenetic analysis*

Mass-independent levels of structural lipids, storage lipids, and intermediate metabolites were independent of species phylogenetic relatedness (Fig. 3.5, for details see Appendix 3.4). The analysis included species that varied from being very closely related (e.g. 0.5, *H. platessoides* and *L. ferruginea*) to distantly related (e.g. 0.042, *M. scorpius*

and *M. villosus,* Fig. 3.1). Closely related species were not significantly more similar to one another in terms of neutral lipid levels than more distantly related species, for all three functional groups.

3.3.5 *Ecological analysis*

The relationship among structural, storage and intermediate metabolite lipids was illustrated by a principal components analysis (Fig. 3.6). The first component appears to separate out *C. lumpus* from larvae developing in pelagic environments. Principal component two appears to be well related to intermediate metabolites and spawning season, with species that spawn in the spring scoring highest and species that spawn in the summer scoring the lowest.

An analysis of covariance corroborated the PCA, with significant effects of ecological factors on most body-mass independent neutral lipid levels in larval fish. There were greater amounts of storage lipids in species with demersal eggs (Fig. 3.7), largely driven by mid-length triacylglycerols (Fig. 3.8), which became more important towards metamorphosis, although large variability obscure this observation. Intermediate metabolite lipids were more abundant in larvae coming from pelagic eggs, reflecting differences in the levels of shorter chain length free fatty acids (18 carbon free fatty acid) (Fig. 3.7, for details see Appendix 3.5). Only *C. lumpus* was consistently classified in the literature as having demersal larvae and had higher levels of structural, storage and intermediate metabolite lipids than larvae developing in a pelagic environment (Fig. 3.9). Differences between *C. lumpus* and pelagic larvae were most pronounced at hatching and did not appear to be driven by any one class of lipids (Fig. 3.10).

Larvae spawned in spring had the lowest levels of structural lipids and summer spawners had the highest levels (Fig. 3.11, for details see Appendix 3.5). Storage lipids were lowest for *M. scorpius* larvae, the only species studied that spawned in winter, and highest in summer spawned larvae. The intermediate metabolite lipids were highest in spring spawners, followed by *M. scorpius* and finally by summer spawners. The differences in lipids among spawning season were not defined by any particular lipid class (Fig. 3.12).

3.4 **Discussion**

3.4.1 *General patterns of lipid dynamics*

In contrast to studies finding significant general relationships that are little influenced by differences among species (e.g. Houde 1989), this study found significant differences among species in the initial amounts of all three lipid functional groups, potentially reflecting different maternal allocations and different embryonic strategies of development. Wiegand (1996), in a review of yolk lipids in 18 species of teleost fish, also noted "considerable variation" in initial lipid content among species, based on a comparison of wild and cultured eggs. Storage materials, where the majority of essential fatty acids are most likely located, show different levels at hatching but not in the mass-dependent accumulation rates among species. These varied initial amounts of lipids suggest that maternal effects or other pre-hatching experiences may have long term consequences for larval development. From the perspective of a general species model, this indicates, at minimum, the need to include a species term.

There were statistically significant differences among species in the rate of change in structural and intermediate metabolite lipids relative to body size, indicative of species-specific developmental strategies. *Cyclopterus lumpus* was particularly different from the other species, possibly as a result of the differing development location compared to other species in this study, but even with this species excluded significant species-specific differences remained. These results are consistent with Rainuzzo and Jorgenson (1992), who noted that a general model of larval lipid utilisation was not supported in their study of four marine fish species.

The information provided by the underlying relationship of all functional lipid groups with dry mass remains useful but species differences were large – an examination of the individual species relationships highlights this, with differences among species in the mean amounts of lipids in each larva (i.e. intercept) ranging in the hundreds of micrograms for both structural and storage lipids. The range in lipid accumulation rates among species is less extreme than in initial differences, with only microgram lipid per milligram estimated dry mass differences among species, but when dealing with animals that change in size by orders of magnitude, small differences in slopes may be biologically relevant. As a result, larval lipids cannot be considered without a species-specific term nor indiscriminately generalised without risking errors in predictions on the order of hundreds of micrograms per individual.

3.4.2 *Species-specific lipid dynamics*

Further investigation of lipid dynamics for individual species demonstrate that values for all lipid groups increased with size, except for metabolic intermediates in *P.*

americanus. Structural and storage lipids showed similar relationships to dry mass in *G. morhua, M. scorpius*, and *P. americanus*, while in *C. lumpus* and *U. subbifurcata* structural lipids increased at a greater rate than storage lipids. *Cyclopterus lumpus* begins with an extremely large amount of storage lipids and along with *U. subbifurcata* appear to be emphasising the accumulation of structural lipids, used predominantly for cellular membranes, over storage lipids. Previous work supports a relationship between structural and storage lipids (e.g. Fraser 1989, Rainuzzo et al. 1997), so this emphasis on structural lipids functionally suggests a need for rapid development, perhaps as a result of factors that relate to larval behaviour or community ecology which were not examined in this study. Although these fish varied in whether they were gathered from captive, wild, stripped or naturally spawned parents and have different recorded temperature ranges, these factors cannot explain the species groupings identified in this study.

Metabolic intermediate lipids had rates of increase with increasing mass that were similar to those of structural lipids in *M. scorpius*, *C. lumpus*, and *U. subbifurcata,* while in *G. morhua* and *P. americanus* metabolic intermediates did not appear to have any relationship with the other lipid groups. *Gadus morhua* and *P. americanus* also exhibited particularly high proportions of intermediate metabolites at hatching. Of the five species examined separately, these two had the smallest larvae. *Pseudopleuronectes americanus* was the only flatfish to be examined in detail, and it also differed from the other species it was the only fish recorded to ingest bivalve larvae as a major dietary source in a review of dietary preference (Pepin and Penney 1997). Metabolic intermediate lipids are usually a sign of either growth or the catabolism of larger lipids (Kattner et al. 2007), and given that the animals were growing well (for details see previous discussion of RNA/DNA

ratios in Chapter 2), the high proportions of intermediate metabolites are most likely indicative of high lipid synthesis rates. Although almost all lipid groups show an increase during larval development, diverse strategies of allocation existing among fish species.

3.4.3 *Phylogeny*

That taxonomically similar species differ as much as more distantly related ones is in contrast with the conclusions from studies of growth and mortality rates carried out by Houde (1989) and Pepin (1991). However, the literature surrounding the effect of phylogeny on larval fish dynamics is mixed. For example, Bochdansky and Leggett (2001) found that phylogeny had a significant, but weak, effect on the relationship between metabolism and body size. In contrast, Fiorin et al. (2007) showed different lipid allocation between liver, muscle and gonads in two sympatric, closely related species of gobies. In this study, larvae were reared in the same environment with the same food, and exhibited intrinsic species effects. Evolutionary rates are known to vary among traits (Rochet 2000) and lipid values react quickly to environment and feeding (Moyle and Cech 1988). The plasticity of lipid allocation within species may allow for a faster rate of adaptation to changing environmental conditions, diminishing the relevance of phylogeny, when contrasted with traits which have previously been shown to exhibit phylogenetic relationships, such as reproductive tactics, parental care and metabolism (Agrawal 2001).

3.4.4 *Ecology*

The patterns of lipid allocation in larval fish were influenced by ecological factors. Species with demersal eggs showed higher levels of lipids than those with pelagic eggs, except in terms of the intermediate metabolite lipids. Upon closer examination, the difference is most evident in terms of the storage components, and amount of lipid allocated overall to the larvae. This is consistent with the tendency for species with demersal eggs and larvae to receive parental care (Smith 1995) and have a lower risk of mortality, possibly as a result of the greater energetic investment by the parents, than in species which disperse their eggs in the upper water column.

Structural and storage lipid content of larval fish were also related to spawning season. Fish that spawn in the summer had the highest storage and structural lipid levels, followed by the spring spawners, while the only winter spawner (*Myoxocephalus scorpius*) had among the lowest storage lipid levels relative to body mass. This likely reflects lipid and energy availability for the adults during the spawning season and the subsequent availability for the larvae (Conover 1992). It could also be an artefact of the experimental design, as the winter species was reared in slightly warmer waters than it would normally experience and *vice versa* for the summer species. However, the temperature chosen for rearing was well within all species' temperature range, and if the summer spawners were suffering at a colder temperature it would be more likely that there would be reduced lipid accumulation (Sogard and Spencer 2004), which was not observed. The concept that ecological factors serve as driving forces in shaping biochemical composition of fish has strong support (e.g. Friedrich 1994, Hagen and Friedrich 2000, Kamler and Rakusa-Suszczewski 2001, Dantagnan et al. 2007). In this

study, ecological designations provided a framework within which larval fish lipids behaved similarly.

3.4.5 *Caveats and conclusions*

Two potential limitations must be considered in interpreting the results of this study. First, polar lipids were not analysed due to time and constraints of the novel lipid analysis method, short-column gas chromatograph (GC). This method provided a detection limit more sensitive than 0.1 ng, approximately three orders of magnitude more accurate than the conventional method of thin layer chromatography/flame ionisation detection. However, the levels of dominant polar lipid groups are known to follow the relationship between sterols and lipids closely so lipid dynamics observed here are likely representative (e.g. Lochmann and Ludwig 2003). Second, I used laboratory raised larval fish (offspring of either wild- or captive-reared parents), which were reared at similar temperatures and fed a cultivated diet. While this may not be fully representative of the natural situation, it did allow me to reduce environmental effects, such as temperature, salinity, light, and food availability which are known to affect lipid content (Dantagnan et al. 2007). As such, the results are more likely to reflect intrinsic differences among the species than those resulting from differences in environmental conditions or resulting from a bias in experimental settings. Furthermore, when I compared laboratory-reared to wild *U. subbifurcata* larvae, the only significant difference found was in intermediate metabolite lipids, which are known to be highly dependent on differences in diet or environmental temperature (Desvilettes et al. 1997). Thus, it is unlikely that either the

exclusive use of non-polar neutral lipids in analyses or the laboratory rearing of larvae would greatly affect the findings reported herein.

Values reported in this study covered a broad range consistent with the findings of other investigators and indicated the larvae were thriving. Lipids of the larval fish species studied were dominated by sterols (similar to turbot and plaice) and triacylglycerols (sterols and triacyglycerols were present in similar proportions in halibut and pike larvae) (Rainuzzo and Jorgenson 1992, Desvilettes et al. 1997). Within a single species, variability in the amount of various lipids among larvae may be more than double the species mean (Zenebe et al. 1998). Among species, total lipid values may range from 3% to 68% of dry mass (Friedrich 1994), which is consistent with the variability observed in this study. The amount of measurement error of individual larval lipid values are more difficult to establish as I applied a new lipid processing technique developed by Parrish, Yang and Hooper (Parrish et al. 1999, Hooper and Parrish 2009), however Yang et al. (1996) suggest that coefficient of variation between 0.5-8.5% for similar standards, with most compounds being <5%. The values of this study were consistent with those found by T. Hooper of *G. morhua* of the same size and reared with the same protocol (pers. comm.). As reported in Chapter 2, larval nucleic acid ratios and observed increases in structural lipids, storage lipids, sterols and mid-length triacylglycerols during development indicate that the larvae were thriving (Fraser 1989, Desvilettes et al. 1997).

As a result of the significant differences in the allocation and accumulation of different functional groups of lipid in larval fish, the conclusion that there is no species-independent model and that that ecology is more important than phylogeny in determining the lipid composition in larval fish has important consequences for broad

scale comparisons. For example, research on how one species responds to changes in lipid availability as a result of environmental changes may be irrelevant to another closely related species. That ecological differences were evident in such a small subsample of the world's ecological niches for fishes encourages further investigation across different ecosystems. Further, ecological classifications should be explored as delineators of larval strategies in other aspects, including growth rate, metabolism and developmental models. If researchers can confirm that larval fish that share ecological characteristics develop in similar manners, future research can focus on developing ecologically driven general models to further the understanding of this significant period of fish development.

3.5 Tables and figures

Table 3.1 Ecological characteristics of the species used in this study. Sources: (1) Scott and Scott, 1988; (2) Froese and Pauly 2007; (3) P. Pepin (Fisheries and Oceans Canada) unpublished data.

Species	Egg development location in the water column	Larval development location in the water column	Spawning season
G. morhua	Pelagic (2)	Planktonic (2)	Spring (1)
M. scorpius	Demersal (2)	Planktonic (2)	Winter (1)
C. lumpus	Demersal (2)	Demersal (2)	Summer (2)
P. americanus	Demersal (2)	Planktonic (2)	Summer (1)
U. subbifurcata	Demersal (2)	Planktonic (2)	Summer (1)
M. villosus	Demersal (2)	Planktonic (2)	Summer (1)
H. platessoides	Pelagic (2)	Planktonic (2)	Spring (3)
L. ferruginea	Pelagic (2)	Planktonic (2)	Summer (3)
L. atlanticus	Demersal (3)	Planktonic (3)	Spring (3)

Table 3.2 Lipid classes, as a mean proportion of each larva's total neutral lipids, among species at either hatching or metamorphosis. For each individual larva, the proportion of lipids that each lipid class comprises was measured. * indicates that value was below detection; n/a indicates that no samples were available. C_{18}FFA = 18 carbon free fatty acid, C_{22}FFA = 22 carbon free fatty acid, C_{32}DAG = 32 acyl carbon diacylglycerol, C_{16}KET = 16 carbon ketone, C19HC = 19 carbon hydrocarbon, C_{16}ALC = 16 carbon alcohol, C_{36}WE = 36 carbon wax ester, C_{27}SE = cholesterol, C_{43}SE = 43 carbon steryl ester, C_{45}SE = 45 carbon steryl ester, C_{48}TAG = 48 acyl carbon triacylglycerol, C_{54}TAG = 54 acyl carbon triacylglycerol, C_{60}TAG = 60 acyl carbon triacylglycerol

	Time of measurement	Intermediate Metabolites			Other lipid classes				Structural lipids			Storage lipids		
		C_{18}FFA	C_{22}FFA	C_{32}DAG	C_{16}KET	C_{19}HC	C_{16}ALC	C_{36}WE	C_{27}SE	C43SE	C_{45}SE	C_{48}TAG	C_{54}TAG	C_{60}TAG
G. morhua	Hatching	0.483	0.046	0.014	0.019	0.007	0.076	0.021	0.269	0.007	0.019	0.011	0.032	0.004
	Metamorphosis	0.031	0.006	0.010	0.006	0.001	0.007	0.002	0.692	0.009	0.035	0.028	0.175	*
M. scorpius	Hatching	0.031	0.001	0.006	*	0.003	0.004	0.006	0.548	0.027	0.125	0.034	0.203	0.016
	Metamorphosis	0.006	*	0.005	0.002	*	0.006	0.003	0.787	0.003	0.034	0.013	0.141	*
C. lumpus	Hatching	0.008	0.006	0.006	*	0.001	*	0.002	0.553	0.017	0.097	0.019	0.251	0.040
	Metamorphosis	0.008	*	0.004	*	0.001	0.011	0.002	0.534	0.002	0.016	0.008	0.409	0.006
P. americanus	Hatching	0.006	0.046	0.001	0.002	0.100	0.218	0.017	0.602	0.026	0.017	0.031	0.030	0.002
	Metamorphosis	0.004	0.012	0.001	0.001	0.002	0.018	*	0.848	*	0.005	0.009	0.093	0.009
U. subifurcata	Hatching	0.008	0.009	0.013	*	0.001	0.118	0.011	0.604	0.038	0.085	0.005	0.092	0.018
	Metamorphosis	*	0.003	0.005	*	*	*	0.005	0.726	0.01	0.035	0.011	0.206	*
M. villosus	Hatching	0.193	0.037	0.003	0.017	0.080	0.040	0.005	0.579	0.03	0.015	0.034	0.038	0.009
	Metamorphosis	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
H. platessoides	Hatching	0.060	0.043	0.044	0.126	0.159	0.092	0.019	0.381	0.028	0.011	0.009	0.025	0.160
	Metamorphosis	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
L. ferruginea	Hatching	0.067	0.156	0.002	0.085	0.017	0.285	0.011	0.262	0.004	0.019	0.026	0.067	0.016
	Metamorphosis	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
L. atlanticus	Hatching	0.060	0.159	0.220	0.033	0.212	0.032	0.005	0.257	0.009	0.030	0.022	0.105	0.067
	Metamorphosis	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

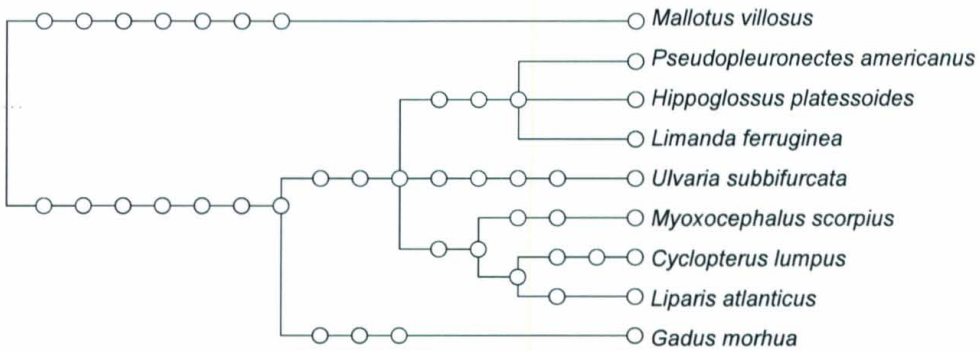


Figure 3.1 A simplified view of the phylogenetic relationship among species in this study. Each dot indicates a taxonomic branching point. Branching points were compiled from the literature (Miya et al. 2003) and online sources (Genbank). To calculate phylogenetic distance, branch length was set to zero (Harvey and Pagel 1991, Rochet 2000) and the phylogenetic distance between two species was calculated as the inverse of the number of branching events where ancestral speciation has occurred (Harvey and Pagel 1991). This scored relatedness of closely related species higher than that of more distantly related ones. With the nine species used in this study, this produced 36 independent species relatedness scores.

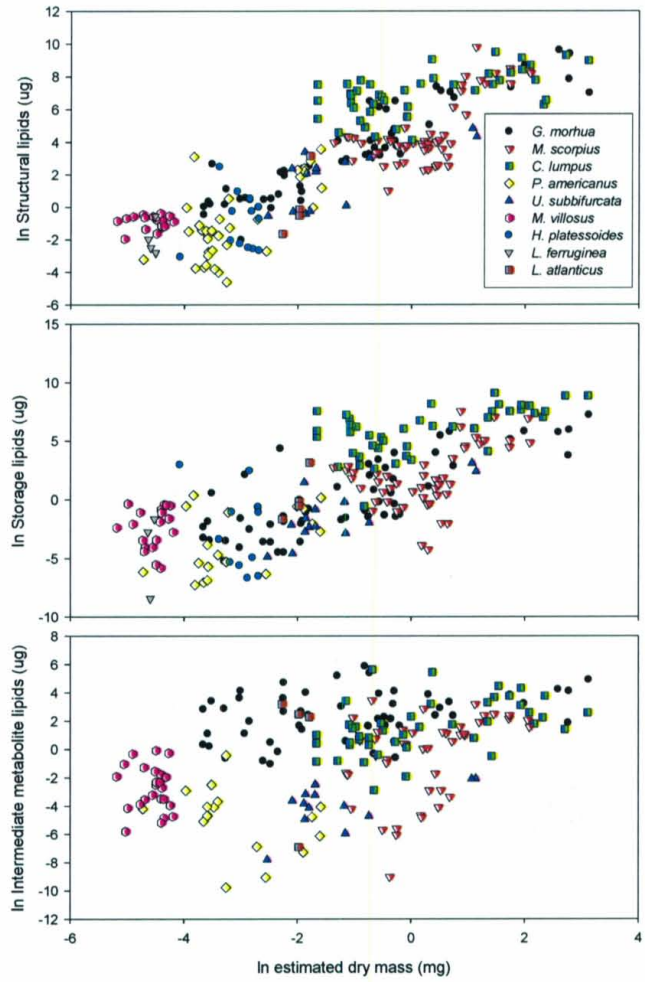


Figure 3.2 Relationship between lipid mass and estimated dry mass among larvae of the nine species used in this study. P-values for all terms (intercept, estimated dry mass, species and interaction term) were <0.001 except the interaction term between species and estimated dry mass for storage lipids and intermediate metabolite lipids (0.375 and 0.020 respectively) and the intercept for intermediate metabolite lipids (0.081). For details see Appendix 3.2.

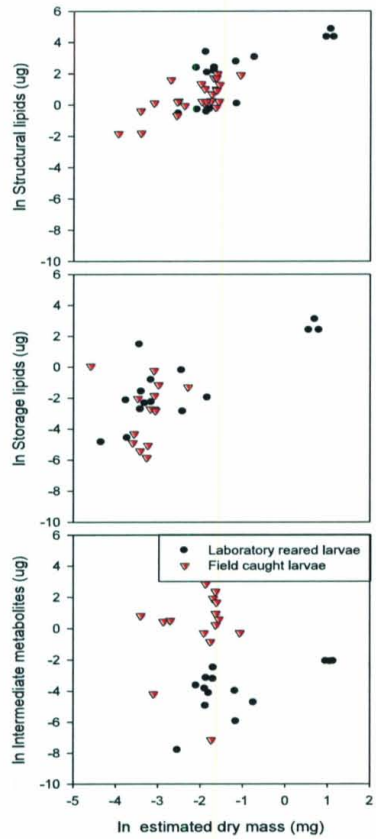


Figure 3.3 Field validation of *Ulvaria subbifurcata* lipids. Effect of rearing environment on structural lipids, storage lipids and intermediate metabolite lipids.

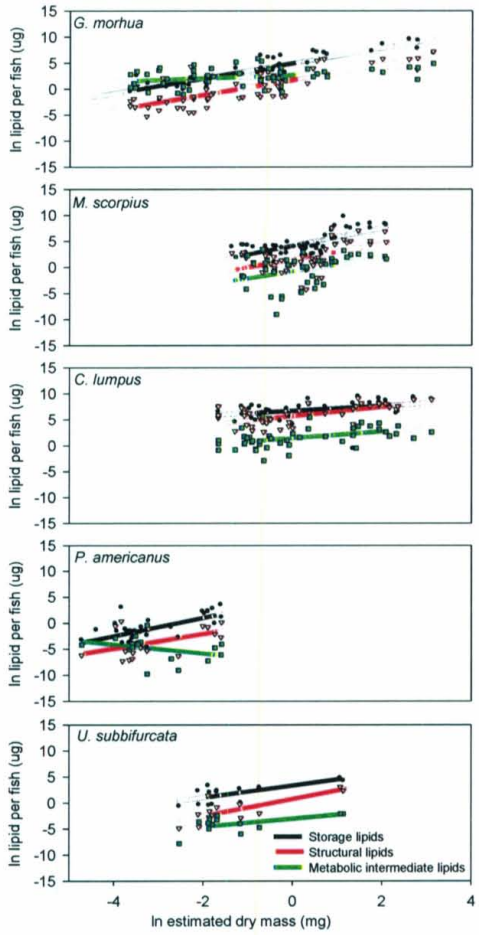


Figure 3.4 The relationships between larval estimated dry mass and the mass of three functional lipid groups for each species where information from hatching to metamorphosis was available.

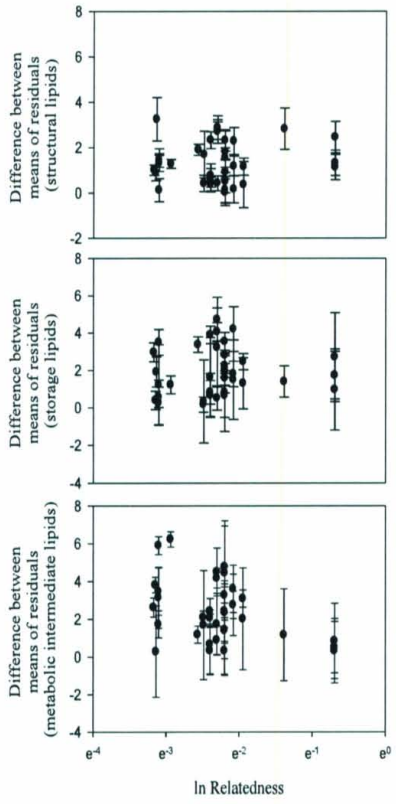


Figure 3.5 Difference between species means of residuals from the species-independent model vs phylogenetic relatedness for structural lipids, storage lipids, and intermediate metabolite lipids (error bars represent ± 1 standard error). Relatedness was calculated as the inverse of the number of branching events where ancestral speciation has occurred (Harvey and Pagel 1991). This estimated relatedness scores closely related species higher than more distantly related ones.

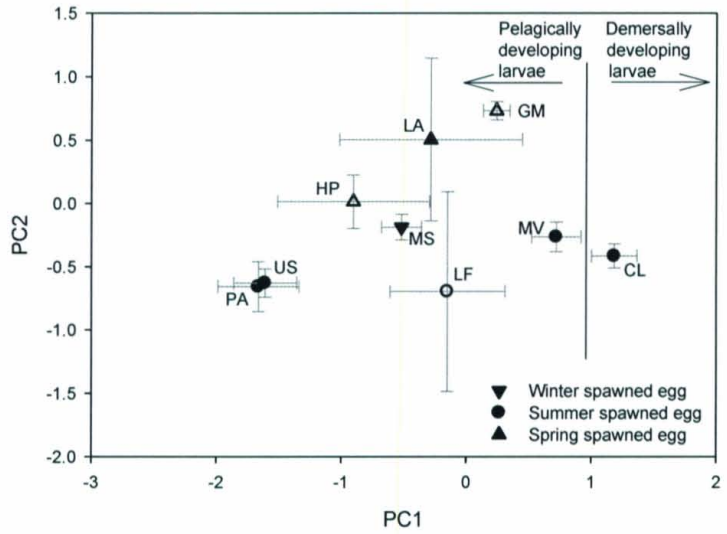


Figure 3.6 Principal components analysis of mass independent residuals of lipid classes (from regression between lipid classes and estimated dry mass). Shown are the means with standard errors. CL = *Cyclopterus lumpus,* GM = *Gadus morhua,* MS = *Myoxocephalus scorpius,* PA = *Pseudopleuronectes americanus,* US = *Ulvaria subbifurcata,* LA = *Liparis atlanticus,* LF = *Limanda ferruginea,* MV = *Mallotus villosus,* HP = *Hippoglossus platessoides.* Open symbols are pelagically developing eggs, closed symbols are demersally developing eggs. The first principal component explains 68.4% of the variation, while the second component explains 22.8%, for a cumulative total of 91.2%. The highest scores on the first principal component were structural (0.612) and storage (0.609) lipids followed by intermediate metabolites (0.505), while the highest score on the second component was intermediate metabolites (0.863), with structural and storage lipids scoring -0.342 and -0.372 respectively.

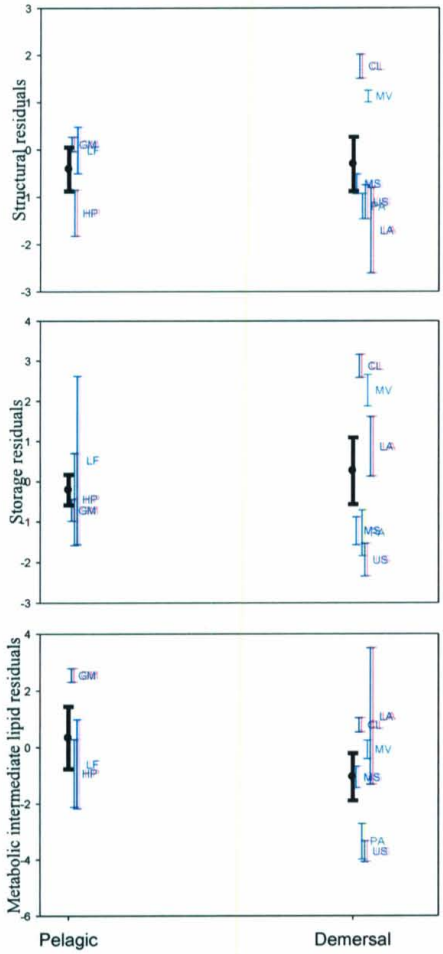


Figure 3.7 Average of species values for egg development location in the water column (shown with standard error) on structural residuals, storage residuals and intermediate metabolite residuals. Residuals derive from the species independent model. The thicker mean and error bars represent the average of all species means and error. GM = *Gadus morhua,* MS = *Myoxocephalus scorpius,* CL = *Cyclopterus lumpus,* PA = *Pseudopleuronectes americanus,* US = *Ulvaria subbifurcata,* MV = *Mallotus villosus,* HP = *Hippoglossus platessoides,* LF = *Limanda ferruginea,* LA = *Liparis atlanticus*

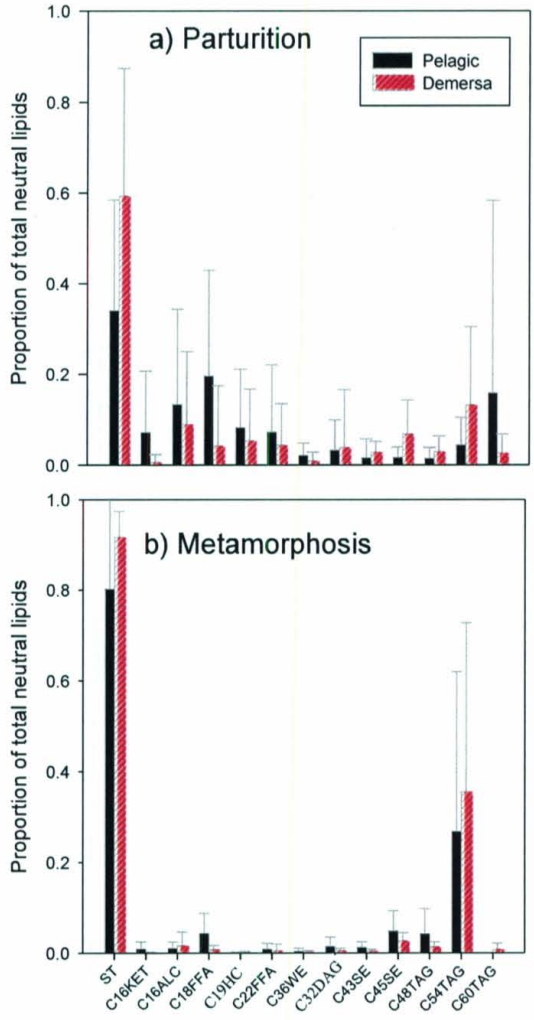


Figure 3.8 Individual larval proportion of total neutral lipids represented by each class according to egg developmental location shown with standard error among individuals at a) hatching and b) metamorphosis in pelagic and demersal eggs. ST = cholesterol, C16KET = 16 carbon ketone, C16ALC = 16 carbon alcohol, C18FFA = 18 carbon free fatty acid, C19HC = 19 carbon hydrocarbon, C22FFA = 22 carbon free fatty acid, C36WE = 36 carbon wax ester, C32DAG = 32 acyl carbon diacylglycerol, C43SE = 43 carbon steryl ester, C45SE = 45 carbon steryl ester, C48TAG = 48 acyl carbon triacylglycerol, C54TAG = 54 acyl carbon triacylglycerol, C60TAG = 60 acyl carbon triacylglycerol.

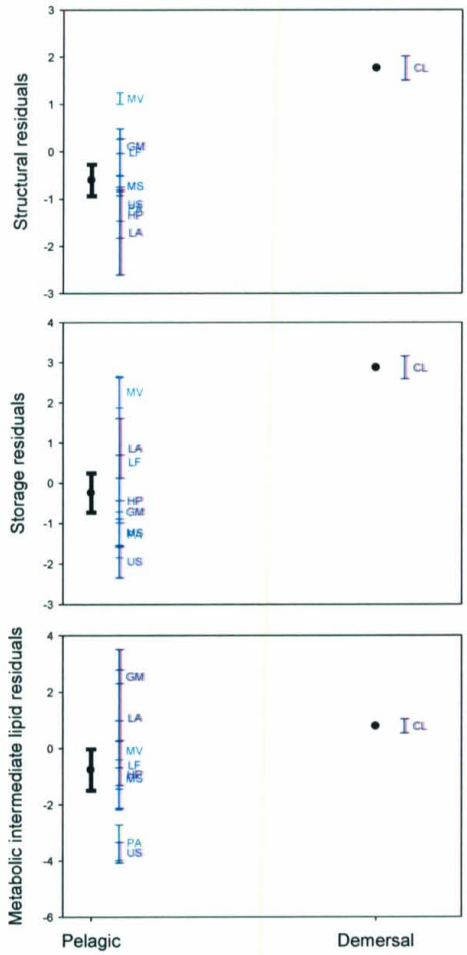


Figure 3.9 Average of species values for larval development location in the water column shown with standard error on structural residuals, storage residuals, and intermediate metabolite residuals. Residuals derive from the species independent model. The thick mean and error bars represent the average of all species means and error. GM = *Gadus morhua,* MS = *Myoxocephalus scorpius,* CL = *Cyclopterus lumpus,* PA = *Pseudopleuronectes americanus,* US = *Ulvaria subbifurcata,* MV = *Mallotus villosus,* HP = *Hippoglossus platessoides,* LF = *Limanda ferruginea,* LA = *Liparis atlanticus.*

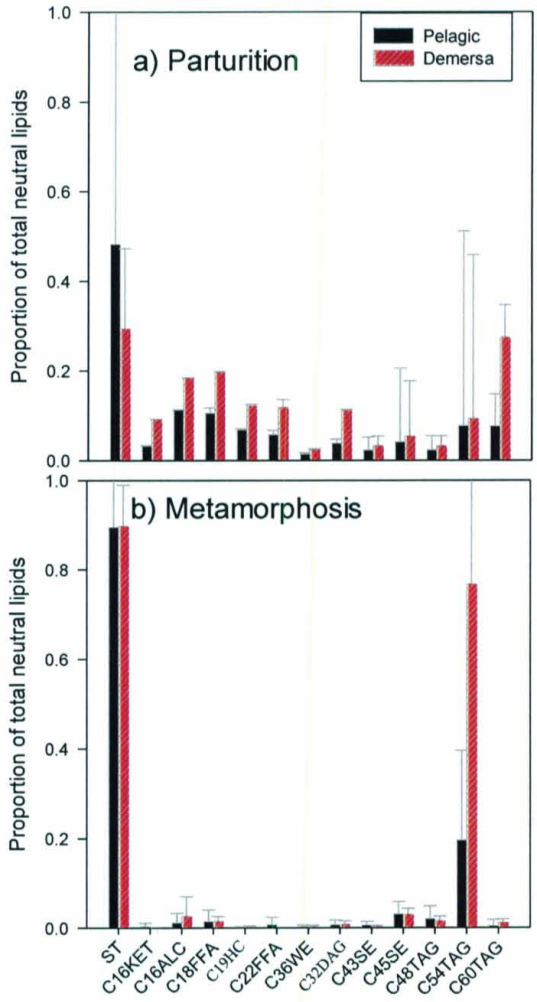


Figure 3.10 Individual larval proportion of total neutral lipids by each class according to larval development location shown with standard error at a) hatching and b) metamorphosis. ST = cholesterol, C16KET = 16 carbon ketone, C16ALC = 16 carbon alcohol, C18FFA = 18 carbon free fatty acid, C19HC = 19 carbon hydrocarbon, C22FFA = 22 carbon free fatty acid, C36WE = 36 carbon wax ester, C32DAG = 32 acyl carbon diacylglycerol, C43SE = 43 carbon steryl ester, C45SE = 45 carbon steryl ester, C48TAG = 48 acyl carbon triacylglycerol, C54TAG = 54 acyl carbon triacylglycerol, C60TAG = 60 acyl carbon triacylglycerol.

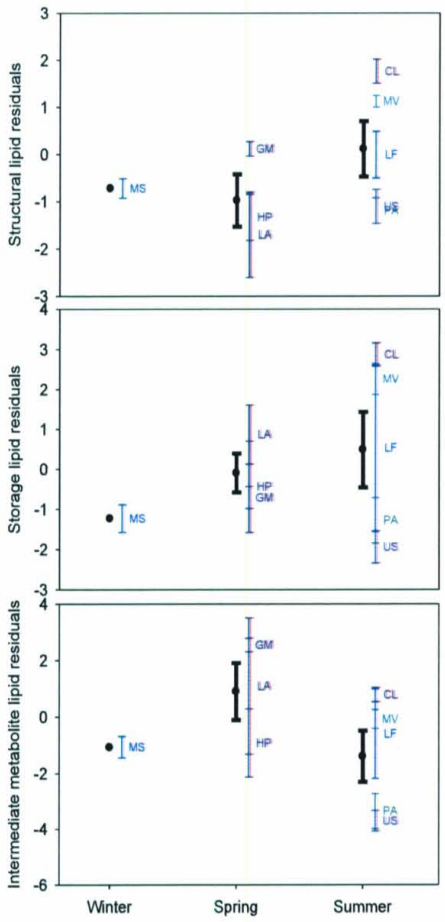


Figure 3.11 Average of species values for spawning season shown with standard error on structural residuals, storage residuals, and intermediate metabolite residuals. Residuals derive from the species independent model. The thick mean and error bars represent the average of all species means and error. GM = *Gadus morhua,* MS = *Myoxocephalus scorpius,* PA = *Pseudopleuronectes americanus,* US = *Ulvaria subbifurcata,* LA = *Liparis atlanticus,* LF = *Limanda ferruginea,* MV = *Mallotus villosus,* HP = *Hippoglossus platessoides*

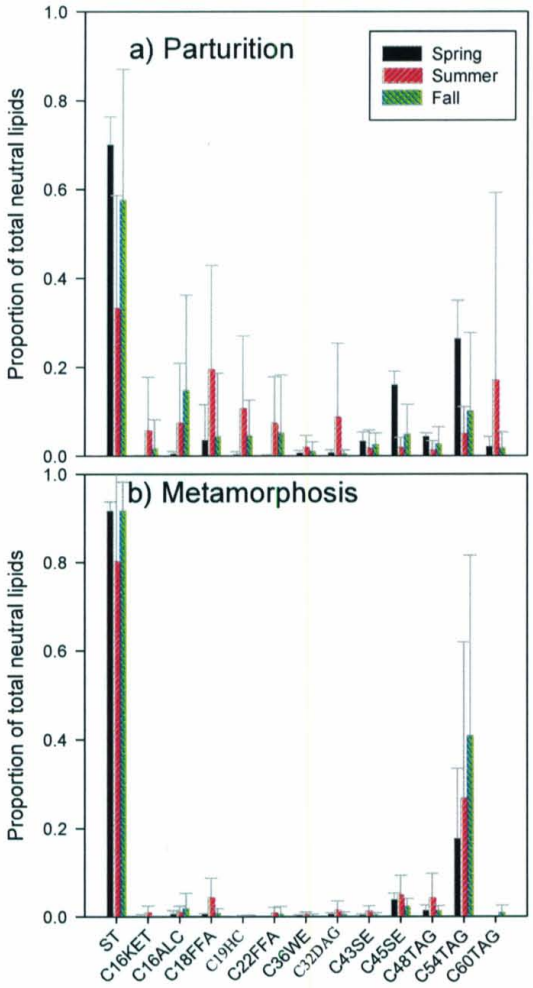


Figure 3.12 Individual larval proportion of total neutral lipids represented by each class shown with standard error at a) hatching and b) metamorphosis in winter, spring, and summer spawned larvae ST = cholesterol, C16KET = 16 carbon ketone, C16ALC = 16 carbon alcohol, C18FFA = 18 carbon free fatty acid, C19HC = 19 carbon hydrocarbon, C22FFA = 22 carbon free fatty acid, C36WE = 36 carbon wax ester, C32DAG = 32 acyl carbon diacylglycerol, C43SE = 43 carbon steryl ester, C45SE = 45 carbon steryl ester, C48TAG = 48 acyl carbon triacylglycerol, C54TAG = 54 acyl carbon triacylglycerol, C60TAG = 60 acyl carbon triacylglycerol.

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3.7 Appendices

Appendix 3.1 Comparison of lipid levels in wild and laboratory reared *Ulvaria subbifurcata*. DF = degrees of freedom, SS = Sum of squares, F = Fisher's F-statistic, p = p-value

		DF	SS	F	p
Structural lipids	Estimated dry mass	1	61.924	67.56	<0.001
	Rearing location	1	1.365	1.49	0.230
	Estimated dry mass* Rearing location	1	0.064	0.07	0.793
	Error	36	32.998		
	Total	39	96.352		
Storage lipids	Estimated dry mass	1	71.276	22.67	<0.001
	Rearing Location	1	4.209	1.34	0.2582
	Estimated dry mass* Rearing Location	1	3.933	1.25	0.2740
	Error	25	78.598		
	Total	28	158.016		
Intermediate metabolite lipids	Estimated dry mass	1	1.200	0.27	0.6085
	Rearing Location	1	119.938	26.89	<0.001
	Estimated dry mass* Rearing Location	1	0.272	0.06	0.8070
	Error	25	111.512		
	Total	28	232.922		

Appendix 3.2 Analysis of covariance results of the species-independent general model of larval fish lipids.

DF = degrees of freedom, SS= sum of squares, F = Fisher's F-statistic, p = p-value.

Dependent variable	Term	DF	SS	F	P
Structural lipids	Intercept	1	2465.054	1337.06	<0.001
R^2 =0.852	Estimated dry mass	1	1717.605	931.64	<0.001
	Species	4	238.310	32.32	<0.001
	Species* Estimated dry mass	4	54.787	7.43	<0.001
	Error	190	350.292		
	Total	200	4826.048		
Storage lipids	Intercept	1	410.258	97.07	<0.001
R^2 =0.750	Estimated dry mass	1	1528.974	361.78	<0.001
	Species	4	557.224	32.96	<0.001
	Species* Estimated dry mass	4	18.019	1.07	0.375
	Error	166	701.562		
	Total	176	3216.038		
Intermediate	Intercept	1	11.596	3.08	0.081
metabolite lipids	Estimated dry mass	1	270.733	71.91	<0.001
R^2 =0.656	Species	4	807.354	53.61	<0.001
	Species* Estimated dry mass	4	45.111	3.00	0.020
	Error	159	598.638		
	Total	169	1733.431		

Appendix 3.3 Individual species regressions of larval fish neutral lipids (μg) on estimated dry mass (mg). DF = degrees of freedom, Parameter estimates (ln y = α +β ln dry mass), F = Fisher's F-statistic, p = p-value.

Species	Lipid group	Source	DF	SS	F	p	R^2
G. morhua	Structural	Model	1	361.644	282072	<0.001	0.845
	α = 4.997 β= 1.460	Error	52	66.516			
		Total	53	428.159			
	Storage	Model	1	358.018	89.45	<0.001	0.632
	α = 1.790 β= 1.452	Error	52	208.130			
		Total	53	566.158			
	Intermediate metabolite	Model	1	17.397	6.68	0.013	0.116
	α = 2.718 β= 0.322	Error	51	132.784			
		Total	52	150.180			
M. scorpius	Structural	Model	1	102.721	50.00	<0.001	0.476
	α = 4.124 β= 1.685	Error	55	113.066			
		Total	56	215.858			
	Storage	Model	1	73.973	13.70	0.001	0.212
	α = 1.648 β= 1.437	Error	51	275.309			
		Total	52	349.282			
	Intermediate metabolite	Model	1	54.328	7.61	0.008	0.139
	α = -0.789 β= 1.279	Error	47	335.605			
		Total	48	389.934			
C. lumpus	Structural	Model	1	17.736	6.4	0.015	0.132
	α = 6.720 β= 0.472	Error	42	116.466			
		Total	43	134.203			
	Storage	Model	1	62.137	22.26	<0.001	0.346
	α = 5.665 β= 0.884	Error	42	117.289			
		Total	43	179.396			
	Intermediate metabolite	Model	1	31.331	11.49	0.002	0.215
	α = 1.436 β= 0.628	Error	42	114.479			
		Total	43	145.810			
P. americanus	Structural	Model	1	75.497	26.75	<0.001	0.426
	α = 4.523 β= 1.755	Error	36	101.599			
		Total	37	177.845			
	Storage	Model	1	30.375	5.00	0.040	0.238
	α = 0.718 β= 1.410	Error	16	97.213			
		Total	17	127.588			
	Intermediate metabolite	Model	1	9.67	1.90	0.189	0.112
	α = -7.581 β= -0.860	Error	15	76.52			
		Total	16	86.198			
U. subbifurcata	Structural	Model	1	27.627	16.65	0.001	0.562
	α = 3.347 β= 1.144	Error	13	21.576			
		Total	14	49.203			
	Storage	Model	1	57.203	25.29	<0.001	0.660
	α = 0.777 β= 1.647	Error	13	29.410			
		Total	14	86.613			
	Intermediate metabolite	Model	1	12.719	7.13	0.020	0.373
	α = -2.995 β= 0.795	Error	12	21.402			
		Total	13	34.121			

Appendix 3.4 Relationships between phylogenetic relatedness and the difference between lipid group residual means of each pair of species as the independent variable. DF = degrees of freedom; SS= sum of squares, F = Fisher's F-statistic, p = p-value.

		DF	SS	F	p	R^2
Structural lipids	Model	1	1.364	0.84	0.366	0.024
	Error	34	55.185			
	Total	35	56.550			
Storage lipids	Model	1	2.151	0.83	0.369	0.023
	Error	34	88.367			
	Total	35	90.518			
Intermediate metabolite lipids	Model	1	0.348	0.11	0.745	0.003
	Error	34	110.156			
	Total	35	110.503			

Appendix 3.5 Analysis of the effect of ecological factors on the lipid content of larval fish. The nested ANOVA was performed on the residuals from the general species-independent model. DF = degrees of freedom, SS = sum of squares, F = Fisher's F-statistic, p = p-value.

			DF	SS	F	p
Structural lipids	Egg development location	Egg development location	1	0.65	0.31	0.575
		Species (egg development location)	7	288.18	19.94	<.001
		Error	231	476.96		
	Spawning season	Spawning season	2	40.62	9.84	<.001
		Species (spawning season)	6	248.21	20.04	<.001
		Error	231	476.96		
Storage lipids	Egg development location	Egg development location	1	51.38	10.71	0.001
		Species (egg development location)	7	590.00	17.57	<.001
		Error	205	983.63		
	Spawning season	Spawning season	2	238.92	24.90	<.001
		Species (spawning season)	6	402.45	13.98	<.001
		Error	205	983.63		
Intermediate metabolite lipids	Egg development location	Egg development location	1	304.32	61.03	<.001
		Species (egg development location)	7	500.36	14.33	<.001
		Error	212	1821.96		
	Spawning season	Spawning season	2	342.80	34.37	<.001
		Species (spawning season)	6	461.87	15.44	<.001
		Error	204	1017.28		

Chapter 4 Developmental tradeoffs in larval fish species of differing ecological backgrounds

4.1 Introduction

To achieve metamorphosis and exit the larval stage, many fish undergo significant morphological changes including organ development, skeletal ossification, and an overall increase in body mass by building muscle and increasing energy stores (Morrison 1987, Hernandez et al 2001, Yamada et al. 2001, Gisbert and Doroshov 2003). While all of these processes require energy, the supply of energy is often limited – first during the endogenous feeding period as a result of finite maternal allocation (Kamler 2008), and then by foraging limitations during exogenous feeding (Houde 1989). In this study, I examine the manner in which larval fish experience tradeoffs as a result of these constraints and whether such tradeoffs show species-specific or ecological patterns.

Physiological tradeoffs deal directly with a concept central to larval fish development – allocation of energy within the individual (Brett 1972) – but only limited research has addressed this topic in larval fish. Physiological tradeoffs are manifestations of the Principle of Allocation (Levins 1968, Sibly and Calow 1986), which states that in energy limited environments, increased allocation of energy to one trait necessarily reduces the available energy to another trait. Research on larval fish tradeoffs is limited by their small size and the difficulty of maintaining them in laboratory conditions. In one of the few studies on tradeoffs in fish larvae, Billerbeck and Conover (2001) demonstrated that laboratory selection for faster growth rate resulted in increased predation on Atlantic silverside *Menidia menidia*, likely resulting from decreased

swimming performance. For juvenile fish, tradeoffs such as a metabolic tradeoff between growth rate and swimming performance have been demonstrated (Arnott et al. 2006). Despite the limited evidence, it is reasonable to suspect that there may also be tradeoffs at the physiological level in fish larvae.

As larval fish development is associated with several factors, including egg development location, larval development location, and spawning season (e.g. Potts and Wootton 1984, Moyle and Cech 1988, Munro et al. 1990), tradeoffs experienced by larval fish may also be associated with these factors. The pelagic eggs of many marine fish species are characteristically small, with limited energy reserves and are characteristically produced in higher numbers than in species with demersal eggs (Potts and Wootton 1984, see review Winemiller and Rose 1993). Species that produce demersal eggs often display parental care and invest heavily in energy per egg at hatch (Sargent and Gross 1987), possibly increasing the likelihood of offspring survival (Conover 1992). Marine fish hatch at various levels of development: Pelagic larvae often experience an extended period as an "eleutheroembryo", e.g. a free embryo which does not exogenously feed and at the extreme is little more than photosensitive pigments with a tail (Potts and Wooton 1984). By contrast, some species of fish with demersal larvae begin their free-swimming stage already possessing the full complement of organs, bones, and morphology (Hall et al. 2004). Spawning season also affects fish larvae, as larvae hatched in the late summer and fall must develop faster than larvae hatched in the spring because over-wintering mortality is often higher in smaller animals (Conover 1992). Given this evidence and pressure, as well as research on rapid evolution by Jones (2004) suggesting that ecology

influences tradeoffs, fish with different life histories will likely allocate energy differently.

Contrary to this logic, in the study of larval fish, many have attempted to develop general relationships to describe growth, mortality, metabolism, and feeding both within and among species (e.g. Giguere and St. Pierre 1988, Miller et al. 1988, Houde 1989, Pepin 1991, Fuiman et al. 1998). While studies of physiological tradeoffs tend to be limited to single species (e.g. Zera and Harshman 2001), life history theory was developed as a general framework within which the qualitative rather than quantitative relationships between traits are species-independent. As an example, a negative relationship between number and size of offspring has been repeatedly identified for many species (see review in Stearns 2000). The generality of this qualitative tradeoff has also been supported by genetic linkages (Roff 1996). Examination of the potential for general tradeoffs in multiple species of larval fish provides an opportunity to guide the development of a multi-species model for larval fish.

To explore potential developmental tradeoffs in larval fish, I address three questions regarding a variety of North Atlantic species: 1. Are there common tradeoffs irrespective of species, or is there a species effect on tradeoffs? 2. What tradeoffs are observed within each species? 3. Is there an effect of ecology on tradeoffs?

4.2 **Materials and methods**

4.2.1 *Rearing, sampling and processing*

Ten species of larval fish were reared under controlled laboratory conditions: *Gadus morhua* (Atlantic cod), *Mallotus villosus* (capelin), *Ulvaria subbifurcata* (radiated

shanny), *Cyclopterus lumpus* (Atlantic lumpfish), *Pseudopleuronectes americanus* (winter flounder), *Myoxocephalus scorpius* (shorthorn sculpin), *Limanda ferruginea* (yellowtail flounder), *Liparis atlanticus* (Atlantic snailfish), *Myoxocephalus aenaeus* (grubby sculpin) and *Hippoglossus platessoides* (American plaice). Ecological characteristics were compiled for each species from available literature or from direct observations (Table 3.1). For rearing, sampling and processing details see Chapter 2, with the addition of *Myoxocephalus aenaeus*. *Myoxocephalus aenaeus* is a species with demersal eggs, pelagic larvae and winter spawning (Froese and Pauly 2007) and was reared in the same manner as *M. scorpius*.

4.2.2 *Analysis*

To preserve the intrinsic variability of larval development, each larva was treated as an independent observation. Eleven state variables were used in the analysis: gill arch count, intestinal epithelial thickness, liver area, degree of ossification, and the amounts of intermediate metabolite lipids, carbon, nitrogen, DNA, RNA, storage lipids and structural lipids in each individual. Carbon gives a measure of structural elements in larval fish that can be used to describe the developing larva. Nitrogen's natural rarity, coupled with its important role in molecules such as proteins, nucleic acids and ATP makes it a major limiting component in nature (Elser et al. 1996). Lipids are an important part of an animal's biochemical makeup as they have many roles including structural functions in membranes, substrates for catabolism, physical protection for organs, insulation, buoyancy, and various functions as chemical messengers (Wiegand 1996). Nucleic acids play a major role in growth and development, as DNA is the carrier of genetic

information, while RNA is the foundation of protein synthesis (Clemmesen 1993). In addition developmental progression was documented through ossification (Morrison 1987, Cahu et al. 2003), the formation of gills for respiration (Morrison 1987, Phillips 1999), intestinal epithelium thickness to increase digestive capacity, and liver size which contributes to digestion, and enzyme synthesis and protein synthesis (Baglole et al. 1997, Fishelson and Becker 2001, Gisbert and Doroshov 2003).

In addition, each larva was analysed for morphometry (for details see Chapter 2) including head length and height, body depth at pectoral fin, body depth at anal fin insertion, eye diameter, yolk sac length and depth, and total length. Dry-mass-to-length relationships were developed for each species to estimate dry mass as dry mass was not available for all animals due to technical requirements of processing (e.g. lipids, detailed in Chapter 2 and Appendix 2.4).

Comparison of state variables from each organism data was accomplished without pooling by using the multiple imputation function in SAS (SAS Institute Inc., North Carolina, USA). Multiple imputation is the process of replacing a missing value with multiple plausible values by combining information from the existing multivariate distribution with information on the variation surrounding these distributions. This allows for uncertainty in the missing values, without altering the underlying relationships. Multiple imputation uses all available information, and to give the most accurate imputation possible, all of the state values and morphometric values were used to predict the missing data. Based on the amount of missing data, multiple imputation was performed 100 times using 500 iterations to calculate each imputation as suggested by Graham et al. (2007). Five species had enough data to impute separately (*G. morhua, M.*

scorpius, C. lumpus, U. subbifurcata, P. americanus). The remaining species were imputed as a group. This process resulted in 100 data sets for each species.

Each resultant data set (100 for each species) was subjected to a principal components analysis (SAS) using all state variables. Principal component one (PC1) was highly correlated with body size measures, so the loading of each variable on principal component two (PC2) was of particular interest. PC2 represents the greatest separation of the individuals based on multivariate relationships independent of body size and therefore the strongest separation in life history strategies (growth trajectories, reproduction strategies, etc.: Stearns 1992) and potential tradeoffs. The resultant relationships obtained by pairwise comparisons of the loadings of variables on PC2 were then analysed for the effect of species using an analysis of covariance (ANCOVA). A significant species term would indicate that a general model of tradeoffs in larval fish is an inaccurate description of individual species.

Each species was also analysed individually for potential tradeoffs by comparing the loadings of the variables on each species specific PC2. Opposite loadings on PC2 represent potential tradeoffs, with the difference between the loadings representing the magnitude of the tradeoff. Variables that have loadings of the same sign on PC2 were interpreted as synergistic physiological variables during development.

The same variable loadings on PC2 were also analysed to determine whether tradeoffs were conserved across ecological groups (Table 3.1). A combined correlation coefficient between physiological variable loadings including all species (e.g. carbon *vs* DNA) for each ecological designation of egg development location, larval development location, and spawning season was calculated and the magnitude and sign compared

using Fisher's z-transformation. Variables belong to two conceptual categories: 1) Developmental progress variables that reflect features which increase in complexity as a larva grows and include ossification, the formation of gills, intestinal epithelium thickness and liver size; and 2) Biochemical variables that represent compositional features of the larva and include total amounts of carbon and nitrogen, structural lipids, storage lipids, intermediate metabolite lipids, RNA and DNA.

4.3 **Results**

4.3.1 *Species independent results*

The data extend across 10 species of larval fish and a dry mass range of 0.010 to 14.438 µg (3 orders of magnitude). In total 2236 fish were included in the analysis. Data were transformed using a natural logarithm to more closely approximate linearity and a normal error distribution. Principal component scores on PC1 were highly related to morphometrics (Appendix 4.1, Eigenvalues PC1 = 4.30, PC2 = 2.54, PC3 = 1.661).

When all species were analysed in one data set, 27 significant negative relations were observed out of the 55 relations examined (Fig. 4.1, detailed in Appendix 4.2). Both RNA and DNA were strongly negatively correlated with the same state variables, with the exception of ossification. The four development characteristics (intestinal epithelial thickness, liver area, gill arch count and ossification) shared very few similarities. Overall, different lipids categories shared a number of similarities in their relations to other variables, as did nucleic acids, while carbon, nitrogen and developmental characteristics did not.

Each variable pair with a significant negative relationship between scores on PC2

was submitted to a general linear model relating the scores for the state variables to each other. The categorical variable of species was added to determine whether there was an effect of species. Tradeoffs were not conserved across species (Fig. 4.2), with significant species interaction terms for all pairs of physiological variables (detailed in Appendix 4.3, $p<0.05$). Both the slope and the intercept were significantly different in all variable pairs.

4.3.2 *Species-specific patterns*

Gadus morhua

Gadus morhua showed 25 significant negative relations among the state variables (Fig. 4.2, detailed in Appendix 4.4). Nucleic acids showed negative relationships with three of the four developmental progress variables: ossification, intestinal epithelial thickness and liver area. Within *G. morhua*, the major groups of tradeoffs involved intermediate metabolite lipids or involved the biochemical variables with intestinal epithelial thickness or liver area.

Myxocephalus scorpius

Twenty-six negative relations were observed in the case of *M. scorpius* (Fig. 4.2, detailed in Appendix 4.5). The two nucleic acids shared all the same significant relations; in addition to carbon and nitrogen, they also showed negative relations with storage lipids, intermediate metabolite lipids, gill arch count and ossification. As with the species independent analysis, the developmental progress variables of intestinal epithelial thickness, liver area, gill arch count and ossification were negatively correlated with each

other, except for liver area with intestinal epithelial thickness and ossification with gill arch count.

Cyclopterus lumpus

Cyclopterus lumpus exhibited only 13 significant negative relations. Only two were shared with the species independent analysis: structural lipids with nitrogen and liver area with storage lipids (Fig. 4.2, detailed in Appendix 4.6). Carbon and nitrogen notably shared no relations with other variables. Developmental progress variables were not negatively related to each other, in contrast to the analysis of all species.

Pseudopleuronectes americanus

For *P. americanus*, I obtained a moderate number (20) of significant negative correlations between variables (Fig. 4.2, detailed in Appendix 4.7). The majority of tradeoffs in *P. americanus* were related to the four developmental progress variables. Ossification was negatively related to seven of the state variables, with the exceptions of intermediate metabolite lipids, intestinal epithelial thickness and gill arch count. Of the six correlations between the developmental progress variables, the only significant negative relations were between intestinal epithelial thickness and ossification with liver area.

Ulvaria subbifurcata

Twenty-four significant negative correlations were observed when *U. subbifurcata* was examined independently (Fig. 4.2, detailed in Appendix 4.8). Both

nucleic acids were negatively related to structural lipids, storage lipids, and gill arch count. Among developmental progress variables, the only negative relation involved gill arch count and intestinal epithelial thickness. Negative correlations were shared between nucleic acids, carbon, nitrogen, and structural lipids with intermediate metabolites lipids. However, apart from this, there were no overarching patterns or groupings in *U. subbifurcata.*

4.3.3 *Ecological effects*

Egg development location, demersal versus pelagic, appeared to have a significant effect on the nature of the tradeoffs (Fig. 4.3, detailed in Appendix 4.9) when the correlation scores were combined and compared using Fisher's Z-transformation. Only six of the 27 variables failed to show a negative correlation in the combined species data set (detailed in Appendix 4.2). Otherwise, all of the negative correlations found in the multi-species analysis were significantly affected by egg development location. Pelagic eggs had 32 significant negative correlations, while demersally developed eggs had 26 negative correlations (detailed in Appendix 4.9). The primary overlap was between nucleic acids and lipids, and gill arch count. Marked differences included the lack of negative correlations for demersal eggs between intermediate metabolite lipids and nitrogen, carbon, and structural and storage lipids. Also, nucleic acids had negative relations with carbon and nitrogen in demersal eggs, while in pelagic eggs they did not. Nucleic acids from pelagic eggs are negatively correlated with intestinal epithelial thickness, while those from demersal eggs are not.

There was a significant difference between the pelagic larvae and *C. lumpus* (the only true demersal larva) in all pairs of variables displaying significant negative relations, except for a few pairs of factors (nitrogen and intermediate metabolite lipids, DNA and gill arches, and ossification with carbon, DNA and liver area, Fig. 4.3, detailed in Appendix 4.10). The tradeoffs unique to pelagic larvae occurred mostly in relations with nucleic acids. In addition, pelagic larvae displayed more negative relations with intermediate metabolite lipids and intestinal epithelial thickness.

When spawning season (winter, spring or summer,) was examined, eight of the 24 pairwise comparisons between spring and summer combined coefficients were non-significant, two between spring and winter, and three between winter and summer hatched larvae (Fig. 4.4, detailed in Appendix 4.11). Winter, spring and summer spawned species exhibited 29, 29, and 23 significant negative correlations, respectively. Of these, 16 were shared despite having different correlation coefficients, five of these between nucleic acids and the other biochemical variables, and ten between developmental progress variables and biochemical variables. Eight, eight and zero negative correlations were unique to spring, winter and summer spawning larvae, respectively, but there was no clear pattern of unique tradeoffs dependent on season.

4.4 **Discussion**

4.4.1 *Species independent results*

This comparative analysis reveals very substantial differences in the nature of tradeoffs of state and developmental variables among a broad range of larval fish species, raising important questions about the value of general models of development and/or

growth based on the combination of information from many species. For example, despite the positive overall relationship between amounts of carbon and nitrogen in each larva based on data from all species, *P. americanus* exhibits a negative relationship between these two variables. Although the natural diet of *P. americanus* differs somewhat from that of the other species examined (Pepin and Penney 1997), it is not merely one species which deviates from general patterns identified in the multispecies analysis; in the overall positive relationship of nitrogen with ossification, both *C. lumpus* and *U. subbifurcata* show a negative relationship. The biological significance of these results in the general evaluation of energy budgeting in larval fish is far reaching: different species of larvae are operating with significantly different energy allocation strategies. As a result, is not justifiable to ignore the effect of species when pursuing general energy allocation models. This is particularly important when considering patterns of growth and vulnerability to starvation because the requirements and patterns of energy allocation may result in considerable differences in the susceptibility and response of different species' larvae to changes in prey availability.

4.4.2 *Species-specific patterns*

While examination of each species individually yielded further support for the species-specific nature of observed physiological tradeoffs, there were also commonalities; in four of five species examined at the individual level, the following developmental biochemical tradeoffs were frequently observed: liver area with storage lipids, intestinal epithelial thickness with storage lipids, liver area with carbon, intestinal epithelial thickness with structural lipids, liver area with nitrogen, and intestinal epithelial

thickness with nitrogen. The negative relation of liver area and intestinal epithelial thickness with storage lipids is counterintuitive as both the liver and the intestinal epithelium are related to storage in adult animals, however there has been previous evidence that these organs provide very little storage in very young animals, and are instead largely producing hormones and digestive enzymes (Ferron and Legget 1994). The remaining common relations (liver area with carbon, intestinal epithelial thickness with structural lipids, liver area with nitrogen, and intestinal epithelial thickness with nitrogen) may be reflective of another unique property of very young animals – the propensity to increase cell size rather than increase the number of cells within a tissue (Wold et al. 2008). These unique properties of early development expand on the previous findings (Billerbeck and Conover 2001, Arnott et al. 2006, and Killen et al. 2007) suggesting developmental strategies are more complicated than prioritizing growth.

This can be alternatively thought of in the context of competition between increasing organ development and size. While this study uses multivariate analysis to largely remove the effects of size and evaluate the changes in allocation as size increases, there remains evidence of energy allocated to increasing size (in the amounts of DNA, RNA, nitrogen structural lipids, and carbon per unit mass). Increasing organ development is represented by liver area, intestinal epithelial thickness, ossification and gill arches. The concept that there is limited energy to allocate and that more energy devoted to increasing body size may result in less energy available for development (and *vice versa*) is also addressed extensively for juvenile animals by comparing size at maturity and total energy allocation to reproduction (Kozlowski 1992). However, it is not commonly considered in the context of larval fish. In larvae, increases in complexity (gills,

ossification, digestive development) are required not just for conferring reproductive benefits but for continued survival and size increases of the organism. There is support in the literature for the hypothesis that young fishes have limited energy to allocate (Post and Lee 1996), resulting in, for example, a tradeoff between growth and aerobic scope (e.g. Arnott et al. 2006) and a tradeoff between growth rate and swimming performance (e.g. Billerbeck and Conover 2001). Larval fish show signs of a tradeoff between size and complexity that likely influences the high mortality rates often observed at this life stage. While increased complexity is invaluable for wild fish to be effective in utilising resources and avoiding predation, in aquaculture animals living without predation, environmental challenges and foraging challenges, manipulating this balance of emphasis between size and complexity may prove valuable.

4.4.3 *Ecological effects*

In a manner consistent with the findings of Jessup and Bohannan (2008), biochemical and developmental tradeoffs were dependent on ecological designations, which significantly affected the strength and direction of observed tradeoffs. Egg development location affected not only the strength of the observed negative correlations, but also their existence. Similarly, the only demersally developing larvae, *C.lumpus*, differed from pelagic larvae. Demersal eggs and the demersally developing larvae *C. lumpus* exhibited fewer tradeoffs than pelagic eggs and larvae, which may reflect greater energetic investment by parents in both the size of their eggs and parental care (Potts and Wootton 1984). This may imply that their total energy budget is greater than that of other taxa considered here, diminishing the requirement for tradeoffs even in their early life

history. The large number of tradeoffs in pelagic larvae were mostly related to nucleic acids. Given that DNA can indicate total cell number while RNA is an indication of protein synthesis (Clemmesen 1993), this provided evidence that cell number and protein synthesis may experience greater allocation competition with other developmental processes in pelagic larvae. Pelagic larvae are limited first by maternally allocated resources in the egg and eleutheroembryo stages, which are generally less than those allocated to demersal larvae, and then further by their underdeveloped state relative to demersal larvae, limiting foraging ability (Kamler 2008). Spawning season also significantly affected observed tradeoffs in physiological measures of larval fish. Summer spawned larvae exhibited the fewest significant tradeoffs, consistent with more and better prey availability (Munro et al. 1990, Conover 1992, Kattner et al. 2007). However, there were no consistent patterns of tradeoffs between variables amongst seasons.

The ecological groupings were not confounded by the spawning method (collection or stripping) or natural wild diet, and all species were reared within their temperature range. If the increased numbers of potential tradeoffs observed in fish with pelagic larvae and eggs, or those spawned in seasons with lower nutrient availability are evidence that larval characteristics represent the outcome of evolution under varying conditions of limited energy availability, then wild larvae are likely to show stronger effects than could be demonstrated under an *ad libitum* laboratory setting because their energy budget would be further affected by predation (Killen et al. 2007) and prey availability (Hjort 1914). Even under the more favourable conditions of a laboratory environment, without the pressures of predation, foraging, and environmental

fluctuations, pelagic eggs and larvae exhibited signs that they are operating under a more limited energy budget than their demersal counterparts.

4.4.4 *Conclusions*

It seems clear that there is no general tradeoff model. As tradeoffs reflect energy allocation, this conclusion has important consequences for the fields of fisheries, ecology, and aquaculture as energy allocation is pertinent to all of these fields. Despite all the fishes examined in this study occurring in the limited environment of coastal North Atlantic waters, different developmental strategies were apparent. This study highlights the need for more integrated multi-species studies of tradeoffs, rather than single-species examinations of presumed representative species (see Zera and Harshman 2001 for review). Future explorations should examine how ecological determinants of life history may interact with the length of the growing season (Teletchea and Fontaine 2010) and temperature effects (Conover 1992). If this research was expanded across a broader range of habitats, as well as other life history traits such as egg size, size at hatch, and growth rates would help organise observed tradeoffs. Future work on wild larvae or larvae reared under nutrient limited conditions will likely show more pronounced tradeoffs. The tradeoffs highlighted here will need to be further established by both selecting on one trait and observing the other, or by manipulating one physiological trait to observe changes in another.

4.5 Tables and figures

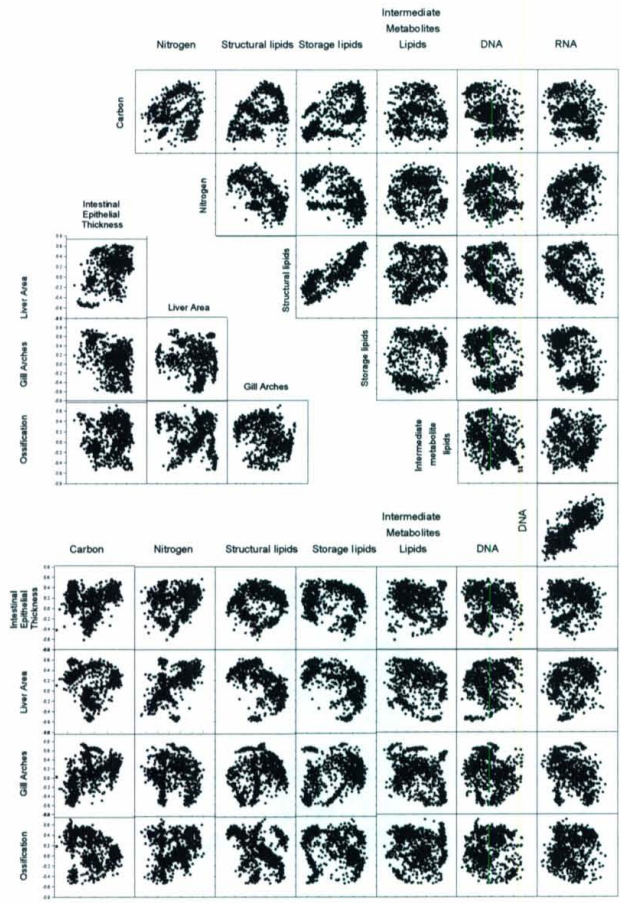

Figure 4.1 Correlation matrix between variable scores (unitless) with the second principal component (PC2) for all species. These variable scores are the result of a principal components analysis using all state variables after multiple imputation of the data for each species. The resultant correlations were generated by comparing the loading of each variable on PC2 to each other variable on PC2 from the data sets.

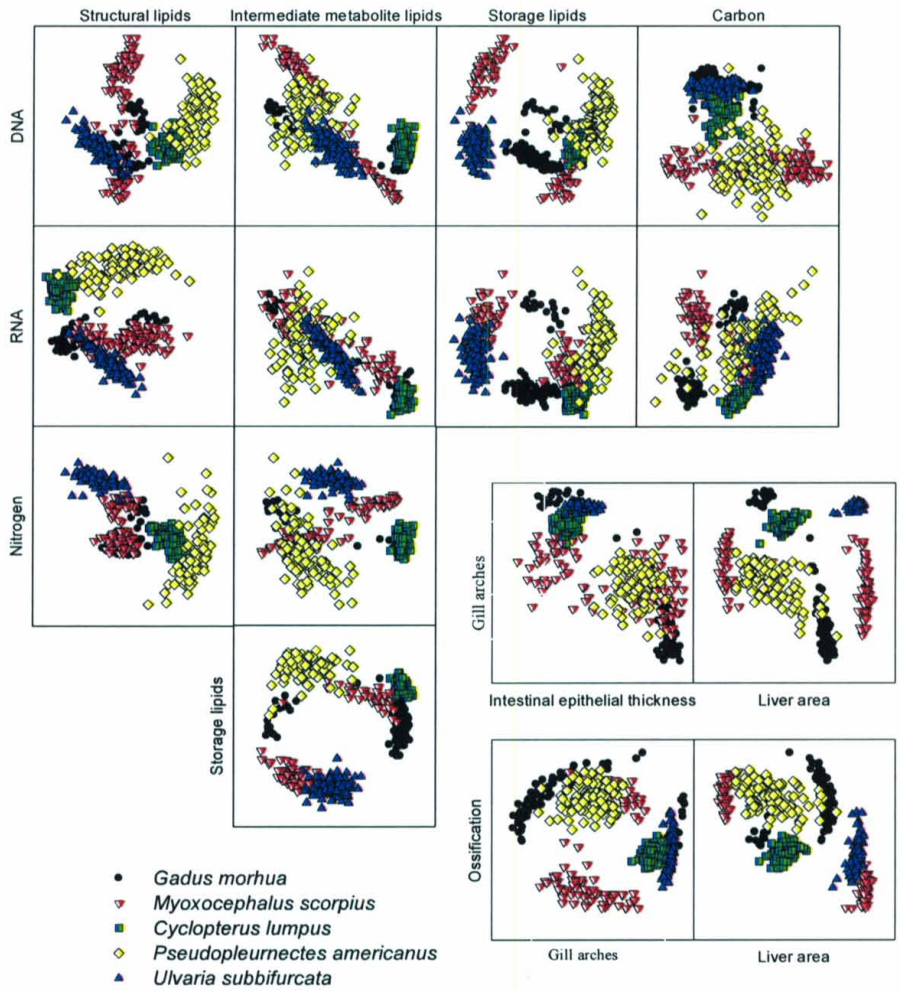

Figure 4.2 Correlation matrix between variable scores (unitless) on PC2 displayed by species for which information from hatching to metamorphosis existed. This represents a subset of data presented in Figure 4.1. Only plots in which negative correlations from the multi-species analysis are shown.

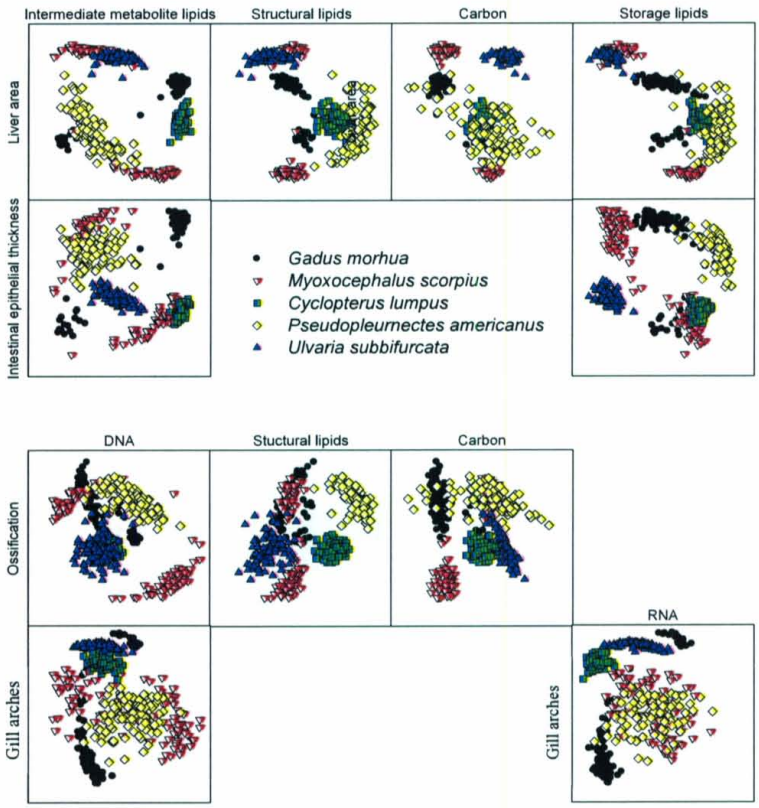

Figure 4.2 (cont.)

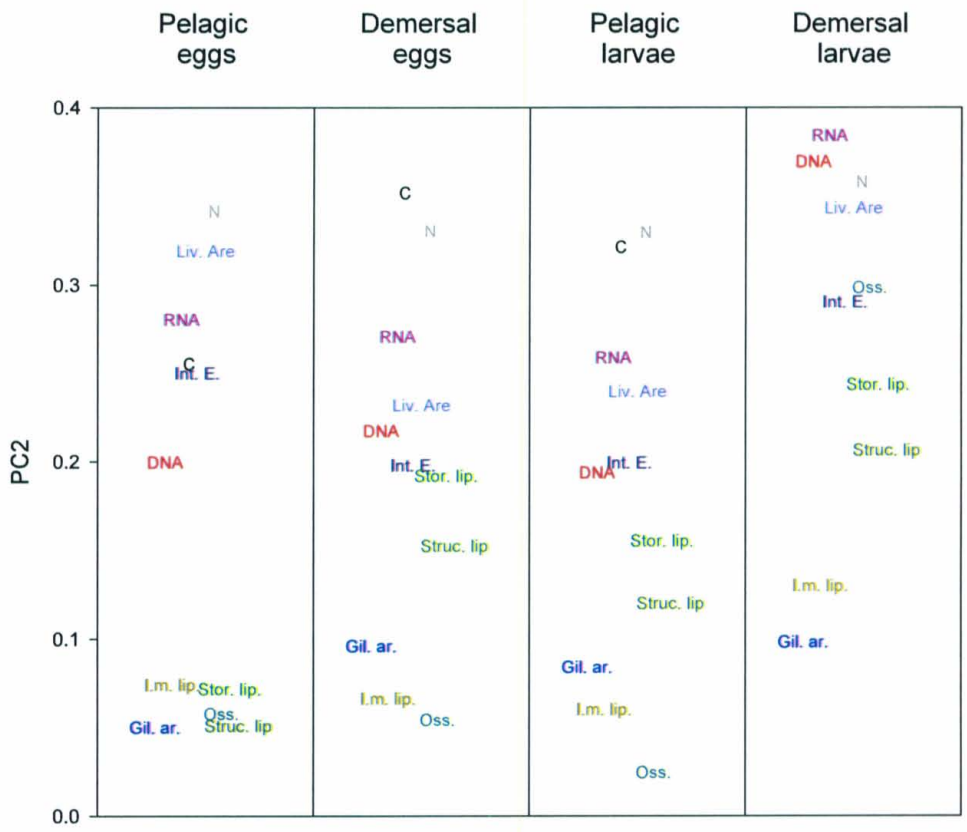

Figure 4.3 Average scores on PC2 of each variable by ecological designation of egg development location and larval development location. Although this figure does not explicitly show the tradeoffs between these variables, it does demonstrate the difference between ecological designations in the relationships among variables. The correlations of these variables through development are further examined using correlation coefficients in Appendix 4.9 (egg location), 4.10 (larvae location) and 4.11 (season). N = nitrogen, C = carbon, Struc. lip = structural lipids, Stor. lip. = storage lipids, I.m. lip = intermediate metabolite lipids, Liv. Are = liver area, Int. E. = intestinal epithelial thickness, Gil. ar. = gill arch count, Oss. = ossification.

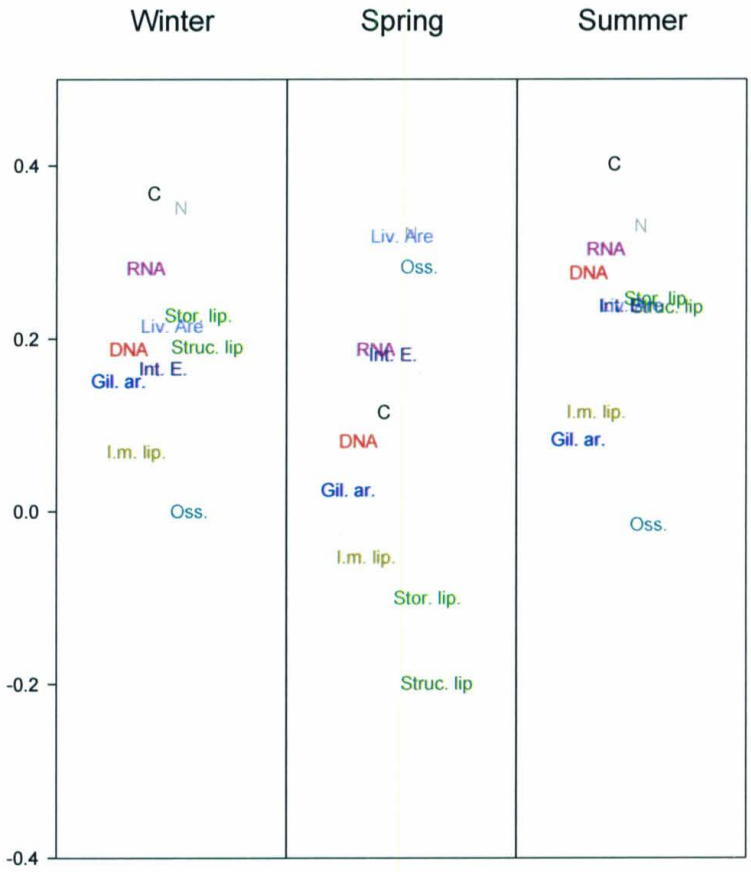

Figure 4.4 Average scores on principal component two of each variable by ecological designation spawning season. Although this figure does not explicitly show the tradeoffs between these variables, it does demonstrate the difference between ecological designations in the relationships between variables. The correlations of these variables through development are further examined using correlation coefficients in Appendices 4.9 (egg location), 4.10 (larvae location) and 4.11 (season). N = nitrogen, C = carbon, Struc. lip. = structural lipids, Stor. lip. = storage lipids, I.m. lip. = intermediate metabolite lipids, Liv. Are = liver area, Int. E. = intestinal epithelial thickness, Gil. ar. = gill arch count, Oss. = ossification.

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4.7 Appendices

Appendix 4.1 Correlation of morphometrics with principal component one. N=226500 (based on imputed data). All correlations were significant ($p<0.05$), but this significance is likely to be inflated as a result of the imputation process.

Species	Dry mass	Total length	Head length	Head height	Body depth at pectoral fin	Body depth at anal fin	Eye diameter
G. morhua	0.984	0.984	0.985	0.956	0.925	0.979	0.986
M. scorpius	0.777	0.777	0.785	0.769	0.772	0.896	0.825
C. lumpus	0.980	0.980	0.903	0.980	0.984	0.972	0.918
P. americanus	0.883	0.883	0.842	0.863	0.856	0.801	0.866
U. subbifurcata	0.844	0.844	0.806	0.818	0.887	0.914	0.724

Appendix 4.2 Pearson correlation between variable scores. Variable scores are from PC2 for all species (N=10 species and N=100 imputations). Areas in grey represent repeated values in the table included for completeness. Numbers in bold are significant negative correlations ($p < 0.05$).

	Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossification
Carbon	1.000	0.254	0.401	0.497	-0.006	**-0.429**	**-0.288**	0.009	**-0.118**	**-0.154**
		<.001	<.001	<.001	0.837	**<.001**	**<.001**	0.767	**<.001**	**<.001**
Nitrogen	0.254	1.000	**-0.589**	**-0.435**	**-0.109**	-0.057	0.382	0.026	0.350	0.296
	<.001		**<.001**	**<.001**	**<.001**	0.060	<.001	0.391	<.001	<.001
Structural lipids	0.401	**-0.589**	1.000	0.845	0.176	**-0.295**	**-0.476**	0.007	**-0.460**	**-0.298**
	<.001	**<.001**		<.001	<.001	**<.001**	**<.001**	0.823	**<.001**	**<.001**
Storage lipids	0.497	**-0.435**	0.845	1.000	0.309	**-0.416**	**-0.501**	**-0.129**	**-0.631**	0.040
	<.001	**<.001**	<.001		<.001	**<.001**	**<.001**	**<.001**	**<.001**	0.188
Intermediate metabolite lipids	-0.006	**-0.109**	0.176	0.309	1.000	**-0.485**	**-0.335**	**-0.242**	**-0.327**	0.020
	0.837	**<.001**	<.001	<.001		**<.001**	**<.001**	**<.001**	**<.001**	0.512
DNA	**-0.429**	-0.057	**-0.295**	**-0.416**	**-0.485**	1.000	0.766	0.085	0.325	**-0.129**
	<.001	0.060	**<.001**	**<.001**	**<.001**		<.001	0.005	<.001	**<.001**
RNA	**-0.288**	0.382	**-0.476**	**-0.501**	**-0.335**	0.766	1.000	0.074	0.266	0.097
	<.001	<.001	**<.001**	**<.001**	**<.001**	<.001		0.014	<.001	0.001
Intestinal epithelial thickness	0.009	0.026	0.007	**-0.129**	**-0.242**	0.085	0.074	1.000	0.471	-0.013
	0.767	0.391	0.823	**<.001**	**<.001**	0.005	0.014		<.001	0.668
Liver area	**-0.118**	0.350	**-0.460**	**-0.631**	**-0.327**	0.325	0.266	0.471	1.000	**-0.242**
	<.001	<.001	**<.001**	**<.001**	**<.001**	<.001	<.001	<.001		**<.001**
Gill arch count	0.490	-0.012	0.156	0.179	**-0.116**	**-0.215**	**-0.339**	**-0.517**	**-0.091**	**-0.282**
	<.001	0.700	<.001	<.001	**<.001**	**<.001**	**<.001**	**<.001**	**0.003**	**<.001**

Appendix 4.3 Analysis of covariance results of the species-independent general model.

Results are reported for all negative relationships. DF error = 980, state variable = 1, species = 9, interaction = 9

		Species term	Species x variable
Carbon	DNA	<0.001	<0.001
Carbon	RNA	<0.001	<0.001
Carbon	Liver area	<0.001	<0.001
Carbon	Ossification	<0.001	<0.001
Nitrogen	Structural lipids	<0.001	<0.001
Nitrogen	Intermediate metabolite lipids	<0.001	<0.001
Structural lipids	DNA	<0.001	<0.001
Structural lipids	RNA	<0.001	<0.001
Structural lipids	Liver area	<0.001	<0.001
Structural lipids	Ossification	<0.001	<0.001
Storage lipids	Intermediate metabolite lipids	<0.001	<0.001
Storage lipids	DNA	<0.001	<0.001
Storage lipids	RNA	<0.001	<0.001
Storage lipids	Intestinal epithelial thickness	<0.001	<0.001
Storage lipids	Liver area	<0.001	<0.001
Intermediate metabolite lipids	DNA	<0.001	<0.001
Intermediate metabolite lipids	RNA	<0.001	<0.001
Intermediate metabolite lipids	Intestinal epithelial thickness	<0.001	<0.001
Intermediate metabolite lipids	Liver area	<0.001	<0.001
Intermediate metabolite lipids	Gill arch count	<0.001	<0.001
DNA	Gill arch count	<0.001	<0.001
DNA	Ossification	<0.001	<0.001
RNA	Gill arch count	<0.001	<0.001
Intestinal epithelial thickness	Gill arch count	<0.001	<0.001
Liver area	Gill arch count	<0.001	<0.001
Liver area	Ossification	<0.001	<0.001
Gill arch count	Ossification	<0.001	<0.001

Appendix 4.4 Pearson correlation and significance between variable scores on PC2 for *Gadus morhua* based on 100 imputations. Areas in grey represent repeated values in the table included for completeness. Numbers in bold are significant negative correlations ($p< 0.05$).

	Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossification
Carbon	1.000	0.982	0.514	-0.001	**-0.940**	0.915	0.928	**-0.918**	**-0.905**	**-0.608**
		<.001	<.001	0.989	**<.001**	<.001	<.001	**<.001**	**<.001**	**<.001**
Nitrogen	0.982	1.000	0.552	0.029	**-0.920**	0.853	0.904	**-0.913**	**-0.890**	**-0.510**
	<.001		<.001	0.777	**<.001**	<.001	<.001	**<.001**	**<.001**	**<.001**
Structural lipids	0.514	0.552	1.000	0.757	**-0.659**	0.391	0.678	**-0.708**	**-0.763**	0.203
	<.001	<.001		<.001	**<.001**	<.001	<.001	**<.001**	**<.001**	0.043
Storage lipids	-0.001	0.029	0.757	1.000	**-0.198**	-0.138	0.171	**-0.283**	**-0.316**	0.606
	0.989	0.777	<.001		**0.049**	0.171	0.089	**0.004**	**0.001**	<.001
Intermediate metabolite lipids	**-0.940**	**-0.920**	**-0.659**	**-0.198**	1.000	**-0.896**	**-0.976**	0.979	0.977	0.492
	<.001	**<.001**	**<.001**	**0.049**		**<.001**	**<.001**	<.001	<.001	<.001
DNA	0.915	0.853	0.391	-0.138	**-0.896**	1.000	0.934	**-0.857**	**-0.831**	**-0.787**
	<.001	<.001	<.001	0.171	**<.001**		<.001	**<.001**	**<.001**	**<.001**
RNA	0.928	0.904	0.678	0.171	**-0.976**	0.934	1.000	**-0.964**	**-0.957**	**-0.534**
	<.001	<.001	<.001	0.089	**<.001**	<.001		**<.001**	**<.001**	**<.001**
Intestinal epithelial thickness	**-0.918**	**-0.913**	**-0.708**	**-0.283**	0.979	**-0.857**	**-0.964**	1.000	0.963	0.422
	<.001	**<.001**	**<.001**	**0.004**	<.001	**<.001**	**<.001**		<.001	<.001
Liver area	**-0.905**	**-0.890**	**-0.763**	**-0.316**	0.977	**-0.831**	**-0.957**	0.963	1.000	0.386
	<.001	**<.001**	**<.001**	**0.001**	<.001	**<.001**	**<.001**	<.001		<.001
Gill arch count	0.797	0.801	0.876	0.557	**-0.914**	0.695	0.893	**-0.942**	**-0.952**	-0.157
	<.001	<.001	<.001	<.001	**<.001**	<.001	<.001	**<.001**	**<.001**	0.118

Appendix 4.5 Pearson correlation and significance between variable scores on PC2 for *Myoxocephalus scorpius* based on 100 imputations. Areas in grey represent repeated values in the table included for completeness. Numbers in bold are significant negative correlations ($p< 0.05$).

	Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossification
Carbon	1.000	0.974	0.107	0.966	0.853	**-0.872**	**-0.882**	**-0.869**	**-0.964**	0.933
		<.001	0.131	<.001	<.001	**<.001**	**<.001**	**<.001**	**<.001**	<.001
Nitrogen	0.974	1.000	0.012	0.900	0.933	**-0.939**	**-0.921**	**-0.789**	**-0.950**	0.861
	<.001		0.862	<.001	<.001	**<.001**	**<.001**	**<.001**	**<.001**	<.001
Structural lipids	0.107	0.012	1.000	0.121	**-0.258**	0.213	-0.040	-0.104	0.013	0.168
	0.131	0.862		0.089	**<.001**	0.003	0.573	0.144	0.856	0.017
Storage lipids	0.966	0.900	0.121	1.000	0.778	**-0.816**	**-0.781**	**-0.945**	**-0.966**	0.987
	<.001	<.001	0.089		<.001	**<.001**	**<.001**	**<.001**	**<.001**	<.001
Intermediate metabolite lipids	0.853	0.933	**-0.258**	0.778	1.000	**-0.992**	**-0.854**	**-0.674**	**-0.893**	0.740
	<.001	<.001	**<.001**	<.001		**<.001**	**<.001**	**<.001**	**<.001**	<.001
DNA	**-0.872**	**-0.939**	0.213	**-0.816**	**-0.992**	1.000	0.859	0.731	0.923	**-0.791**
	<.001	**<.001**	0.003	**<.001**	**<.001**		<.001	<.001	<.001	**<.001**
RNA	**-0.882**	**-0.921**	-0.040	**-0.781**	**-0.854**	0.859	1.000	0.651	0.858	**-0.761**
	<.001	**<.001**	0.573	**<.001**	**<.001**	<.001		<.001	<.001	**<.001**
Intestinal epithelial thickness	**-0.869**	**-0.789**	-0.104	**-0.945**	**-0.674**	0.731	0.651	1.000	0.919	**-0.966**
	<.001	**<.001**	0.144	**<.001**	**<.001**	<.001	<.001		<.001	**<.001**
Liver area	**-0.964**	**-0.950**	0.013	**-0.966**	**-0.893**	0.923	0.858	0.919	1.000	**-0.959**
	<.001	**<.001**	0.856	**<.001**	**<.001**	<.001	<.001	<.001		**<.001**
Gill arch count	0.345	0.431	**-0.534**	0.328	0.569	**-0.573**	**-0.381**	**-0.473**	**-0.498**	0.348
	<.001	<.001	**<.001**	<.001	<.001	**<.001**	**<.001**	**<.001**	**<.001**	<.001

Appendix 4.6 Pearson correlation and significance between variable scores on PC2 for *Cyclopterus lumpus* based on 100 imputations. Areas in grey represent repeated values in the table included for completeness. Numbers in bold are significant negative correlations ($p < 0.05$).

	Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossification
Carbon	1.000	0.199 0.049	0.270 0.007	0.686 <.001	**-0.260** **0.009**	0.034 0.737	0.717 <.001	0.078 0.445	0.305 0.002	-0.192 0.057
Nitrogen	0.199 0.049	1.000	**-0.524** **<.001**	0.132 0.192	0.128 0.206	0.481 <.001	0.463 <.001	**-0.424** **<.001**	**-0.255** **0.011**	**-0.252** **0.012**
Structural lipids	0.270 0.007	**-0.524** **<.001**	1.000	0.593 <.001	**-0.432** **<.001**	0.207 0.040	0.052 0.609	0.453 <.001	-0.152 0.133	0.037 0.718
Storage lipids	0.686 <.001	0.132 0.192	0.593 <.001	1.000	**-0.507** **<.001**	0.434 <.001	0.436 <.001	0.104 0.305	**-0.270** **0.007**	**-0.387** **<.001**
Intermediate metabolite lipids	**-0.260** **0.009**	0.128 0.206	**-0.432** **<.001**	**-0.507** **<.001**	1.000	0.032 0.751	0.262 0.009	0.459 <.001	0.377 <.001	0.682 <.001
DNA	0.034 0.737	0.481 <.001	0.207 0.040	0.434 <.001	0.032 0.751	1.000	0.293 0.003	0.238 0.018	**-0.586** **<.001**	-0.115 0.257
RNA	0.717 <.001	0.463 <.001	0.052 0.609	0.436 <.001	0.262 0.009	0.293 0.003	1.000	0.279 0.005	0.408 <.001	0.080 0.432
Intestinal epithelial thickness	0.078 0.445	**-0.424** **<.001**	0.453 <.001	0.104 0.305	0.459 <.001	0.238 0.018	0.279 0.005	1.000	0.255 0.011	0.587 <.001
Liver area	0.305 0.002	**-0.255** **0.011**	-0.152 0.133	**-0.270** **0.007**	0.377 <.001	**-0.586** **<.001**	0.408 <.001	0.255 0.011	1.000	0.306 0.002
Gill arch count	**-0.236** **0.019**	0.363 <.001	**-0.772** **<.001**	**-0.690** **<.001**	0.626 <.001	-0.184 0.069	0.168 0.097	-0.053 0.603	0.446 <.001	0.319 0.001

Appendix 4.7 Pearson correlation and significance between variable scores on PC2 for *Pseudoplueronectes americanus* based on 100 imputations.

Areas in grey represent repeated values in the table included for completeness. Numbers in bold are significant negative correlations ($p < 0.05$).

	Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossification
Carbon	1.000	**-0.358**	0.014	0.194	0.501	0.732	0.742	0.126	**-0.573**	**-0.370**
		<.001	0.890	0.054	<.001	<.001	<.001	0.213	**<.001**	**<.001**
Nitrogen	**-0.358**	1.000	0.432	**-0.113**	**-0.600**	0.170	0.200	**-0.237**	0.784	**-0.500**
	<.001		<.001	**0.264**	**<.001**	0.092	0.047	**0.018**	<.001	**<.001**
Structural lipids	0.014	0.432	1.000	0.788	**-0.294**	0.359	0.329	**-0.549**	0.477	**-0.571**
	0.890	<.001		<.001	**0.003**	<.001	0.001	**<.001**	<.001	**<.001**
Storage lipids	0.194	**-0.113**	0.788	1.000	0.126	0.273	0.247	**-0.536**	0.069	**-0.362**
	0.054	**0.264**	<.001		0.213	0.006	0.014	**<.001**	0.495	**<.001**
Intermediate metabolite lipids	0.501	**-0.600**	**-0.294**	0.126	1.000	0.203	0.162	-0.038	**-0.778**	0.124
	<.001	**<.001**	**0.003**	0.213		0.044	0.109	0.709	**<.001**	0.220
DNA	0.732	0.170	0.359	0.273	0.203	1.000	0.976	-0.080	-0.179	**-0.666**
	<.001	0.092	<.001	0.006	0.044		<.001	0.429	0.077	**<.001**
RNA	0.742	0.200	0.329	0.247	0.162	0.976	1.000	<.001	-0.109	**-0.718**
	<.001	0.047	0.001	0.014	0.109	<.001		0.999	0.281	**<.001**
Intestinal epithelial thickness	0.126	**-0.237**	**-0.549**	**-0.536**	-0.038	-0.080	0.000	1.000	**-0.306**	0.425
	0.213	**0.018**	**<.001**	**<.001**	0.709	0.429	0.999		**0.002**	<.001
Liver area	**-0.573**	0.784	0.477	0.069	**-0.778**	-0.179	-0.109	**-0.306**	1.000	**-0.420**
	<.001	<.001	<.001	0.495	**<.001**	0.077	0.281	**0.002**		**<.001**
Gill arch count	0.465	**-0.789**	**-0.253**	0.270	0.527	0.207	0.194	-0.006	**-0.640**	0.113
	<.001	**<.001**	**0.012**	0.007	<.001	0.039	0.055	0.950	**<.001**	0.264

Appendix 4.8 Pearson correlation and significance between variable scores on PC2 for *Ulvaria subbifurcata* based on 100 imputations. Areas in grey represent repeated values in the table included for completeness. Numbers in bold are significant negative correlations ($p < 0.05$).

	Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossif-ication
Carbon	1.000	0.985	**-0.460**	0.822	**-0.333**	0.157	0.339	0.018	**-0.315**	**-0.853**
		<.001	**<.001**	<.001	**0.001**	0.120	0.001	0.862	**0.001**	**<.001**
Nitrogen	0.985	1.000	**-0.490**	0.819	**-0.284**	0.213	0.332	-0.095	**-0.370**	**-0.834**
	<.001		**<.001**	<.001	**0.004**	0.034	0.001	0.347	**<.001**	**<.001**
Structural lipids	**-0.460**	**-0.490**	1.000	**-0.111**	0.843	**-0.882**	**-0.912**	**-0.355**	0.202	0.274
	<.001	**<.001**		**0.270**	<.001	**<.001**	**<.001**	**<.001**	0.044	0.006
Storage lipids	0.822	0.819	**-0.111**	1.000	0.079	-0.166	-0.086	**-0.365**	**-0.594**	**-0.889**
	<.001	<.001	**0.270**		0.436	0.098	0.396	**<.001**	**<.001**	**<.001**
Intermediate metabolite lipids	**-0.333**	**-0.284**	0.843	0.079	1.000	**-0.735**	**-0.919**	**-0.702**	-0.078	0.156
	0.001	**0.004**	<.001	0.436		**<.001**	**<.001**	**<.001**	0.440	0.121
DNA	0.157	0.213	**-0.882**	-0.166	**-0.735**	1.000	0.903	0.297	-0.027	0.112
	0.120	0.034	**<.001**	0.098	**<.001**		<.001	0.003	0.787	0.267
RNA	0.339	0.332	**-0.912**	-0.086	**-0.919**	0.903	1.000	0.611	0.117	-0.060
	0.001	0.001	**<.001**	0.396	**<.001**	<.001		<.001	0.248	0.556
Intestinal epithelial thickness	0.018	-0.095	**-0.355**	**-0.365**	**-0.702**	0.297	0.611	1.000	0.548	0.157
	0.862	0.347	**<.001**	**<.001**	**<.001**	0.003	<.001		<.001	0.118
Liver area	**-0.315**	**-0.370**	0.202	**-0.594**	-0.078	-0.027	0.117	0.548	1.000	0.534
	0.001	**<.001**	0.044	**<.001**	0.440	0.787	0.248	<.001		<.001
Gill arch count	**-0.454**	**-0.400**	0.557	**-0.438**	0.593	**-0.355**	**-0.495**	**-0.324**	0.454	0.511
	<.001	**<.001**	<.001	**<.001**	<.001	**<.001**	**<.001**	**0.001**	<.001	<.001

Appendix 4.9 Pearson correlation between variable scores on PC2 for pelagic and demersal eggs. Areas in grey represent repeated values in the table included for completeness. Numbers in bold are significant negative correlations in the species-independent model ($p < 0.05$). Correlation coefficients that are not significantly different using Fisher's Z-test between pelagic and demersal eggs are underlined. Numbers marked with (*) are **not** significantly different from zero.

		Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossif-icat-ion
Carbon	**Pelagic**	1.00	0.46	0.76	0.70	-0.51	**0.19**	***0.07**	-0.34	**-0.55**	**-0.64**
	Demersal		0.70	0.27	0.79	0.33	**-0.39**	**-0.26**	*-0.06	**-0.56**	**0.02**
Nitrogen	**Pelagic**	0.46	1.00	**-0.31**	-0.58	**-0.43**	0.61	0.82	-0.15	0.27	0.45
	Demersal	0.70		**-0.31**	0.30	**0.17**	-0.25	-0.10	-0.19	*-0.05	0.39
Structural lipids	**Pelagic**	0.76	**-0.31**	1.00	0.88	-0.15	**-0.36**	**-0.36**	-0.23	**-0.62**	**-0.62**
	Demersal	0.27	**-0.31**		0.66	0.27	**-0.34**	**-0.38**	*0.00	**-0.19**	**-0.46**
Storage lipids	**Pelagic**	0.70	-0.58	0.88	1.00	***-0.01**	**-0.48**	**-0.61**	**-0.14**	**-0.38**	-0.39
	Demersal	0.79	0.30	0.66		**0.47**	**-0.41**	**-0.38**	**-0.51**	**-0.66**	*0.02
Intermediate metabolite lipids	**Pelagic**	-0.51	**-0.43**	-0.15	***-0.01**	1.00	**-0.48**	**-0.50**	**0.60**	**0.58**	*0.02
	Demersal	0.33	**0.17**	0.27	**0.47**		**-0.71**	**-0.38**	**-0.35**	**-0.57**	0.10
DNA	**Pelagic**	**0.19**	0.61	**-0.36**	**-0.48**	**-0.48**	1.00	0.86	-0.59	-0.28	**-0.19**
	Demersal	**-0.39**	-0.25	**-0.34**	**-0.41**	**-0.71**		0.86	0.08	0.40	**-0.20**
RNA	**Pelagic**	***0.07**	0.82	**-0.36**	**-0.61**	**-0.50**	0.86	1.00	-0.59	-0.37	0.16
	Demersal	**-0.26**	-0.10	**-0.38**	**-0.38**	**-0.38**	0.86		0.19	0.46	-0.23
Intestinal epithelial thickness	**Pelagic**	-0.34	-0.15	-0.23	**-0.14**	**0.60**	-0.59	-0.59	1.00	0.75	0.29
	Demersal	*-0.06	-0.19	*0.00	**-0.51**	**-0.35**	0.08	0.19		0.50	-0.32
Liver area	**Pelagic**	**-0.55**	0.27	**-0.62**	**-0.38**	**0.58**	-0.28	-0.37	0.75	1.00	**0.66**
	Demersal	**-0.56**	*-0.05	**-0.19**	**-0.66**	**-0.57**	0.40	0.46	0.50		**0.07**
Gill arch count	**Pelagic**	0.70	-0.20	0.71	0.71	**-0.72**	***0.10**	**-0.14**	**-0.61**	**-0.66**	**-0.40**
	Demersal	*0.27	*-0.06	-0.22	*0.01	**0.15**	**-0.19**	**-0.28**	***-0.12**	**-0.06**	**0.22**

Appendix 4.10 Pearson correlation between variable scores on PC2 for pelagic and demersal larvae. Areas in grey represent repeated values in the table included for completeness. Numbers in bold are significant negative correlations ($p < 0.05$) in the species-independent model. Correlation coefficients that are not significantly different using Fisher's Z-test between pelagic and demersal eggs are underlined. Numbers marked with (*) are **not** significantly different from zero.

		Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossif-icat-ion
Carbon	**Pelagic**	1.00	0.67	0.46	0.77	0.13	**-0.27**	**-0.27**	-0.16	**-0.62**	**-0.19**
	Demersal	1.00	0.20	0.27	0.69	-0.26	***0.03**	**0.72**	*0.08	**0.31**	**-0.19**
Nitrogen	**Pelagic**	0.67	1.00	**-0.29**	*0.03	***-0.01**	*-0.05	0.21	-0.15	**0.07**	0.46
	Demersal	0.20	1.00	**-0.52**	0.13	**0.13**	0.48	0.46	-0.42	**-0.25**	-0.25
Structural lipids	**Pelagic**	0.46	**-0.29**	1.00	0.76	0.22	**-0.40**	**-0.41**	-0.12	**-0.34**	**-0.55**
	Demersal	0.27	**-0.52**	1.00	0.59	-0.43	**0.21**	***0.05**	0.45	**-0.15**	***0.04**
Storage lipids	**Pelagic**	0.77	*0.03	0.76	1.00	**0.43**	**-0.50**	**-0.52**	**-0.46**	**-0.62**	-0.07
	Demersal	0.69	0.13	0.59	1.00	**-0.51**	**0.43**	**0.44**	***0.10**	**-0.27**	-0.39
Intermediate metabolite lipids	**Pelagic**	0.13	***-0.01**	0.22	**0.43**	1.00	**-0.70**	**-0.47**	**-0.13**	**-0.34**	*0.00
	Demersal	-0.26	**0.13**	-0.43	**-0.51**	1.00	***0.03**	**0.26**	**0.46**	**0.38**	0.68
DNA	**Pelagic**	**-0.27**	*-0.05	**-0.40**	**-0.50**	**-0.70**	1.00	0.88	-0.16	0.31	**-0.21**
	Demersal	***0.03**	0.48	**0.21**	**0.43**	***0.03**	1.00	0.29	0.24	-0.59	***-0.11**
RNA	**Pelagic**	**-0.27**	0.21	**-0.41**	**-0.52**	**-0.47**	0.88	1.00	-0.08	0.23	-0.14
	Demersal	**0.72**	0.46	***0.05**	**0.44**	**0.26**	0.29	1.00	0.28	0.41	*0.08
Intestinal epithelial thickness	**Pelagic**	-0.16	-0.15	-0.12	**-0.46**	**-0.13**	-0.16	-0.08	1.00	0.61	-0.24
	Demersal	*0.08	-0.42	0.45	***0.10**	**0.46**	0.24	0.28	1.00	0.25	0.59
Liver area	**Pelagic**	**-0.62**	**0.07**	**-0.34**	**-0.62**	**-0.34**	0.31	0.23	0.61	1.00	**0.26**
	Demersal	**0.31**	**-0.25**	**-0.15**	**-0.27**	**0.38**	-0.59	0.41	0.25	1.00	**0.31**
Gill arch count	**Pelagic**	0.47	-0.15	0.19	0.35	**-0.22**	**-0.11**	**-0.28**	**-0.30**	**-0.32**	***0.02**
	Demersal	-0.24	0.36	-0.77	*-0.69	**0.63**	**-0.18**	**0.17**	***-0.05**	**0.45**	**0.32**

Appendix 4.11 Pearson correlation between variable scores on PC2 for larvae spawned in different seasons. Areas in grey represent repeated values in the table included for completeness. Numbers in bold are significant negative correlations ($p < 0.05$) in the species-independent model. Numbers marked with (*) are **not** significantly different from zero.

		Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossif-ication
Carbon	**Spring**		0.61	0.71	0.61	-0.49	***0.01**	***0.06**	*-0.11	**-0.49**	**-0.56**
	Summer		0.24	0.38	0.72	*0.01	***0.08**	**0.19**	0.16	**-0.24**	**-0.59**
	Winter		0.93	0.19	0.91	0.69	**-0.78**	**-0.77**	-0.56	**-0.88**	**0.77**
Nitrogen	**Spring**	0.61		***-0.05**	-0.25	**-0.46**	0.41	0.69	-0.18	0.27	0.39
	Summer	0.24		**-0.52**	-0.25	**-0.24**	0.44	0.58	*-0.02	0.51	*0.07
	Winter	0.93		**-0.18**	0.67	**0.72**	-0.83	-0.78	-0.42	-0.79	0.78
Structural lipids	**Spring**	0.71	***-0.05**		0.81	*-0.05	**-0.49**	**-0.25**	*-0.02	**-0.57**	**-0.56**
	Summer	0.38	**-0.52**		0.79	0.26	**-0.44**	**-0.55**	*-0.08	**-0.21**	**-0.55**
	Winter	0.19	**-0.18**		0.56	0.18	***0.00**	**-0.14**	*-0.08	**-0.23**	**-0.35**
Storage lipids	**Spring**	0.61	-0.25	0.81		**0.26**	**-0.42**	**-0.35**	***-0.09**	**-0.26**	-0.31
	Summer	0.72	-0.25	0.79		***0.04**	**-0.20**	**-0.33**	**-0.19**	**-0.40**	-0.70
	Winter	0.91	0.67	0.56		**0.76**	**-0.72**	**-0.68**	**-0.84**	**-0.91**	0.88
Intermediate metabolite lipids	**Spring**	-0.49	**-0.46**	*-0.05	**0.26**		**-0.53**	**-0.44**	**0.55**	**0.56**	*-0.04
	Summer	*0.01	**-0.24**	0.26	***0.04**		**-0.22**	**-0.19**	**-0.15**	**-0.30**	0.00
	Winter	0.69	**0.72**	0.18	**0.76**		**-0.96**	**-0.68**	**-0.57**	**-0.82**	0.30
DNA	**Spring**	***0.01**	0.41	**-0.49**	**-0.42**	**-0.53**		0.82	-0.73	-0.32	***-0.12**
	Summer	***0.08**	0.44	**-0.44**	**-0.20**	**-0.22**		0.87	*-0.01	*-0.02	**-0.01**
	Winter	**-0.78**	-0.83	**0.00**	**-0.72**	**-0.96**		0.87	0.45	0.84	**-0.54**
RNA	**Spring**	***0.06**	0.69	**-0.25**	**-0.35**	**-0.44**	0.82		-0.72	-0.45	*0.08
	Summer	**0.19**	0.58	**-0.55**	**-0.33**	**-0.19**	0.87		0.16	0.26	*0.03
	Winter	**-0.77**	-0.78	**-0.14**	**-0.68**	**-0.68**	0.87		0.43	0.75	-0.52

Appendix 4.11 (cont.)

		Carbon	Nitrogen	Structural lipids	Storage lipids	Intermediate metabolite lipids	DNA	RNA	Intestinal epithelial thickness	Liver area	Ossification
Intestinal epithelial thickness	**Spring**	-0.11	-0.18	*-0.02	***-0.09**	**0.55**	-0.73	-0.72		0.71	0.20
	Summer	0.16	*-0.02	*-0.08	**-0.19**	**-0.15**	*-0.01	0.16		0.22	0.31
	Winter	-0.56	-0.42	*-0.08	**-0.84**	**-0.57**	0.45	0.43		0.82	-0.86
Liver area	**Spring**	**-0.49**	0.27	**-0.57**	**-0.26**	**0.56**	-0.32	-0.45	0.71		**0.69**
	Summer	**-0.24**	0.51	**-0.21**	**-0.40**	**-0.30**	*-0.02	0.26	0.22		**0.54**
	Winter	**-0.88**	-0.79	**-0.23**	**-0.91**	**-0.82**	0.84	0.75	0.82		**-0.70**
Gill arch count	**Spring**	0.62	*-0.02	0.54	0.39	**-0.77**	**0.28**	***-0.01**	**-0.53**	**-0.60**	**-0.26**
	Summer	0.27	-0.44	0.10	0.15	**0.16**	**-0.15**	**-0.32**	**-0.10**	**0.00**	***0.07**
	Winter	0.40	0.40	-0.42	0.25	**0.24**	**-0.42**	**-0.34**	**-0.27**	**-0.26**	**0.30**

Chapter 5 What makes a good larva? Using survival to infer the importance of physiological priorities in the early development of multiple species of fish

5.1 Introduction

The larval period of many fish is one of extremely high mortality, often with more than 99% of individuals failing to reach metamorphosis (Benoit et al. 2000). Even in an aquaculture setting where predation, environmental variability, and foraging costs are minimised, survival rates exceeding 40% are considered exceptional (Brown and Puvanendran 2003). Because the larval period is considered a strong predictor of future cohort success (Houde 1989), understanding the factors that affect the vulnerability of individuals to mortality has major biological and economic implications both in the wild and in aquaculture settings (Leggett and Deblois 1994). The earliest hypothesis regarding high larval mortality was that it primarily resulted from poor feeding success during a critical period of development (Hjort 1914). This was further developed by Cushing and Harris (1973) with the "match-mismatch" hypothesis in which the synchronisation of the occurrence of larval fish with cycles of food sources was essential for larval survival. Other major sources of loss that have been investigated include oceanic transport (Norcross and Shaw 1984), developmental bottlenecks (e.g. Balon 1979), predation (e.g. Ware 1975, Rice et al. 1993, Bailey et al. 2003), and size-dependent mortality (e.g. Pepin 1993, Meekan and Fortier 1996). From an individual perspective, some characteristics, or combination thereof, may allow selection of a few of these fish to survive, metamorphose,

and subsequently reproduce. The question of larval mortality can thus be rephrased - are there certain characteristics of fish larvae that increase their likelihood of surviving?

Selective forces act on variation among individuals, and many fish species display a naturally high intrinsic level of variability in developmental characteristics (Fuiman et al. 2005). This variation is often ignored when data are analysed using average measures of state rather than their distribution(s) (Chambers et al. 1988, Benoit et al. 2000, Pepin et al. 1999, Peacor et al. 2007). As ontogeny progresses many larvae die, potentially changing the distribution of this variation. By examining the distribution surrounding the mean as a function of the progression to metamorphosis, trait values can be discerned that are potentially optimal or beneficial for a fish to possess in order to survive through the larval period. As an example, previous work by Pepin et al. (1999) examined variation surrounding instantaneous growth rate (as represented by RNA/DNA) of larval fish in the field and found that the range of variation in growth rates decreased with increasing size, narrowing toward a higher growth rate. This suggests that if there is serial correlation (e.g. when values are very dependent upon adjacent values) in condition then individuals with high growth rates are more likely to survive. Previous examination of otoliths has shown that serial correlation in growth rate is substantial (Gallego et al. 1996, Pepin et al. 2001), and it is likely that this applies to other condition measures. Examination of variation surrounding growth rate has been studied, not only on instantaneous growth rate using nucleic acids, but also otolith increments (e.g. Buckley et al. 2006) and length (e.g. Erzini 1994, Benoit et al. 2000).

High growth rates are not likely the only feature of a successful larva. Similar to growth rate, it is likely that there are ideal developmental trajectories in other variables

that are associated with successful metamorphosis. One potential variable is lipid content. Lipids in larval fish are a major source of nutrition and structure (Wiegand 1996). The amount of lipid an animal possesses affects its ability to survive periods of food deprivation (Rainuzzo et al. 1997), its swimming performance (Kamler 2008) and generally its available energy (Shulman and Love 1999). Protein is also linked to survival of larval fish (Pangle et al. 2005) because it allows the maintenance and repair of the body, is a source of energy and has been used as an indicator of long-term growth (on the scale of weeks to months) (Weber et al. 2003, Rosa and Nunes 2004). The combined examination of the variation and trajectory of nucleic acid ratios, lipids and protein in age- and/or size-dependent analysis can establish what factors are key to a successful larva. This analysis of a successful larva can establish whether some physiological elements are more important for some species than others, all of which may in turn improve our understanding of recruitment processes (Rice et al. 1993).

In this laboratory analysis, the extrinsic mortality sources of the field (e.g., predation, starvation, environmental effects) are minimised, allowing intrinsic developmental variation to be isolated and quantified. This study explicitly addresses four questions: (1) Is there intrinsic selection in the absence of known selective pressures (e.g., predation) or is the pattern of loss among larval fish apparently random? (2) What is the direction of this selection? (3) Does the intensity or rate of selection differ among species? (4) Does the direction of selection vary among species? I will address these questions about how the distribution of three variables (RNA/DNA ratio, and the amounts of protein and lipids relative to body size) changes as animals approach metamorphosis when extrinsic pressures are standardised in a laboratory setting. By quantifying how the

variation changes among different measures of state through development, features that are associated with greater survival in the absence of strong selective forces can be identified. This could serve as a basis against which to contrast the intensity of selection measured in cohorts of larval fish in the field.

5.2 **Materials and methods**

5.2.1 *Rearing, sampling and processing*

Five species of larval fish were reared under controlled laboratory conditions: *Gadus morhua* (Atlantic cod), *Ulvaria subbifurcata* (radiated shanny), *Cyclopterus lumpus* (Atlantic lumpfish), *Pseudopleuronectes americanus* (winter flounder), and *Myoxocephalus scorpius* (shorthorn sculpin). For rearing, sampling and processing details see Chapter 2.

5.2.2 *Analysis*

All variables were ln-transformed to achieve a near-normal error distribution. The ratios of nitrogen/carbon, storage lipids/dry mass, structural lipids/dry mass, intermediate metabolite lipids/dry mass and RNA/DNA were estimated for each larva. Each larva was treated as an independent data point and was not grouped according to sampling date. This allowed the individual variability of the larvae to be available for analysis without the masking that would have resulted from pooling observations.

The distributions of each of these ratios were investigated separately for each species using the non-parametric methods described by Evans and Rice (1988) and summarised herein. A cumulative distribution function (CDF) using kernel smoothing

was created allowing local weighting (Davison and Hinkley, 1997, eq. 7.24) to provide a continuous representation of the change in the distribution of a variable in relation to another metric, in this case body mass. Bandwidth, the parameter defining the extent of local influence, was estimated for each variable for each species using leave-one-out cross-validation methods: when each observation was deleted in turn, the rest of the data were used to predict the missing value. This process results in residuals, for which the sum of squared differences of all observations was computed and values of sums of squares which minimised bandwidth were determined. When the bandwidth for each variable had been explored for each species, the average bandwidth for all species was calculated and used to develop a new CDF for each species. This enabled robust comparison among species regardless of different sample sizes for each species. The 10%, 50% and 90% cumulative probabilities were estimated, and the difference between the 10% and 90% percentiles (hereafter referred to as scatter) was used as a measure of variability.

To answer the first question of whether there is intrinsic selection, one must evaluate whether the change in scatter over the interval from hatch to metamorphosis is significantly different from that which would be found in random data. Using residuals from the estimated median of each ratio and dry mass, 500 synthetic random data sets were created by recombining the two variables in random pairs. Scatter at a given dry mass was compared to the randomised data sets. The observed scatter at that particular size was considered significantly different from random if the scatter was greater than 97.5% or less than 2.5% of the scatters for randomised data sets (see Pepin et al. 1999).

The change in the medians of each dependent variable in relation to dry mass using a regression was used to distinguish if the selection was stabilizing or had a direction of selection. The sign of the slope in the regression indicated the direction of selection.

To determine whether the magnitude of selection differed among species, the rate of change in scatter relative to dry mass served as an estimate of the intensity of selection. The difference in the strength in selection between species was quantified using an analysis of covariance (ANCOVA), with mass and species as continuous and categorical variables respectively. A significant interaction term (mass×species) indicated a significant difference in the strength of selection among species.

Similarly, to determine whether any direction of selection differed among species, an ANCOVA was also applied. The medians for each bandwidth step of the creation of the CDF were combined for each species of each ratio and examined for an effect on/from dry mass, species, and the interaction of body size and species. Significance of this interaction term indicated a difference in the relationship of the medians to dry mass among species.

5.3 **Results**

Dry mass of species presented both a range and an overlap large enough to allow comparison among species (Table 5.1). For descriptive details of ratios see Appendix 5.1. The bandwidth (ln[dry mass]) used for all analyses was set at 0.153. See the results section of Chapter 2 for details on data reliability.

5.3.1 *Evidence of intrinsic selection*

The evidence for intrinsic selection was mixed, depending on the state variable and the species examined. The slope of scatter of nitrogen/carbon relative to dry mass was significant for *G. morhua, C. lumpus, M. scorpius* and *P. americanus,* but not for *U. subbifurcata* (original data -Fig. 5.1, scatter only - Fig. 5.2, for details see Appendix 5.2). When the scatter was compared to a randomised data set, the distribution was significantly different from random for *G. morhua, C. lumpus,* and *P. Americanus*, with the initial scatter being greater than the final scatter relative to the randomised data sets. *Ulvaria subbifurcata* and *M. scorpius* exhibited a different pattern, with significantly greater scatter in the middle of the range of dry mass (Fig. 5.2).

The slope of scatter in the standardised amount of intermediate metabolite lipids relative to dry mass was significantly negative for *G. morhua*, *M. scorpius*, and *U. subbifurcata*, whereas the slope was not significant for either *C. lumpus* or *P. americanus* (original data - Fig. 5.3, scatter only - Fig. 5.4, for details see Appendix 5.2). *Myoxocephalus scorpius* and *U. subbifurcata* began development with significantly greater scatter, and at metamorphosis there was significantly less than random scatter (Fig. 5.4). *Gadus morhua* and *C. lumpus* both exhibited a significantly higher degree of scatter at the beginning of development, but the apparent decline as the animals approached metamorphosis could not be differentiated from a randomization of the data. *Pseudopleuronectes americanus* did not follow either of these patterns and was not significantly different from a random distribution during the majority of development.

There was a significant decrease in the amount of scatter in storage lipids in relation to dry mass as animals grew for all species, except *M. scorpius* (original data –

Fig. 5.5,scatter only Fig. 5.6, for details see Appendix 5.2). The lack of a significant decrease in scatter with development in *M. scorpius* is the result of a significant increase in variability in storage lipids at intermediate body sizes.

The ratio of structural lipids to dry mass showed a significant negative slope in scatter in only one species, *U. subbifurcata*, while there was a significantly positive slope in scatter in *G. morhua* (original data - Fig. 5.7, scatter only - Fig. 5.8, for details see Appendix 5.2). Comparison with randomly generated data sets were for the most part without significance, except for an increase in scatter during the middle of development for *M. scorpius* and *C. lumpus* (Fig. 5.8).

Finally, the change in scatter of RNA/DNA as larvae grew also differed among species. All species, except *C. lumpus*, showed a slope significantly different from zero for the change in scatter relative to dry mass (original data Fig. 5.9, scatter only Fig. 5.10, for details see Appendix 5.2). When the distribution was compared to randomly generated data sets, there were no significant differences in scatter, although scatter decreased in *P. americanus* and *U. subbifurcata* (Fig. 5.10).

5.3.2 *Direction of selection*

When the medians of the ratios were examined for the direction of change in relation to body size, the direction of selection was consistent among all species only for the ratio of intermediate metabolite lipids to dry mass, where there was a consistent decrease (Fig. 5.3, for details see Appendix 5.4). The ratio of storage lipids to dry mass increased with body size in four of five species, with a significant decrease in *C. lumpus* (Fig. 5.5, for details see Appendix 5.4). Structural lipids to dry mass was highly variable

with slopes ranging from -0.491 in *C. lumpus* to 0.381 for *P. americanus*, with only *G. morhua* and *C. lumpus* having significant slopes (Fig. 5.7, for details see Appendix 5.4). The median of the nitrogen/carbon ratio increased significantly with body size for *G. morhua*, *M. scorpius*, and *C. lumpus*, but decreased significantly for *P. americanus* and *U. subbifurcata* (Fig. 5.1, for details see Appendix 5.4). For RNA/DNA the median increased significantly for all species with the exception of *P. americanus* where the slope was not significantly different from zero (Fig. 5.9, for details see Appendix 5.4).

5.3.3 *Species effects on intensity of selection*

There was a significant effect of species on the strength of the overall relationship between scatter and body size for all ratios examined (Fig. 5.11, for details see Appendix 5.6). The species that demonstrated the strongest change in scatter differed across the five physiological metrics considered in this chapter.

The rate of change in variance in intermediate metabolite lipids relative to body size was greatest for *M. scorpius*, followed by *U. subbifurcata. Gadus morhua*, *C. lumpus* and *P. americanus* did not show strong evidence of intrinsic selection. RNA/DNA exhibited the greatest variation in the strength of selection among species, with *P. americanus* showing the strongest selective loss of variability among individuals, while *G. morhua* and *U. subbifurcata* showed relatively weak selection. In contrast, *M. scorpius* demonstrated a strong increase in the scatter of RNA/DNA, which is suggestive of a distinct lack of stabilising selection.

5.3.4 *Species effects on direction of selection*

There were also significant species effects on the direction of selection when the change in the median was evaluated as animals grew (Fig. 5.12, for details see Appendix 5.7). Differences among species in all ratios under study were evidenced through significant interactions when subjected to an ANCOVA. Although all species showed a negative trend in the median relative to body size for intermediate metabolite lipids, *P. americanus* displayed a much greater slope than other species at -1.269. *Gadus morhua, C. lumpus*, and *U. subbifurcata* had similar slopes and these were greater than that for *M. scorpius.* The slopes of the medians for structural lipids were even more varied than that for the other lipid classes, with *C. lumpus* and *U. subbifurcata* showing decreasing trends with increased size while all other species increased. Finally, the median of RNA/DNA increased with body size, except in the case of *P. americanus,* in which there was no significant change with increasing body size. *Myoxocephalus scorpius* and *C. lumpus* had the greatest slopes.

5.4 **Discussion**

The variation among individuals changed as animals increased in size, indicating non-random selection, which varied in intensity among species and which is consistent with Peacor et al.'s (2007) study of variability in animal size during development. In the cases of intermediate metabolite lipids, storage lipids and nitrogen/carbon ratios (excluding *M. scorpius*), there were decreases in scatter as the larvae grew. Of the species examined, *G. morhua, P. americanus* and *U. subbifurcata* showed the most evidence of contraction in variation of variables under study as body size increased. This decrease in

the variation as size increased suggests non-random survival, and thus selection was occurring for preferred trait values, given the assumption of serial correlation. In contrast, an increase in variation among individuals as a cohort develops, such as that seen in RNA:DNA for *M. scorpius*, has been suggested to result from stochastic processes (DeAngelis et al. 1993, Ludsin and DeVries 1997) or learning (Dukas and Bernay 2000).

5.4.1 *Evidence of intrinsic selection*

Field studies have previously shown that the distribution of growth rates or condition of larval fish contracts towards faster development rates with increasing body size in the larval period, as demonstrated by the changes in RNA/DNA (Pepin et al. 1999) and otolith increment width (e.g. Post and Prankevicius 1987, Meekan and Fortier 1996). This was largely confirmed for the species in this laboratory study, with the exception of *P. americanus*, which did not show a significant change in the median. However, the ranges of RNA/DNA ratios in this study for *P. americanus* and *U. subbifurcata* were similar to values found by Pepin et al. (1999) under natural conditions, supporting not only the reliability of this study but also strongly supporting intrinsic selection.

The general consistency in the patterns of change in nucleic acid ratios in the two species common to the field and laboratory studies is contrary to expectations. A broader range of variation in condition and a lower rate of change with increasing body size would have been expected than would be observed in the field because the laboratory largely isolates animals from many extrinsic forces including starvation, predation and temperature variability. Although a comparison based on only two species is restrictive, the similarity between outcomes from laboratory and field settings suggests that the

underlying mortality rates of larval fish in the field may reflect an intrinsic potential for failure more than environmental effects on year class strength.

5.4.2 *Species effects on intrinsic selection*

Direction and strength of intrinsic selection were significantly different among species for all ratios examined, as has been found previously (Pepin et al. 1999). For instance, the relative amounts of nitrogen/carbon, storage lipids/dry mass, structural lipids/dry mass, and RNA/DNA increased during development while intermediate metabolites/dry mass decreased in *G. morhua* and *M. scorpius*. In contrast, *P. americanus* showed decreases in nitrogen/carbon, RNA/DNA, and intermediate metabolite lipids/dry mass from hatch to metamorphosis, while displaying increasing trends in storage lipids/dry mass and structural lipids/dry mass. In terms of both storage and structural lipids, *C. lumpus* stands out as the species with the greatest decrease in the median value of lipids to dry mass. The higher starting value for storage and structural lipids of *C. lumpus* relative to the other species may account for the lack of an increase through the larval period and this higher starting value may be reflective of the unique life history of *C. lumpus* relative to the other species in the study. *Cyclopterus lumpus* is the only species studied here with a larva that develops almost entirely in a demersal environment possibly allowing for greater parental care and selecting for greater initial investment in energetically costly lipids given the higher survival chances of each larva. Although *C. lumpus* was the extreme example, many species differed in direction and strength of selection, suggesting that species experience intrinsic selection uniquely.

There was consistent contraction to the lower end of the distribution of intermediate metabolite lipids among all species, although this was not significant in *C. lumpus* and *P. americanus*. Intermediate metabolites are small lipid moieties of larger storage lipids (Weigand 1996) or, alternatively, they are a product of breakdown of storage lipids when energy demands are greater than the energy consumed by the animal (Desvilettes et al. 1997). The possible interpretations are that animals which have a low retention time for small lipid classes before incorporating them into larger lipid classes are more successful, or alternatively, that successful animals are not breaking down larger lipids to use as energy. With the exception of *C. lumpus*, larvae with a higher concentration of storage lipids are making up more of the population as the animals progress towards metamorphosis. The simultaneous accumulation of storage lipids seen in this study is also indicative of the larvae being in good condition (Fraser 1989), suggesting that the former of the two interpretations is more likely than the latter. The larger animals in the distribution tended to have plenty of storage lipids, showing that energy intake likely exceeded energy requirements during this experimental study. Thus, it appears that animals that have low retention times for small lipid classes before incorporating them into larger lipid classes are more successful.

As with storage and intermediate metabolite lipids, many of the ratios in this study are functionally interrelated. Intermediate metabolite lipids are related to structural lipids in much the same way as storage lipids, because they represent building blocks for more complex structures (Kattner et al. 2007). The ratio of nitrogen to carbon is an indicator of protein content, and the ratio of RNA to DNA is functionally related to protein synthesis. Similar results across species are expected for intermediate metabolite lipids and

structural lipids, protein and nucleic acids, and as already mentioned intermediate metabolite lipids and storage lipids. In contrast, while intermediate metabolite lipids decrease in all species, and storage lipids generally increase, the relationship with structural lipids was inconsistent. This may be an artefact of methodological limitations, which precluded the inclusion of phospholipids, which are a major component of structural lipids. As a result, in the study of food chain dynamics and feed development, the structural lipids that were included in this study (steryl esters, sterols and their components) may not be as important as other elements in determining larval success. The other functional relationship observed in this study, between nucleic acid ratios and nitrogen/carbon ratios, was relatively well supported. Results were as expected in all species with the exception of *U. subbifurcata*, where the nitrogen/carbon ratio decreased while the nucleic acid ratio increased. However, this inconsistency found in *U. subbifurcata* was not statistically significant.

5.4.3 *Conclusion*

The repeated reduction in variation as development progresses addresses a fundamental question in reproductive ecology about the number and quality of offspring, by suggesting that a number of these larvae were unlikely to survive as a result of intrinsic selection irrespective of environmental forces. The source of this variation at hatch, and the subsequently unsuccessful larvae, could be random genetic variation, but in most organisms' variation in offspring traits is the result of phenotypic plasticity, rather than alternative genotypes (Moran 1992) implying polyphenism, bet-hedging or some other mechanism. Polyphenism is favoured by temporal variation in environments, as

may be experienced by marine fishes (McGinley and Geber 1987, Moran 1992). This could also be an allocation of limited resources into a form of diversified bet-hedging, where a small proportion of larvae receive an unequal amount of investment, making them superior to other larvae from the same spawning (Philippi and Seger 1989). Regardless of the mechanism, the results of this study suggest that selection irrespective of external factors is likely occurring.

This study of North Atlantic fishes has shown that the survival of these larvae is often non-random, with considerable variability in the inherent potential for survival of offspring produced among different species. There is intrinsic selection occurring on different aspects of larval investment and not just on growth rate. Other factors, such as various lipids types, also show a reduction in the variability among individuals as larvae develop, indicating that selective forces are acting on a variety of traits simultaneously. However, the magnitude and direction of selection may differ significantly among species. The similarity of the nucleic acid results of this laboratory study to the field observations by Pepin et al. (1999) suggests that there are underlying intrinsic selective forces affecting larval fish development.

5.5 **Tables and figures**

Table 5.1 Species, range and number of animals used for the analyses.

Species	Dry mass (mg)[1]		
	Minimum	Maximum	N
G. morhua	0.015	22.886	203
M. scorpius	0.206	12.544	213
C. lumpus	0.189	22.332	183
P. americanus	0.009	0.205	82
U. subbifurcata	0.007	3.134	43

[1] For analyses, dry mass was measured in µg for unit agreement with other variables

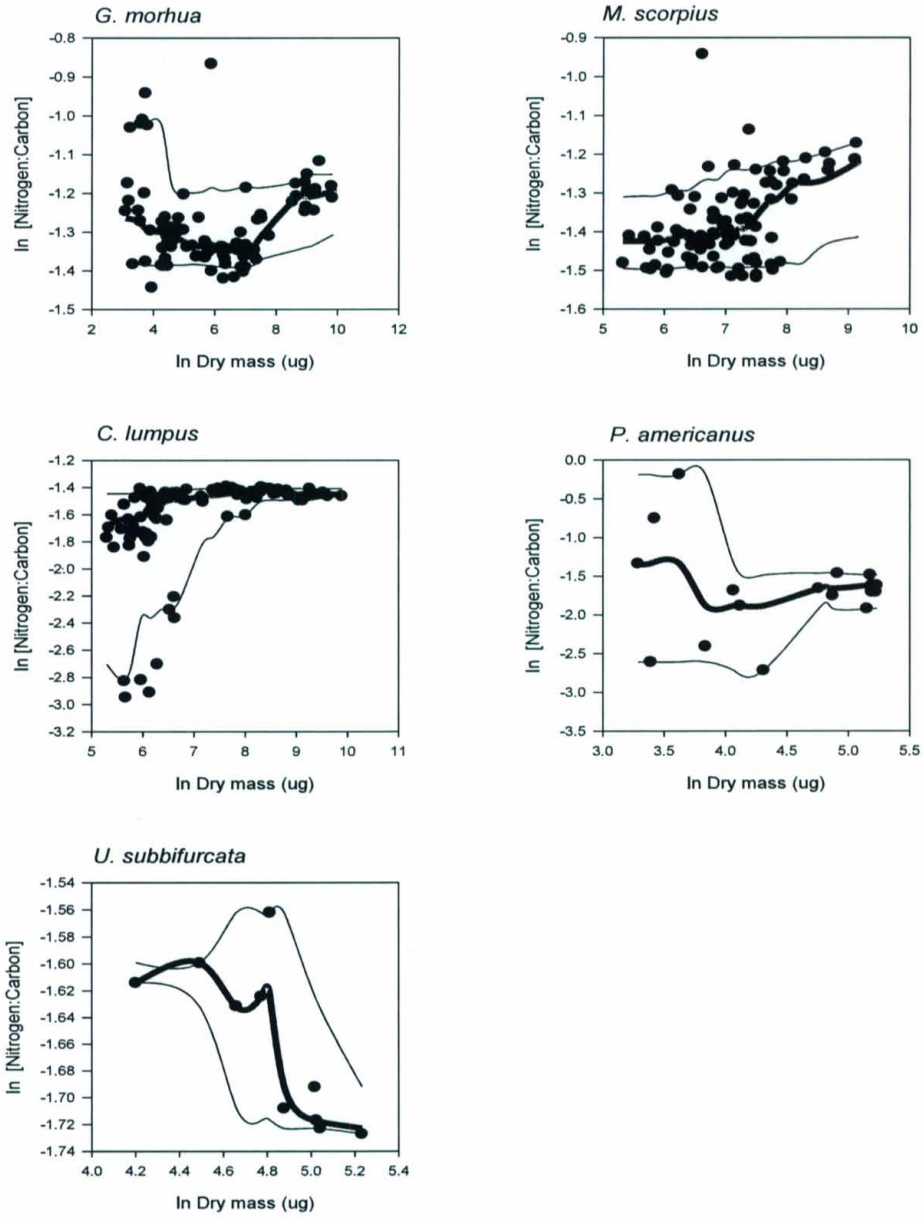

Figure 5.1 Observed nitrogen:carbon in relation to dry mass. Estimated 10^{th}, 50^{th}, and 90^{th} percentiles are shown (solid lines). The heavy line represents the 50^{th} percentile.

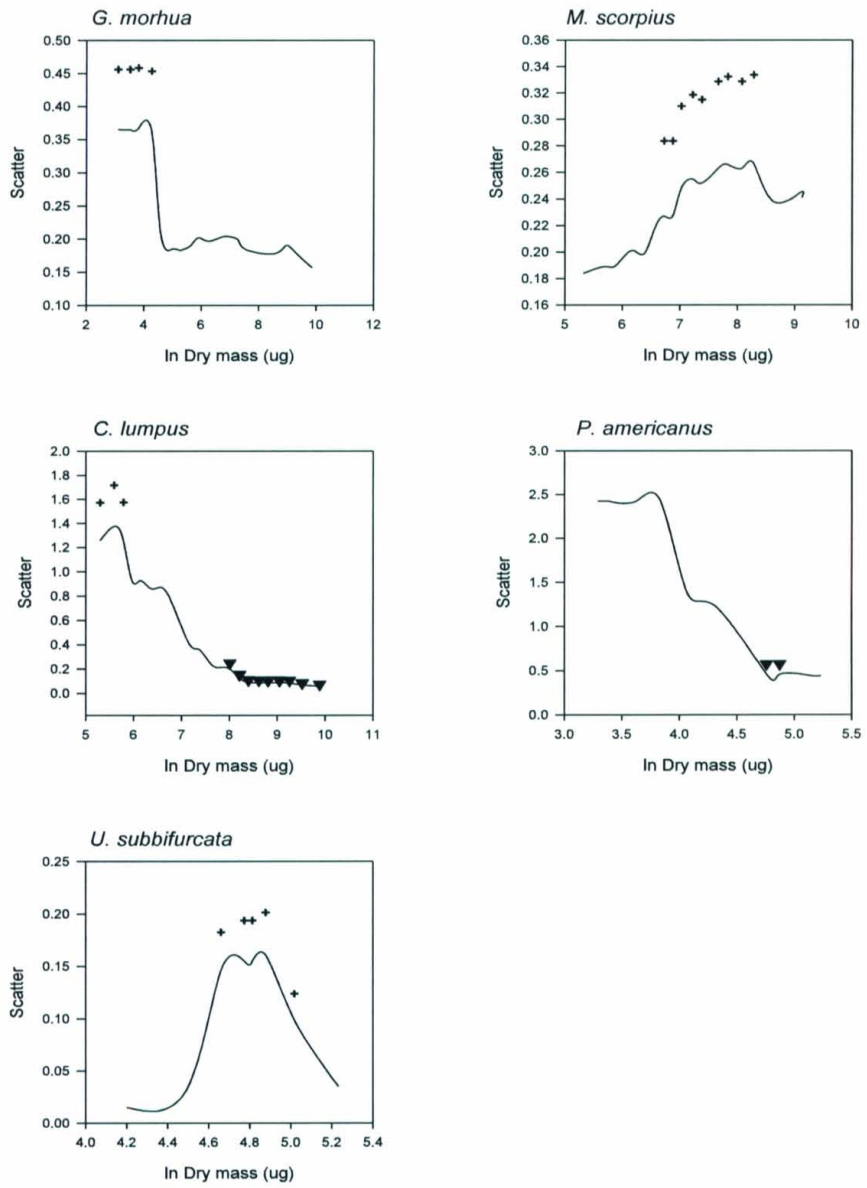

Figure 5.2 Scatter between 10th and 90th percentiles for nitrogen:carbon ratio in relation to dry mass: + indicates where the scatter was greater than 97.5% of the scatters for randomised data sets; ▼indicates where the scatter was less than 2.5% of the scatters for randomised data sets.

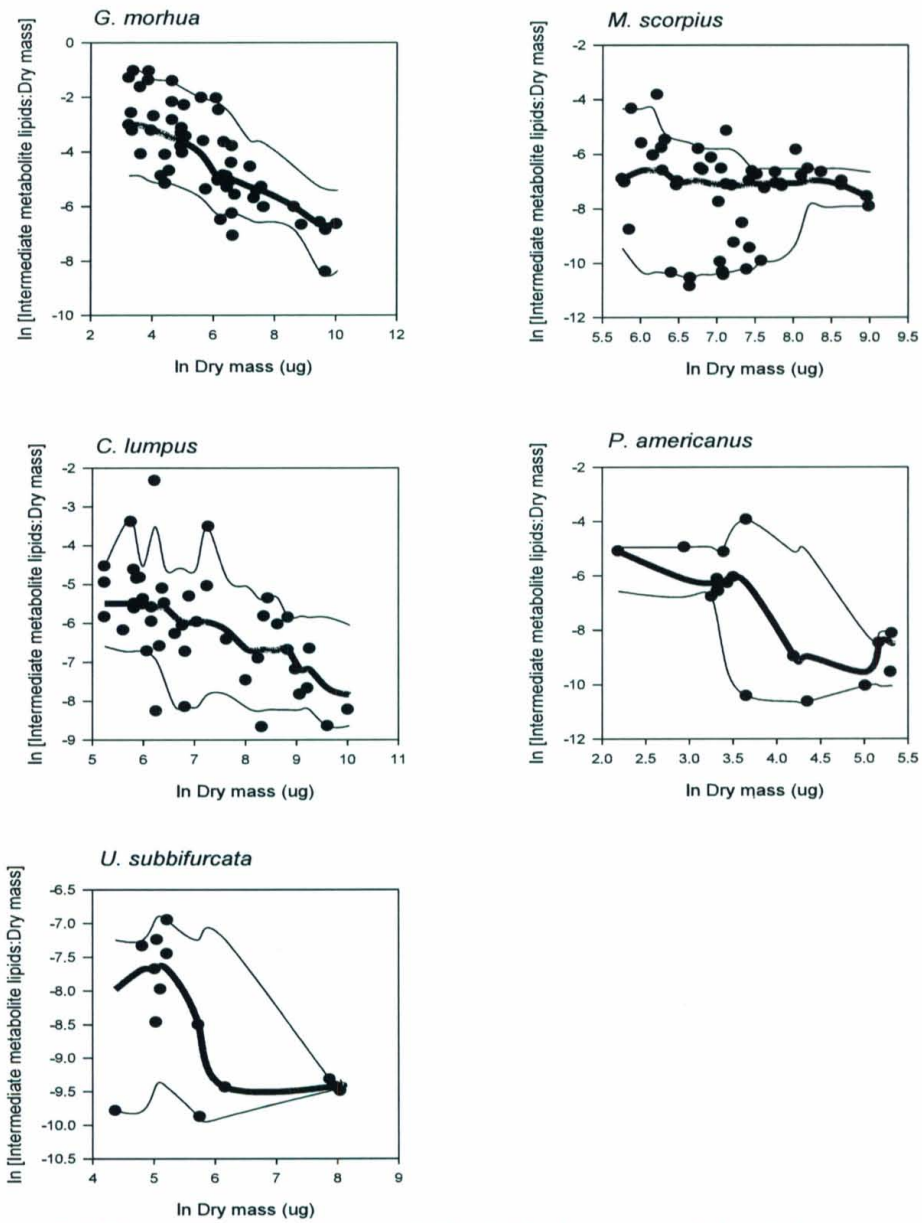

Figure 5.3 Observed intermediate metabolite lipids:dry mass ratio in relation to dry mass. Estimated 10th, 50th, and 90th percentiles are shown (solid lines). The heavy line represents the 50th percentile.

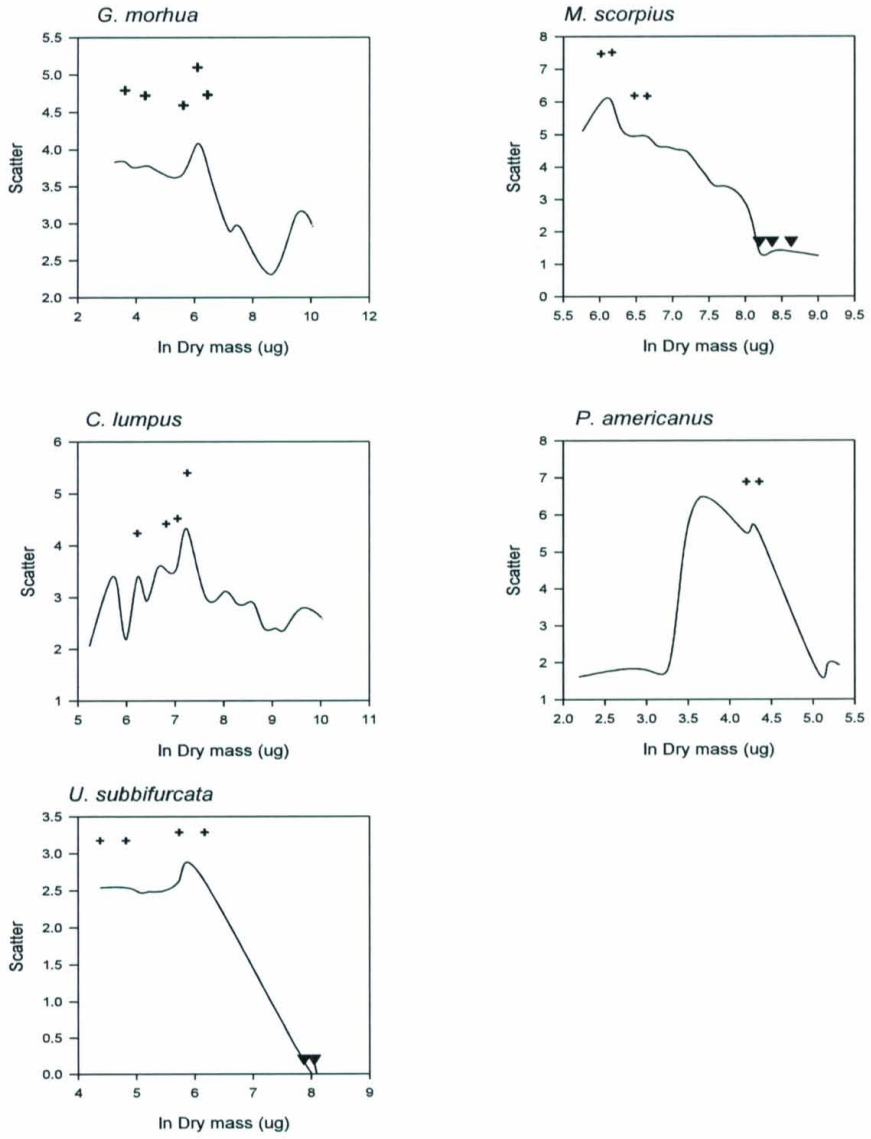

Figure 5.4 Scatter between 10th and 90th percentiles for intermediate metabolite lipids: dry mass ratio in relation to dry mass: + indicates where the scatter was greater than 97.5% of the scatters for randomised data sets; ▼indicates where the scatter was less than 2.5% of the scatters for randomised data sets.

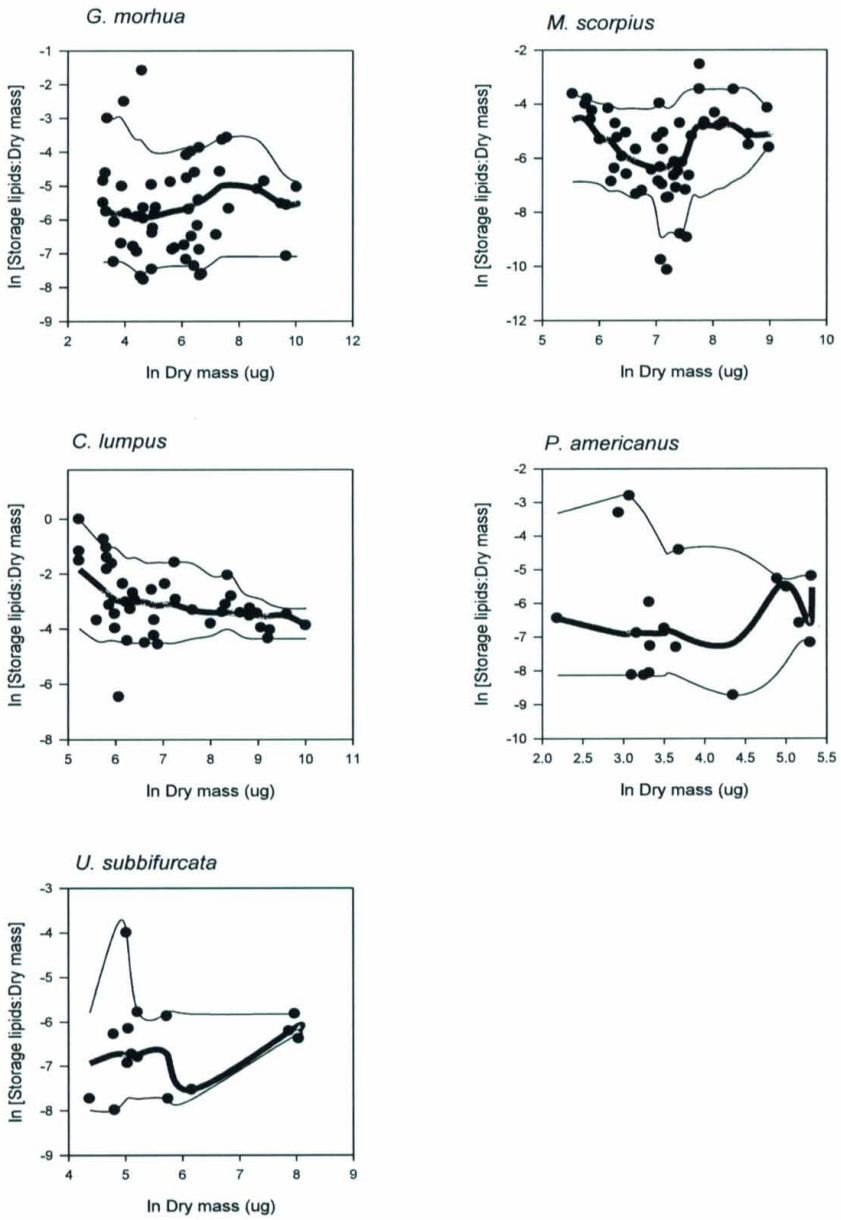

Figure 5.5 Observed storage lipid: dry mass ratio in relation to dry mass. Estimated 10th, 50th, and 90th percentiles are shown (solid lines). The heavy line represents the 50th percentile.

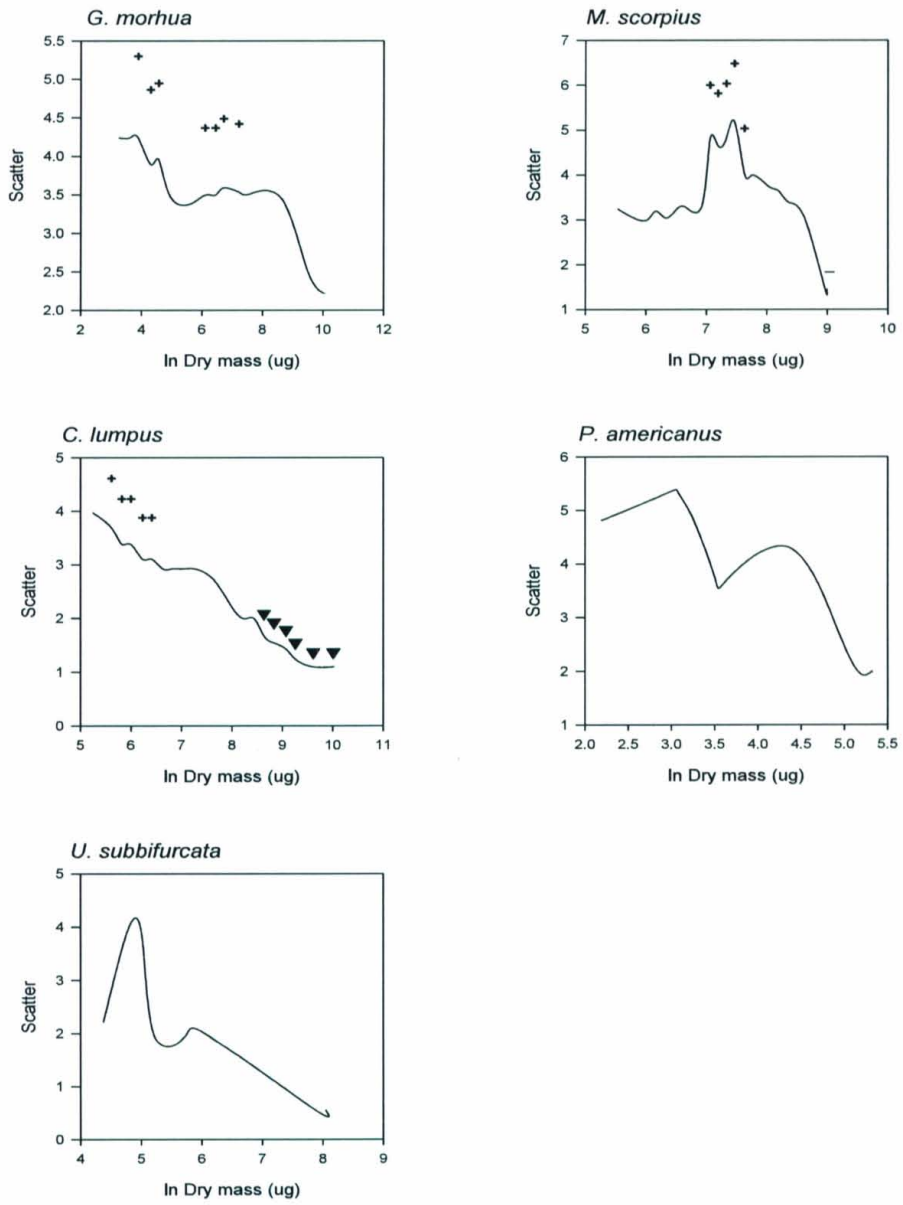

Figure 5.6 Scatter between 10th and 90th percentiles for storage lipids: dry mass ratio in relation to dry mass: + indicates where the scatter was greater than 97.5% of the scatters for randomised data sets; ▼indicates where the scatter was less than 2.5% of the scatters for randomised data sets.

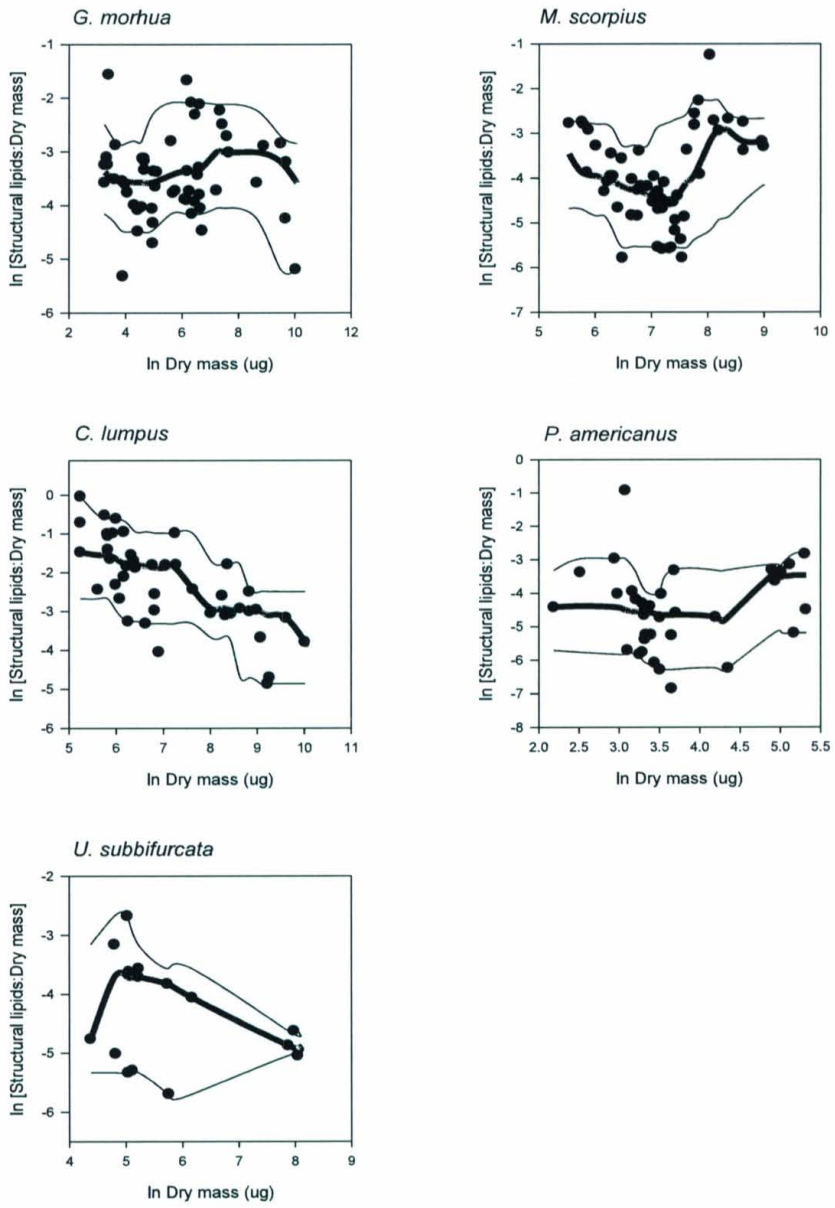

Figure 5.7 Observed structural lipid: dry mass ratio in relation to dry mass. Estimated 10^{th}, 50^{th}, and 90^{th} percentiles are shown (solid lines). The heavy line represents the 50^{th} percentile.

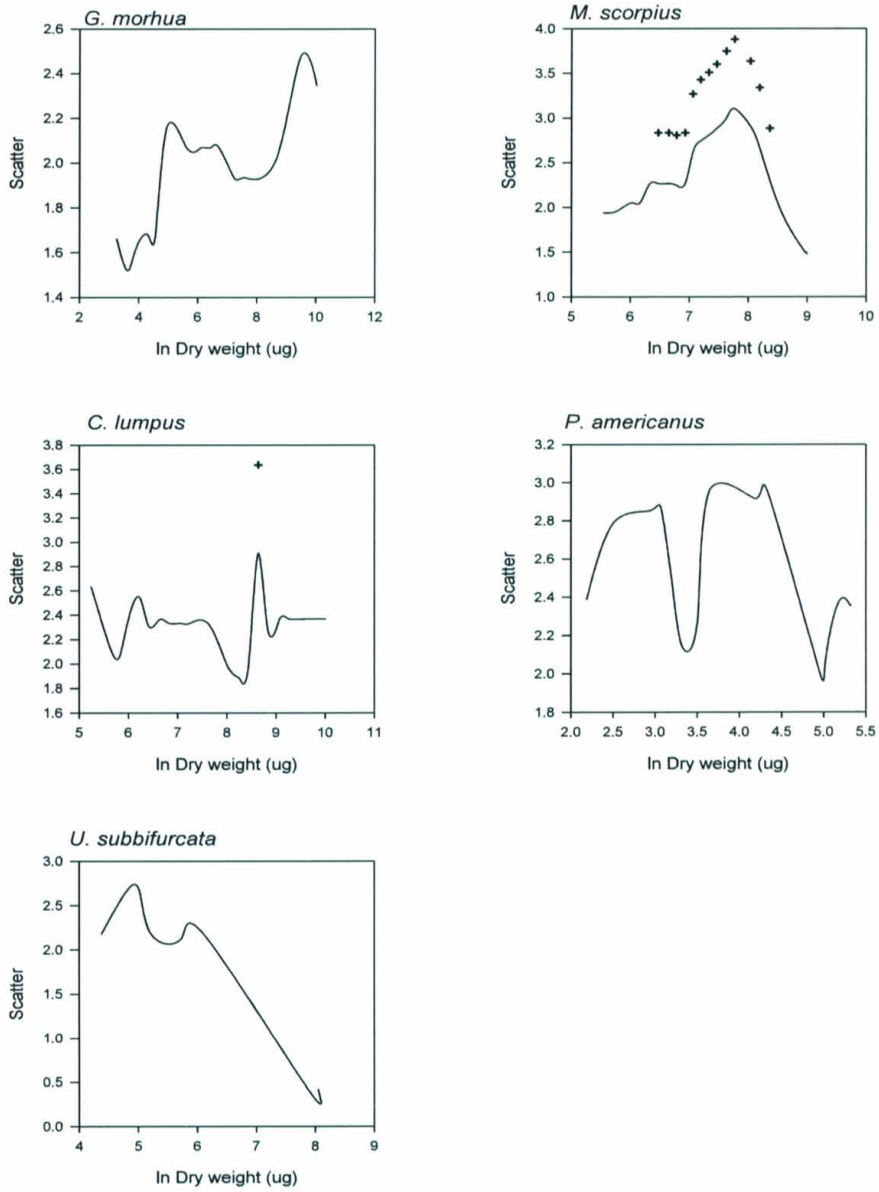

Figure 5.8 Scatter between 10th and 90th percentiles for structural lipids: dry mass ratio in relation to dry mass: + indicates where the scatter scatter was greater than 97.5% of the scatters for randomised data sets; ▼indicates where the scatter was less than 2.5% of the scatters for randomised data sets.

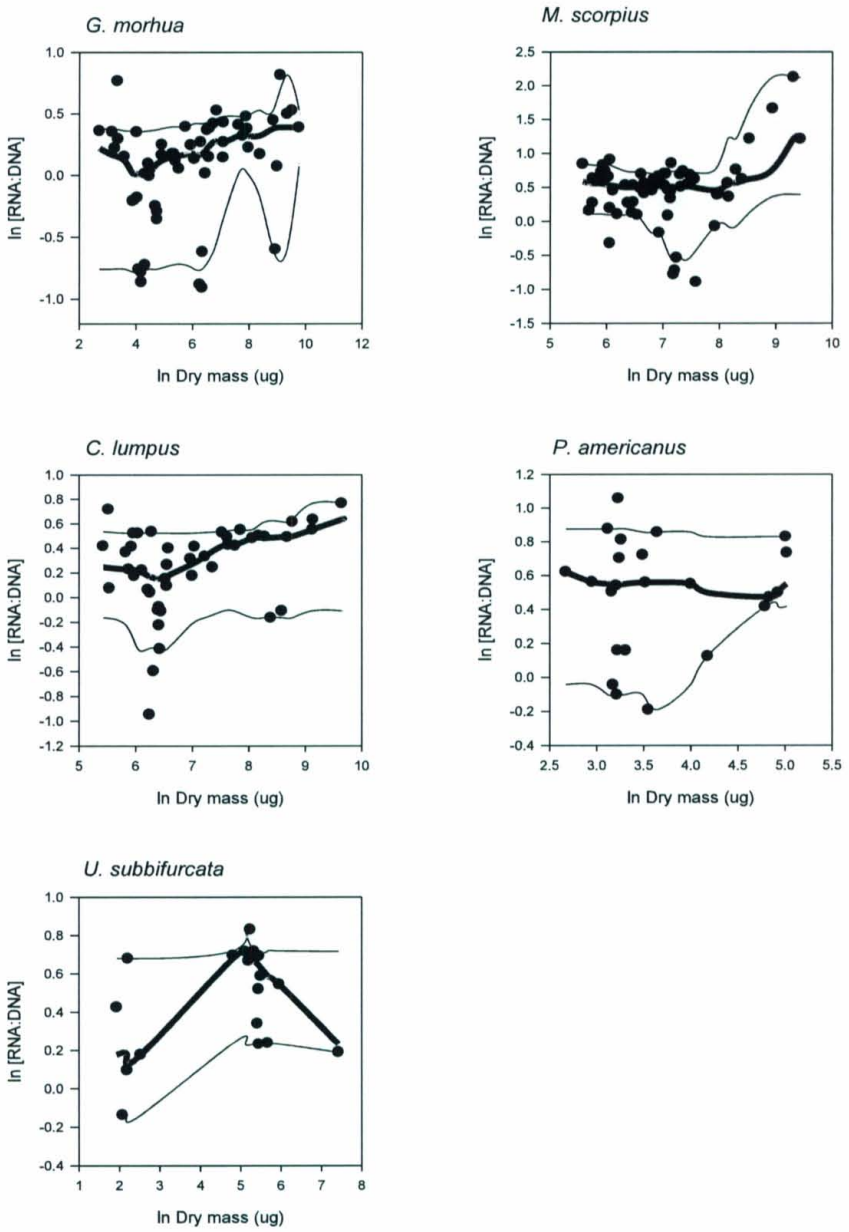

Figure 5.9 Observed nucleic acid (RNA: DNA) ratio in relation to dry mass. Estimated 10^{th}, 50^{th}, and 90^{th} percentiles are shown (solid lines). The heavy line represents the 50^{th} percentile.

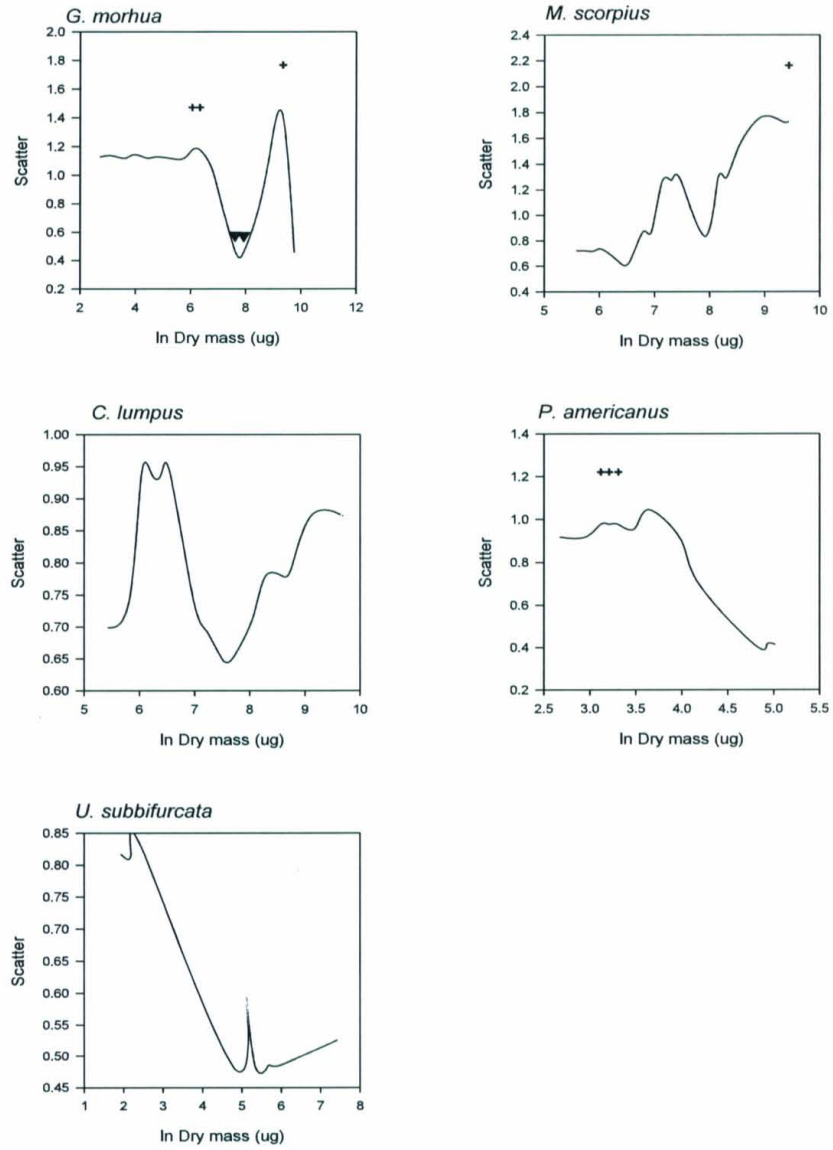

Figure 5.10 Scatter between 10th and 90th percentiles for nucleic acid (RNA: DNA) ratio in relation to dry mass: + indicates where the scatter was greater than 97.5% of the scatters for randomised data sets; ▼indicates where the scatter was less than 2.5% of the scatters for randomised data sets.

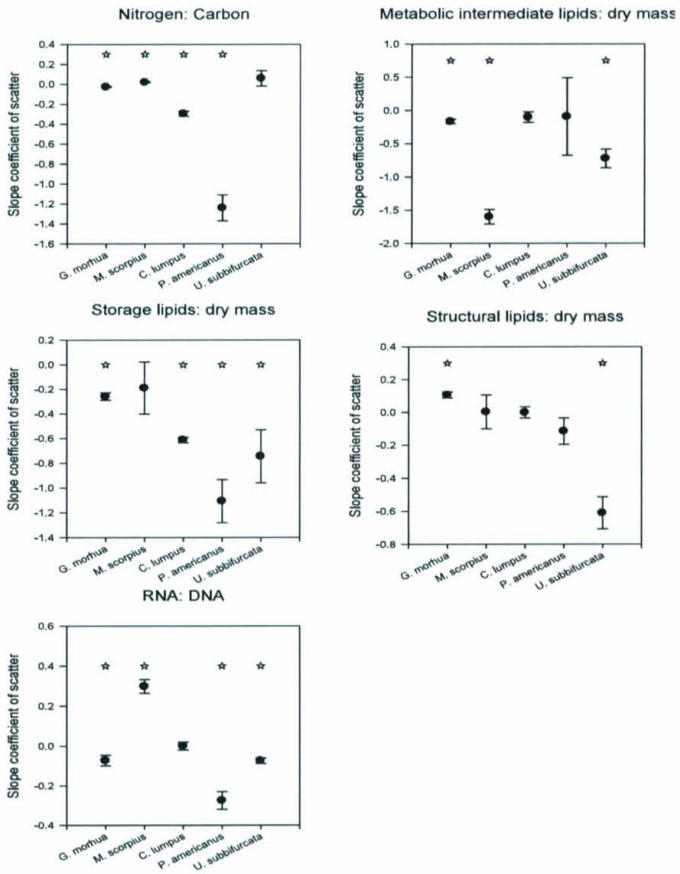

Figure 5.11 Slope of strength of selection (the decrease in scatter as body size increases) shown with confidence intervals. Starred slopes are significantly (p<0.01) different from zero.

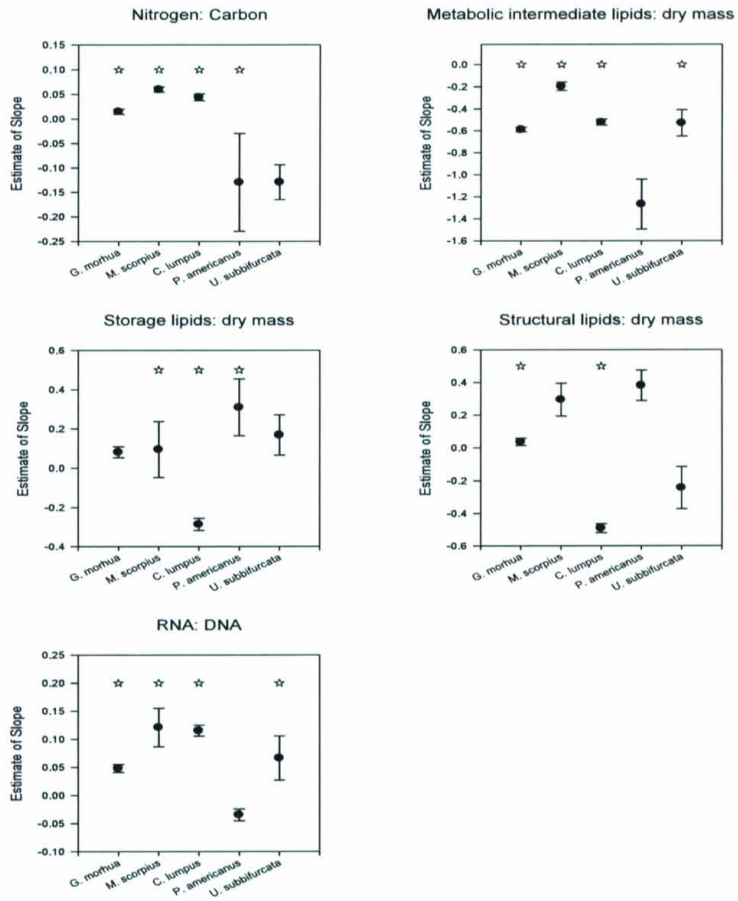

Figure 5.12 Slope of direction of selection (change in median value as body size increases) shown with confidence intervals. Starred slopes are significantly (p<0.01) different from zero.

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5.7 **Appendix**

Appendix 5.1 Variables and sample characteristics for the analyses of this study

		G. morhua	*M. scorpius*	*C. lumpus*	*P. americanus*	*U. subbifurcata*
Nitrogen: carbon	n	19	19	22	10	8
	mean	0.226	0.230	0.474	1.410	0.100
	min	0.159	0.184	0.059	0.438	0.015
	max	0.367	0.267	1.375	2.423	0.161
Intermediate metabolite lipids:dry mass	n	16	20	21	11	8
	mean	3.425	3.725	2.948	3.135	1.957
	min	2.310	1.266	2.056	1.616	0.171
	max	4.081	6.013	4.320	6.483	2.630
Storage lipids:dry mass	n	16	20	21	11	8
	mean	3.465	3.475	2.399	3.711	2.109
	min	2.224	1.464	1.096	1.973	0.558
	max	4.239	5.183	3.974	5.333	3.992
Structural lipids:dry mass	n	16	21	21	15	8
	mean	1.978	2.342	2.317	2.520	1.846
	min	1.519	1.500	1.885	2.045	0.422
	max	2.480	3.104	2.909	2.966	2.660
RNA:DNA	n	20	18	17	12	9
	mean	0.974	1.105	0.803	0.801	0.612
	min	0.455	0.632	0.646	0.412	0.480
	max	1.414	1.764	0.939	1.046	0.817

Appendix 5.2 Regression of scatter for each ratio on dry mass. Scatter is calculated as difference between the 10% and 90% cumulative probabilities of each ratio. Significant values are shown in bold. Degrees of freedom, sum of squares, Fisher's *F*-statistics and *p*-values are available in Appendix 5.3

Variable		*G. morhua*	*M. scorpius*	*C. lumpus*	*P. americanus*	*U. subbifurcata*
Nitrogen: carbon	Intercept	<0.001	<0.001	<0.001	<0.001	0.005
	Slope	<0.001	<0.001	<0.001	<0.001	0.483
Intermediate metabolite lipids:dry mass	Intercept	<0.001	<0.001	<0.001	<0.001	<0.001
	Slope	<0.001	<0.001	0.214	0.874	0.002
Storage lipids: dry mass	Intercept	<0.001	<0.001	<0.001	<0.001	<0.001
	Slope	<0.001	<0.001	<0.001	<0.001	0.013
Structural lipids:dry mass	Intercept	<0.001	<0.001	<0.001	<0.001	<0.001
	Slope	<0.001	0.976	0.978	0.176	<0.001
RNA:DNA	Intercept	<0.001	<0.001	<0.001	<0.001	<0.001
	Slope	0.012	<0.001	0.995	<0.001	0.001

Appendix 5.3 Extension of Appendix 5.2 Regression of scatter for each ratio on dry mass. Scatter is calculated as difference between 10% and 90% cumulative probabilities. Sum of squares, Fisher's *F*-statistics and *p*-values are shown for intercept and dry mass (degrees of freedom = 1 in all cases.) Degrees of freedom and sum of squares are shown for error terms.

		G. morhua	*M. scorpius*	*C. lumpus*	*P. americanus*	*U. subbifurcata*
Nitrogen: carbon		10.967	1.005	4.952	19.875	0.08
		380.850	3091.074	167.461	250.456	18.674
	Intercept	<0.001	<0.001	<0.001	<0.001	0.005
		0.059	0.01	3.871	7.208	0.002
		23.077	29.819	130.883	90.829	0.560
	Dry mass	<0.001	<0.001	<0.001	<0.001	0.483
		17	17	20	8	6
	Error	0.043	0.006	0.591	0.635	0.026
Intermediate metabolite lipids:dry mass		187.738	277.453	182.517	108.123	30.647
		1839.358	1275.076	630.288	28.040	117.952
	Intercept	<0.001	<0.001	<0.001	<0.001	<0.001
		2.181	46.383	0.478	0.102	6.970
		21.373	213.16	1.651	0.027	26.828
	Dry mass	<0.001	<0.001	0.214	0.874	0.002
		14	18	19	9	6
	Error	1.429	3.917	5.502	34.704	1.559
Storage lipids:dry mass		192.127	241.450	120.874	151.524	35.583
		2640.57	256.068	5583.168	421.017	57.994
	Intercept	<0.001	<0.001	<0.001	<0.001	<0.001
		5.234	0.749	16.912	14.572	7.426
		71.941	0.794	781.181	40.489	12.103
	Dry mass	<0.001	0.385	<0.001	<0.001	0.013
		14	18	19	9	6
	Error	1.019	16.972	0.411	3.239	3.681
Structural lipids:dry mass		62.584	115.203	112.701	95.276	27.269
		2568.549	503.884	2092.218	940.872	216.659
	Intercept	<0.001	<0.001	<0.001	<0.001	<0.001
		0.875	0	0	0.207	5.001
		35.920	0.001	0.001	2.048	39.736
	Dry mass	<0.001	0.976	0.978	0.176	0.001
		14	19	19	13	6
	Error	0.341	4.344	1.023	1.316	0.755
RNA:DNA		18.970	21.959	10.954	7.694	3.366
		290.987	733.435	952.591	534.928	580.32
	Intercept	<0.001	<0.001	<0.001	<0.001	<0.001
		0.504	2.275	0	0.534	0.159
		7.734	75.981	0	37.111	27.426
	Dry mass	0.012	<0.001	0.995	<0.001	0.001
		18	16	15	10	7
	Error	1.173	0.479	0.172	0.144	0.041

Appendix 5.4 Slope of the change in the medians of each ratio in relation to dry mass. A regression was used to determine the direction of selection. The estimate of the slope in the regression indicates the direction of selection. Degrees of freedom, sum of squares, Fisher's *F*-statistics and *p*-values are available in Appendix 5.5. Numbers in bold indicate a change which is significantly different from 0.

	G. morhua	*M. scorpius*	*C. lumpus*	*P. americanus*	*U. subbifurcata*
Variable	Slope (Standard Error)				
Nitrogen:carbon	**0.015 (0.005)**	**0.060 (0.005)**	**0.044 (0.007)**	**-0.130 (0.100)**	-0.129 (0.036)
Intermediate metabolite lipids: dry mass	**-0.588 (0.022)**	**-0.196 (0.038)**	**-0.521 (0.027)**	-1.269 (0.225)	-0.529 (0.121)
Storage lipids: dry mass	0.081 (0.028)	**0.095 (0.142)**	**-0.286 (0.031)**	**0.309 (0.145)**	0.168 (0.103)
Structural lipids: dry mass	**0.036 (0.023)**	0.295 (0.101)	**-0.491 (0.027)**	0.381 (0.094)	-0.244 (0.128)
RNA:DNA	**0.048 (0.007)**	**0.121 (0.034)**	**0.115 (0.010)**	-0.035 (0.010)	0.066 (0.039)

Appendix 5.5 Extension of Appendix 5.4. Change in the medians of each ratio in relation to dry mass. A regression was used to determine the direction of selection. The estimate of the slope in the regression indicates the direction of selection. Degrees of freedom, sum of squares, Fisher's *F*-statistic, standard error and *p*-value are shown.

	G. morhua		*M. scorpius*		*C. lumpus*		*P. americanus*		*U. subbifurcata*	
	Estimate	Df	Estimate	Df	Estimate	Df	Estimate	Df	Estimate	Df
	(SE)	SS	(SE)	SS	(SE)	SS	(SE)	SS	(SE)	SS
		F		F		F		F		F
Variable		Prob		Prob		Prob		Prob		Prob
		1		1		1		1		1
		0.227		0.376		0.385		0.008		0.128
	0.015	48.859	0.060	12.425	0.044	137.065	-0.130	11.056	-0.129	2.837
Nitrogen:carbon	(0.005)	**<0.001**	(0.005)	**0.003**	(0.007)	**<0.001**	(0.100)	**0.008**	(0.036)	0.136
		1		1		1		1		1
Intermediate		2.030		1.416		3.415		0.01		0.386
metabolite	-0.588	622.736	-0.196	49.715	-0.521	494.256	-1.269	0.133	-0.529	19.684
lipids:dry mass	(0.022)	**<0.001**	(0.038)	**<0.001**	(0.027)	**<0.001**	(0.225)	0.725	(0.121)	**0.004**
		1		1		1		1		1
		0.101		1.839		10.843		2.269		0.796
Storage lipids:	0.081	2.536	0.095	8.471	-0.286	330.286	0.309	16.369	0.168	3.621
dry mass	(0.028)	0.134	(0.142)	**0.009**	(0.031)	**<0.001**	(0.145)	**0.001**	(0.103)	0.106
		1		1		1		1		1
		0.509		0.188		3.675		1.138		0.378
Structural lipids	0.036	8.384	0.295	0.449	-0.491	85.873	0.381	4.556	-0.244	2.668
:dry mass	(0.023)	**0.012**	(0.101)	0.511	(0.027)	**<0.001**	(0.094)	0.062	(0.128)	0.154
		1		1		1		1		1
		0.019		0.084		0.086		0.079		0.012
	0.048	8.824	0.121	135.122	0.115	41.322	-0.035	1.673	0.066	13.122
RNA:DNA	(0.007)	**0.009**	(0.034)	**<0.001**	(0.010)	**<0.001**	(0.010)	0.232	(0.039)	**0.011**

Appendix 5.6 ANCOVA of scatter for each ratio on dry mass by species. Scatter is calculated as the difference between the 10% and 90% cumulative probabilities of each ratio. Degrees of freedom: dry mass = 1, species = 4, interaction = 4. F = Fisher's F-statistic, p = p-value.

Ratio	Effect	F	p
Nitrogen:carbon (n = 68)	Dry mass	66.950	<0.001
	Species	144.160	<0.001
	Interaction	110.240	<0.001
Intermediate metabolite lipids:dry mass (n=66)	Dry mass	40.030	<0.001
	Species	13.820	<0.001
	Interaction	12.690	<0.001
Storage lipids: dry mass (n=66)	Dry mass	91.540	<0.001
	Species	3.800	0.008
	Interaction	7.640	<0.001
Structural lipids: dry mass (n = 71)	Dry mass	15.770	<0.001
	Species	13.180	<0.001
	Interaction	13.840	<0.001
RNA:DNA (n=66)	Dry mass	1.900	0.172
	Species	22.640	<0.001
	Interaction	27.990	<0.001

Appendix 5.7 ANCOVA of the change in the medians of each ratio in relation to dry mass. The estimate of the slope in the regression indicates the direction of selection. Degrees of freedom: dry mass = 1 species = 4, interaction = 4. F = Fisher's F-statistic, p = p-value

Ratio	Effect	F	p
Nitrogen:carbon (n = 68)	Dry mass	1.640	0.205
	Species	7.050	<0.001
	Interaction	6.570	<0.001
Intermediate metabolite lipids:dry mass (n=66)	Dry mass	314.950	<0.001
	Species	21.030	<0.001
	Interaction	17.080	<0.001
Storage lipids: dry mass (n=66)	Dry mass	0.020	0.889
	Species	51.280	<0.001
	Interaction	32.070	<0.001
Structural lipids:dry mass (n = 71)	Dry mass	2.990	0.089
	Species	28.230	<0.001
	Interaction	7.880	<0.001
RNA:DNA (n=66)	Dry mass	27.310	<0.001
	Species	7.180	<0.001
	Interaction	4.400	0.003

Chapter 6 Overlooked influences on larval fish development: A multi-species analysis of larval fish

6.1 Introduction

It has become increasingly clear that mortality in the early life history of marine fish larvae plays a central role in determining year class strength (Wang et al. 1997, Van der Meer 2006, Miller 2007), with the factors affecting development being key to our understanding of mortality processes. Modelling of such factors may allow biologists and oceanographers to explore how complex biological and biophysical relationships may affect year class strength of fishes (Roff 1983, Brandt and Hartman 1993, Miller 2007, Daewal et al. 2008). Temperature, food density, and hydrodynamics are just a few of the external factors that have been modelled in attempts to understand year class strength (e.g. Campana 1996, Werner et al. 1996, Hinrichsen et al. 2002, Lough et al. 2005, Lett et al. 2010). However limitations when modelling larval fish have often arisen due to the primary value of interest to fisheries being the available adult biomass of commercially valuable species, resulting in: 1) extrapolation of larval biomass from other life stages, 2) extrapolation of information beyond the focal species (Ney 1993, Brandt and Hartman 1993, Pepin and Miller 1993, Peterson et al. 2008, Miller 2007), and 3) use of growth (as represented by mass) as the focus of the models, with a subjective addition of external variables (Brandt and Hartman 1993, Kooijman 2000). In this study, I explore explicitly the influence of intrinsic developmental features on larval fish body mass using a suite of biochemical and developmental variables. Ecological influences of the location of egg and larval development (e.g. habitat) and spawning season, which are commonly used to

group larval development patterns (e.g. Sargent and Gross 1987, Moyle and Cech 1988), are then examined as potential explanations for the patterns of species-specific differences in larval fish development.

Sometimes the challenges incumbent in rearing and sampling the larval stage require extrapolation of models across life stages or among species. However, not surprisingly, many features of this stage indicate that larvae grow in a different way than other life stages. Fish mass increases by a factor of 10^5 - 10^7 from fertilised egg to adult (Houde 1989), with a thousand-fold increase in the larval stage alone. During this period (weeks to months), larvae shift from relying on endogenous energy sources provided by the parent (such as the yolk) to exogenous sources acquired independently, often in a new habitat that corresponds with the individual's trophic status (Houde 1997, Hall and Wake 1999, Benoit et al. 2000). As examined in Chapter 4 of this thesis, larvae must trade off increases in size with energetic investment in complexity. Models developed from older life stages have repeatedly been shown to be inappropriate for use in studying the dynamics of the larval stage (Post 1990, Madon and Culver 1993, Karjalainen et al. 1997, Post and Parkinson 2001) and may introduce substantial error in any predictions derived from such applications (Ney 1993). The effects of the unique energetic demands associated with increasing developmental complexity may help to explain this discord between larval models and those developed for other stages in the life of fish.

Physiological constraints of metabolism give hope that a species-independent model of larval development may exist (Clarke and Johnston 1999, Kooijman 2000, Bochdansky and Leggett 2001), but previous chapters of this thesis and other authors recommend caution about extrapolation (Pepin and Miller 1993). Species-specific

differences were observed in the relationships between morphometrics and internal measures of state and development (Chapter 2), lipids dynamics (Chapter 3), tradeoffs (Chapter 4), and intrinsic selection (Chapter 5). The majority of development and growth models developed are species-specific (e.g. Wang et al. 1997, Post and Parkinson 2001, Whitledge et al. 2003) and most are for commercially important gadoid species (Miller 2007). In addition, when information on the focal species was limited, "species borrowing" was applied, by which parameters from one species were extrapolated to another because it was the "best available" information (Brandt and Hartman 1993, Ney 1993, Lett et al. 2010). While some degree of extrapolation among taxa may be unavoidable to gain an understanding of processes that affect a greater range of species, it can lead to significant misinterpretation because of differences among species (Fox 1991, Clarke and Johnston 1999) and needs to be applied cautiously (Pepin and Miller 1993). Similarly, models that are developed using one species and then are used as templates for general models (e.g. Roff 1983, Breck 2008) require testing before they are extrapolated to other species. In order to pursue more general models, patterns of species differences need to be understood.

Developmental modelling is a careful balance of including enough variables to allow for accuracy while remaining within the bounds of available information (Brandt and Hartman 1993, Kooijman 2000). Many models are developed as a specific response to a unique situation (e.g. Wang et al. 1997). Parsimony suggests that including the minimal number of variables to explain observed variation is ideal, but this might limit our understanding of larval fish development. For example, in a review of individually-based, coupled physical biological models (ICBM) for larvae (Miller 2007), only 36.7 %

of studies included a specific growth term, and then only as an explicit function of temperature or of surplus energy after metabolic requirements had been met. Very few models have included a variable representing development (Van der Meer 2006), even though it is likely to be highly relevant in understanding patterns of growth (e.g. Kooijman 2000). The development of organs and skeletal structures are energetically demanding undertakings, which represent features that change significantly during the larval stage and vary considerably among species (Baglole et al. 1997, Fishelson and Becker 2001, Gisbert and Doroshov 2003). Modellers must balance between the desire for accurate prediction in specific situations and the desire for a model that can be applied in multiple situations and species. As reviewed and tested in previous chapters of this thesis, many current models are insufficient for interspecific analysis. Variable selection (specifically the lack of developmental terms) may not only reveal the source of species-specific differences, but also address how mass increase in larval fish deviates from that at other life stages.

A potential way to organise species differences when developing more general concepts of larval development is to explore ecological designations (Jones and Ellner 2004). Specifically, the common ecological designations of egg development location, larval development location, and spawning season have been shown in Chapter 3 of this thesis to be relevant (e.g. Potts and Wootton 1984, Moyle and Cech 1988, Munro et al. 1990). Pelagic eggs and larvae are characteristically smaller, have less and different kinds of energy reserves (Potts and Wootton 1984), and are produced in higher numbers by females (Moyle and Cech 1988) than demersal eggs and larvae. Pelagic and demersal larvae hatch along a developmental spectrum that ranges from an eleutheroembryo to a

highly precocious larva (Hall et al. 2004). Similarly, spawning season affects larvae not only in the quality, type and abundance of prey available, but also the length of growing season before winter (Conover 1992). As specific aspects of larval development, such as lipid allocation, have been shown to be similar among common ecological designations, it is possible that the influences on growth of larval fish are guided by ecological designations.

Chapter 4 of this thesis demonstrated that there is a balance of components within larvae, which might not be captured in a simple mass-based analysis. In this chapter, I explore the effects of a number of relevant developmental variables introduced in previous chapters of this thesis on biomass using path analysis. Path analysis (Wright 1934) is a form of structural modelling that estimates correlation between measured variables, as well as known, unmeasured variables. This type of analysis is ideally suited for studies of development, as it allows for the inference of cause without experimentation that is functionally difficult in energy allocation studies (Kooijman 2000). Mass is the focus of this analysis, in keeping with existing physiological models of larval development. By applying the same path analysis to data from each species, we can evaluate and compare the patterns of energy allocation affecting larval mass within and among them. The drawback to this approach is that the multispecies model applied to each species might not be the most accurate representation of the influence of state variable influences on mass. Using the same model for all species is a conservative approach to the question, underestimating species-specific effects on mass. As the analysis was exploratory by nature and I am using path analysis in a novel manner, the conservative approach was taken whenever possible.

First, an *a priori* path model was applied to data including 10 species of larval fish to approximate a general model. The magnitude of the coefficient of each state variable on body size described the magnitude of influence of each variable in a general model of larval fish. Second, the identical path model was applied to each of five species' data sets independently. Finally, to complement the exploratory path analysis, the most influential relationships highlighted by the path analysis were tested individually using bivariate analysis to explore the presence of ecological effects. As outlined in previous chapters, environment has been shown to affect larval fish, so all data for this analysis were derived from fish larvae reared in the laboratory under controlled, standardised conditions.

6.2 **Materials and methods**

6.2.1 *Rearing and sampling*

Ten species of larval fish were reared under controlled laboratory conditions: *Gadus morhua* (Atlantic cod), *Mallotus villosus* (capelin), *Ulvaria subbifurcata* (radiated shanny), *Cyclopterus lumpus* (Atlantic lumpfish), *Pseudopleuronectes americanus* (winter flounder), *Myoxocephalus scorpius* (shorthorn sculpin), *Limanda ferruginea* (yellowtail flounder), *Liparis atlanticus* (Atlantic snailfish), *Myoxocephalus aenaeus* (grubby sculpin) and *Hippoglossus platessoides* (American plaice). For rearing, sampling and processing details please see Chapter 2. *Myoxocephalus aenaeus* was reared identically to *M. scorpius.* Dry mass to length relationships were developed using the ten larvae sampled for CHN analysis and applied to the other study organisms to estimate dry mass (for details see Appendix 2.4) for all species, except *M. aenaeus* where there was

insufficient information. Ecological characteristics were compiled for each species from available literature or from direct observations (Table 3.1).

6.2.2 *Model development*

Species differ from each other in many aspects (previous chapters, this thesis), so all variables studied in this thesis were included in the path analysis model to provide a comprehensive exploration of the potential sources of species differences in mass accumulation. The internal state variables of carbon (structural element), nitrogen (limiting resource), nucleic acids (protein synthesis), ossification (increasing structure and complexity), organogenesis (increasing complexity), and lipids (structure, energy) were included. Their respective biological relevance to larval fish has been covered in detail in previous chapters.

Most of these variables were assessed in the model for their direct influence on larval mass (Fig. 6.1), except for intermediate metabolite lipids, RNA, nitrogen and DNA. These indirect variables were included through causal relationships on other variables (DNA on organogenesis, RNA and nitrogen on protein), or assumed to be in co-relational relationships where direction of action was unknown and strong correlations suspected (structural lipids with intermediate metabolite lipids, storage lipids with intermediate metabolite lipids, and RNA with intermediate metabolite lipids). These correlations are based on the knowledge that there is a relationship between intermediate metabolite lipids with both structural and storage lipids, but that it is not necessarily directional (Kattner et al. 2007), and that both intermediate metabolite lipids and RNA can represent synthesis of larger molecules (lipids and proteins, respectively). Unfortunately, the number of co-

relationships that can be evaluated is limited by the analysis, so only three could be included here, although more certainly exist (Chapter 4, this thesis). Protein, which was not measured directly in this study but is known to be relevant to larval mass, was included as a latent variable, estimated from nitrogen and RNA through the path model. Sixteen path coefficients were estimated along with 9 error terms, one for each endogenous variable (those variables directly relating to the focal variable of mass). The resultant analysis has 25 degrees of freedom and was implemented using PROC CALIS in SAS (SAS Institute Inc, North Carolina, USA) with double dog-leg estimation. The unweighted least squares estimation was used because it is robust to multivariate non-normality and the distribution of variables is unknown in this exploratory analysis (www.support.sas.com).

6.2.3 *Analyses*

Three analyses were undertaken: 1) a general species-independent path analysis, 2) a species-specific path analysis, and 3) a nested ANCOVA of relevant relationships from the path analysis examining ecological influences. To preserve the intrinsic variability of larval development, each larva was treated as an independent observation. Eleven physiological variables were used in the analysis: carbon, DNA, gill arch count, intestinal epithelial thickness, liver area, intermediate metabolite lipids, nitrogen, ossification, RNA, storage lipids and structural lipids (Fig. 6.1). All variables were ln-transformed to approximate a normal error distribution and to reflect allometric aspects of growth and development.

As the measurement of some variables precluded others (e.g. nucleic acids and lipids), missing values needed to be predicted from known information. As no single morphometric effectively represents these variables among species (Chapter 2), a multivariate prediction incorporating all available information as well as scatter was performed using multiple imputation. Multiple imputation is the process of replacing a missing value with multiple plausible values. These plausible values are derived from the existing distribution, representing the uncertainty surrounding the missing value. Multiple imputation allows a comparison of developmental variables on different organisms without pooling data. Multiple imputation (using the multiple imputation function in SAS) was performed 100 times using 500 iterations to calculate each imputation as suggested by Graham et al. (2007) based on the amount of missing data. Five species had enough data to impute separately (*G. morhua, M. scorpius, C. lumpus, U. subbifurcata, P. americanus)*. The remaining species were imputed as a group, which will result in underestimation of species and ecological differences. To give the most accurate imputation possible, all of the developmental values and morphometric values were used to predict the missing data. This resulted in 100 data sets for each species.

For the first analysis, all species were combined into one data set, and a path analysis was performed to determine which relationships were highlighted by a species-independent analysis. Path analysis was carried out independently for each of the 100 imputation data sets to avoid pseudoreplication and allow accurate estimation of error. Standard error for each term within each imputation was estimated using jackknifing. The path coefficient estimates for each term were averaged across all imputations, and the standard error across imputations estimated using a correction that takes into account the

uncertainty arising from imputations (Schafer 1998). The probability that each path coefficient was significantly different from zero was estimated by dividing the path coefficient by the standard error and calculating the probability according to a *z*-distribution.

The second analysis examined individual species to allow a comparison of information gained in species-independent analyses versus species-specific analyses, and to assess whether there were notable differences among species. For the five species where information was available from hatch to metamorphosis (*G. morhua*, *M. scorpius*, *C. lumpus*, *P. americanus* and *U. subbifurcata*), the species were evaluated separately. Standard errors were calculated in the same manner as in the previous analysis. Pairwise *z*-tests were then used to evaluate between species differences for each of the estimated parameters.

Finally, the third analysis used original (unimputed) data to examine the relationships highlighted by the path analysis as having significant effects on mass. These relationships were evaluated for ecological (location of egg development in the water column, location of larval development in the water column, season of spawning) and species effects. Because the original data were used, only relationships where both variables could be measured on the same larva could be examined. The final analysis allows further, more precise, investigation of the species differences among the variables, by removing the error introduced through the imputation and jackknifing procedures. A general regression was applied across all species relating the absolute value between the variables connected by the coefficients of the path model. The resultant residual patterns indicated how each species differed from an averaged relationship across species.

Residuals were contrasted to determine if there was a significant effect of egg development location, larval development location, or spawning season using an ANCOVA. The significance level used was $p < 0.05$.

6.3 **Results**

The data set contained 2226 observations, based on information from ten species. Five of these species were sampled from hatch to metamorphosis allowing independent species analysis for 522 *G. morhua* observations, 548 *M. scorpius* observations, 495 *C. lumpus* observations, 209 *P. americanus* observations, and 90 *U. subbifurcata* observations.

6.3.1 *Species-independent model*

In the first analysis, where data from all species were combined, the resultant path model had an N of 2226, and a goodness of fit index of 0.99 indicating good fit (Fig. 6.2, for details see Appendix 6.1). Of the sixteen coefficients estimated, nine were statistically significantly different from zero ($p<0.05$) based on a z-test. These included three direct effects on dry mass, including intestinal epithelial thickness, liver area, and the latent variable of protein. The coefficients connecting DNA with the three indices of organogenesis were all significantly different from zero, as were the three bidirectional coefficients relating intermediate metabolite lipids with structural lipids, storage lipids, and RNA. The largest coefficient estimated was the latent variable of protein on dry mass (0.897).

6.3.2 *Gadus morhua*

When species were examined independently, a goodness of fit index of 0.94 was achieved for *G. morhua* (Fig. 6.3, for details see Appendix 6.1). Six estimated coefficients were significantly different from zero, including the effect of liver area on dry mass, all relationships describing the effect of DNA on organogenesis, and the correlations of structural and storage lipids with intermediate metabolite lipids. The largest coefficient was again the latent variable of protein on dry mass (1.003), although the estimated standard error was large (0.861), which caused this path coefficient to not be significantly different from zero.

6.3.3 *Myoxocephalus scorpius*

The path analysis of *M. scorpius* included 549 observations with a goodness of fit index of 0.91 (Fig. 6.3, for details see Appendix 6.1). Only four variables had coefficients that were significantly different from zero, including the effects of intestinal epithelial thickness and liver area on dry mass, as well as the correlation between structural lipids and storage lipids with intermediate metabolite lipids. The path coefficient of structural lipids on dry mass was the largest (1.046) in the *M. scorpius* analysis, but the standard error was large (0.424) and the value was not statistically significant.

6.3.4 *Cyclopterus lumpus*

The path model for *C. lumpus* was based on 495 samples, which yielded a goodness of fit index of 0.90 (Fig. 6.3, for details see Appendix 6.1). All correlations estimated were significantly different from zero, as were the effects of protein on dry

mass, nitrogen on protein, and DNA on both intestinal epithelial thickness and liver area. The latent variable of protein had an estimated path coefficient of 0.701 and standard error of 0.153, making it the largest path coefficient estimated for *C. lumpus* and significantly different from zero.

6.3.5 *Pseudopleuronectes americanus*

The path analysis of *P. americanus* yielded the lowest goodness of fit index of all the species, at only 0.80, based on a sample size of 209 observations (Fig. 6.3, for details see Appendix 6.1). Only one estimated coefficient was significantly different from zero in this model, the effect of the latent variable protein on dry mass with a value of 0.64. This was not the largest estimated effect on dry mass, that being the effect of carbon on dry mass with an estimated effect of 0.739. However, in the case of carbon the estimated standard error after jackknifing and corrections for imputation was very large (2.247).

6.3.6 *Ulvaria subbifurcata*

Ulvaria subbifurcata samples numbered only 90, but the path model still had a reasonably good fit (0.86) despite the small sample size (Fig. 6.3, for details see Appendix 6.1). Five of the coefficients estimated were significantly different from zero. This included two direct effects on dry mass (intestinal epithelial thickness and liver area), the effect of DNA on liver area, and the correlation effects between intermediate metabolite lipids and the other two lipid groups of structural and storage lipids. The largest effect was the same as in *P. americanus* (carbon, 2.759), but the standard error was extreme (14.25).

Although the results among species varied considerably, the conservative method of using a jackknife standard error and a correction for imputation resulted in very large standard errors for many of the estimates. Only the relationships of structural lipids with dry mass and carbon with dry mass showed significantly different path coefficients among species. However, substantial non-significant differences were observed for all variables among species, even disregarding the smaller samples of *U. subbifurcata* and *P. americanus*. The sign of the coefficient varied for at least one species for all variables, except for the relationship between DNA and gill arches, and those of protein, intestinal epithelial thickness and liver area on dry mass.

6.3.7 *Species and ecological effects*

The original data were regressed in a general model relating the variables included in the path analysis and the residuals examined for species and ecological effects using a nested ANCOVA. This yielded sufficient data to investigate seven relationships with dry mass (storage lipids, structural lipids, ossification, gill arches, intestinal epithelial thickness, liver area, carbon) and two relationships with intermediate metabolite lipids (storage lipids, structural lipids) (Fig. 6.4, for details see Appendix 6.2). The two variables that exhibited detectable species effects in the path analysis (structural lipids and carbon with dry mass) also showed significant species effects when egg development location and larval development location were nested in the species effect. The relationship between structural lipids and dry mass was also significantly affected by spawning season, with larvae produced in the summer having the greatest amounts of structural lipids. This difference appears to be largely driven by a single species (*C.*

lumpus). Carbon was not significantly affected by spawning season. In addition, this direct analysis of the data also detected significant species effects on the relationships of storage lipids with intermediate metabolite lipids, structural lipids with intermediate metabolite lipids, as well as storage lipids, ossification, and liver area in relation to dry mass.

Cyclopterus lumpus, the only species developing in a demersal environment was different in seven of the nine relationships examined. Egg development location was only significant in the relationships among storage lipids, structural lipids and metabolic intermediate lipids. Spawning location, in addition to a significant effect on structural lipids, also significantly affected the relationships for storage lipids with dry mass, ossification with dry mass, storage lipids with intermediate metabolite lipids and structural lipids with intermediate metabolite lipids. Summer larvae had the greatest amounts of storage and structural lipids relative to dry mass, while winter spawners had more pronounced ossification in relation to dry mass, as well as storage and structural lipids relative to metabolic intermediate lipids. The dry mass relationships with the number of gill arches and the thickness of intestinal epithelial cells were unique in that no significant effects were detected in the nested ANCOVA models incorporating species and ecology.

6.4 **Discussion**

Earlier multi-species modelling studies (Fox 1991, Clarke and Johnston 1999) and preceding chapters in this thesis consistently imply that species-independent models should be applied with caution. When all the species in this study were examined together

the greatest influence on dry mass was protein content. This species-independent result is consistent with the species-independent relationship between RNA:DNA (as an indicator of protein synthesis) and growth developed by Buckley and Dilman (1982). Dry mass in *C. lumpus* and *P. americanus* was significantly influenced only by protein, which has been shown to be related to survival and growth (Pangle et al. 2005, Buckley et al. 2008). In contrast to *C. lumpus* and *P. americanus,* organogenesis (liver area and intestinal epithelial thickness) demonstrated a more important influence on changes in mass for *G. morhua, M. scorpius* and *U. subbifurcata.* Measures of developmental progress also feature prominently in the species-independent model, with both liver area and intestinal epithelial thickness having a larger influence on mass than expected, given their lack in earlier models of larval development. This suggests that not only do the relationships between developmental variables vary among species, but the relative importance of developmental processes (e.g. histological development) in the overall energy budget do as well.

Although pairwise tests of the path coefficients displayed significant differences between species only in the case of the effect of carbon and structural lipids on dry mass, visual examination of the data suggests that this may be an artefact of the conservative treatment of the error and imputation used in this analysis (discussed below). When the original data were examined, the relationships between variables were species-specific in the majority of variables measured, including the relationships of storage lipids, structural lipids, ossification, liver area and carbon with body mass. Developmental investments such as gill arches, intestinal epithelial thickness, liver area and ossification directly reflect an increased complexity, while simultaneously allowing for more rapid growth

through the functional effects they provide. Inclusion of developmental variables suggests that differential energetic allocation to ontogenetic development appears to explain some of the differences in growth previously observed among species.

Despite differences among species, there are still some inferences that can be derived from this multi-species analysis. Protein was important not only in the species-independent model, but also in the individual species models of *C. lumpus* and *P. americanus*. Protein availability has been linked to growth and survival of larval fish (Pangle et al. 2005, Buckley et al. 2008), because it allows for the maintenance and repair of structure, and is a source of energy (Weber et al. 2003, Rosa and Nunes 2004). While not measured directly in this analysis, protein is included as a latent variable influenced by nitrogen and RNA. RNA is functionally the foundation of protein synthesis (Clemmesen 1993, Bergeron 1997), while nitrogen is one of the major limiting components in marine ecosystems (Elser et al. 1996). Nitrogen does not have a high explicit energetic value, so models based on energetics undervalue the limiting nature of nitrogen (Buckley and Dillman 1982). The exceptions to the undervaluing of nitrogen are nutrient limitation models (e.g. Kooijman 2000) and mass balance models (e.g. Buckley and Dillman 1982). Because of its direct influence on protein synthesis, nitrogen may be of greater importance to larval fish growth and development than is currently considered in energy-focused models.

In addition to protein, histological measures of liver area and intestinal epithelial thickness also showed a significant influence on body size among species, although the magnitude of this effect varied. Liver area and intestinal epithelial thickness are important to digestion in fish, enabling them to more efficiently process energy (Baglole et al. 1997,

Fishelson and Becker 2001, Gisbert and Doroshov 2003). Because larval fish are developing new organs, the energy required is likely a greater fraction of their total energy usage than that required for the maintenance of organs in adult and juvenile fish. The influences of organogenesis on growth explored in this study are relatively large in some cases and demonstrate a more consistent pattern of significance and importance than the other variables examined, particularly in *U. subbifurcata* and *M. scorpius*. This is consistent with the information from Chapter 4, which suggested a greater interaction (or balance in terms of energy limitation) between developmental variables and more commonly used indices of conditions, such as biochemical composition. Although the inclusion of an increasing complexity or maturation term is not well represented in larval development models (Van der Meer 2006), it is not novel. For example, *K*-rule dynamic energy budgets (Koojiman 2000) attempt to take into account energy partitioning within larvae between growth, maintenance and maturation. Unfortunately, the coefficient of energy partitioning (*k*) theoretically proposed in dynamic energy budgeting cannot be measured directly through traditional manipulative experiments (Koojiman 2000), and this strategy of modelling has remained limited (Van der Meer 2006).

Histological and developmental complexity variables are often overlooked in larval fish for many reasons: 1) significant labour is required for accurate analysis; 2) these measures are resistant to automation; 3) limited information is available about the effects of histological measures on energy allocation and 4) histological indices of condition and other measures of complexity are often reported qualitatively (e.g. Govoni et al. 1986, Baglole et al. 1997, Gisbert and Doroshov 2003), rather than quantitatively, making them difficult to translate into numerical or energetic models. Despite this,

organogenesis is something that represents a significant energy demand unique to larval fish because of the extensive changes that occur during this life stage. The exception to this is the development of sexual organs in juveniles and adults, which is recognised as requiring significant energy allocation (Roff 1983). Development continuity and consistency in larval fish is potentially a source of variation in growth and survival that may be grossly overlooked. It represents an area of research and understanding that requires further examination to determine the possible differences observed among species and individuals.

The strong signal in the model from the developmental variables raises questions about the normally continuous representation of larval fish growth. Organogenesis does not proceed in a continuous fashion throughout the larval period, but is actually a series of ontogenetic events (e.g. Fuiman et al. 1998, Hernandez et al. 2001, Infante and Cahu 2001). For example, the formation of gills normally occurs when metabolic demands exceed cutaneous respiration – before the larval period has completed, gill development is complete (Phillips 1999). While there may be continuing maintenance and size increase of the organs, it is possible that the initial investment in this type of structure represents a more significant proportion of the energy budget than the ongoing maintenance and growth of organs. However, it is difficult to isolate and identify energy allocation specific to organogenesis. In general terms energy requirements for the development of individual organs are more finite than increases in mass, occurring over finite periods of time that are likely shorter than the larval period. This places limitations on the more common models of energy allocation, which consider each element as more or less a continuous demand throughout much of the early life history. Alternatively, there could be a constant

amount of surplus energy beyond the metabolic requirement of the larvae, which is devoted sequentially to different one-time energy investments. The results from Chapter 4 of this thesis, exploring potential tradeoffs, suggest that there is unequal energy allocation among developmental processes (such as ossification), rather than all processes proceeding at a reduced rate. Furthermore, organogenesis affects a larva's ability to grow and develop continuously – a half-formed swimbladder is unlikely to improve the ability of larvae to forage as much as a fully formed swimbladder. Therefore, the benefit(s) accrued by developmental events may occur in a step-wise manner that can result in substantial departures from models based on the assumption of continuous processes.

Because of the exploratory nature of the present study and the variability among individual larval fish (Pepin et al. 1999, Fuiman et al. 2005), the analyses were treated with caution. I conserved the variability contained within the data through the use of multiple imputation and the treatment of the larvae as independent observations wherever possible. The result of multiple imputation and subsequent analyses is likely an overestimation of error rather than an underestimation, and there are correspondingly high uncertainty in the parameter estimates (e.g. Fig. 6.3). As discussed in Chapter 5, and also by Pepin et al. (1999), there is information contained within this variability. The developmental variables (ossification progress, gill arch count, and intestinal epithelial thickness) regularly showed much less variability than the biochemical variables (e.g. carbon, lipids, and protein) and this is reflected in the variables that are found to be significant in the path analysis. This likely reflects some of the limitations that developmental progress puts on growth, as discussed above and that are subject to greater measurement error. As increased variation makes it less likely for variables to exhibit

significant effects, it only further emphasises the influence that developmental variables may have on growth, and how that influence differs between species.

As explanatory variables of differences among species, ecological designations (egg development location, larval development location, and spawning season) were largely not successful. Much of the evidence for the effects of season and developmental location on growth has also been found in analyses of the patterns of lipid allocation in various species of fish (e.g. Conover 1992, Rainuzzo and Jorgensen 1992, Sargent 1995, Chapter 3). Briefly, larvae and eggs from demersal evironments as represented by the single species *C. lumpus* in this study, had higher lipid values than those developing in the water column, likely reflecting the greater parental investment and lower mortality risk often observed in demersal eggs and larvae (Smith 1995). Larvae spawned in the summer displayed the highest mass-specific lipid levels, coinciding with the highest amount of available zooplankton prey (Conover 1992, Kattner et al. 2007). Even where the effects of ecology were significant in the bivariate analyses, partitioning of the variance suggests that the differences among species were much greater than the effects of ecology on the relationships included in the path analysis. The exceptions were the effect of larval development location on the relationships of storage lipids with dry mass, structural lipids with dry mass, liver area with dry mass, and storage lipids with metabolic intermediate lipids. Although influences of ecological variables were limited in this analysis to lipids, lipids are valuable high energy compounds used for long-term energy storage, as structural components and to fuel metabolic processes (Rainuzzo and Jorgensen 1992, Sargent 1995, Wiegand 1996). Increased understanding of lipid

dynamics through ecological designations still contributed substantially to the understanding of larval fish developmental strategies.

Previous chapters of this thesis have demonstrated through analysis of lipids and growth that the fish reared in this study were not only growing well, but were comparable to wild fish. The results of this exploratory study were consistent with the current understanding of driving forces (such as protein acquisition) on larval fish development. However, increasing developmental complexity appears to be an important contributing factor to the differences in development observed among the species studied here. The use of path analysis and multiple imputation allows some numerical exploration of the hypothesised increasing complexity term suggested by Koojiman (2000). In addition to known biomass influences, addressing the energetic requirements of increasing complexity of organ development might improve the understanding and subsequent model development of larval fish growth and development. Fine scale assessment of the energy required for increasing complexity could further improve the support for the possibility that larval fish energetic needs may not be continuous.

6.5 Tables and figures

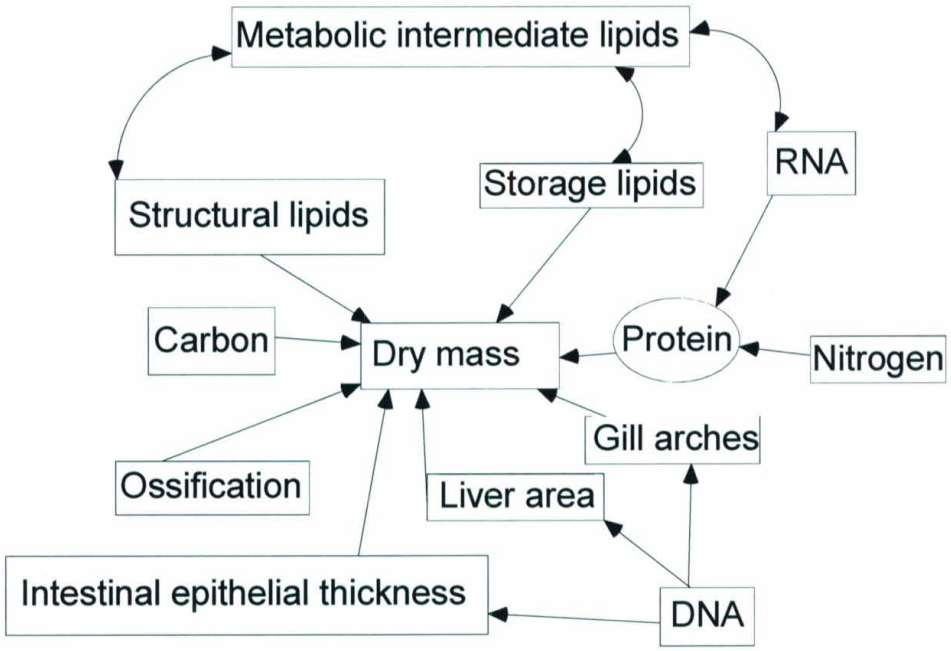

Figure 6.1 Path analysis. Also included in the path analysis were error terms for endogenous variables dry mass, carbon, RNA, nitrogen, DNA, gill arches, liver area, intestinal epithelial thickness and ossification. Rectangular boxes indicate known variables, while the oval term is a latent variable. Single headed arrows indicate suspected causation, while double headed arrows indicate a suspected correlation.

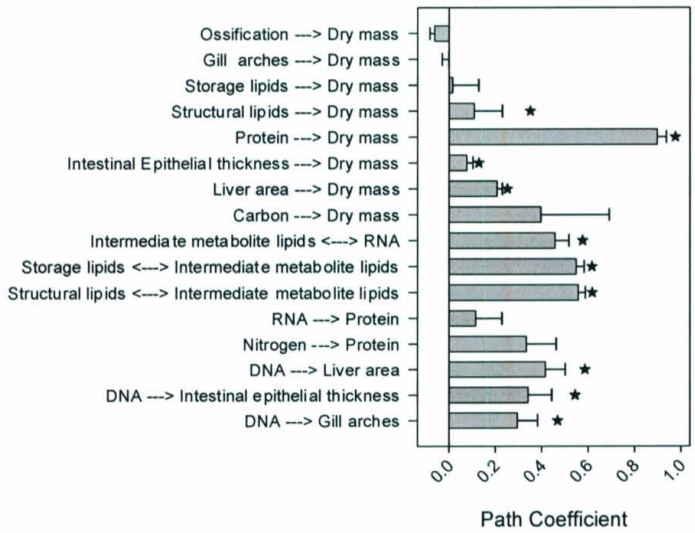

Figure 6.2 Path coefficients from the model diagrammed in Figure 6.1. All species are analysed together. Error bars indicate jackknifed standard error corrected for multiple imputation. Stars indicate path coefficients significantly different from zero.

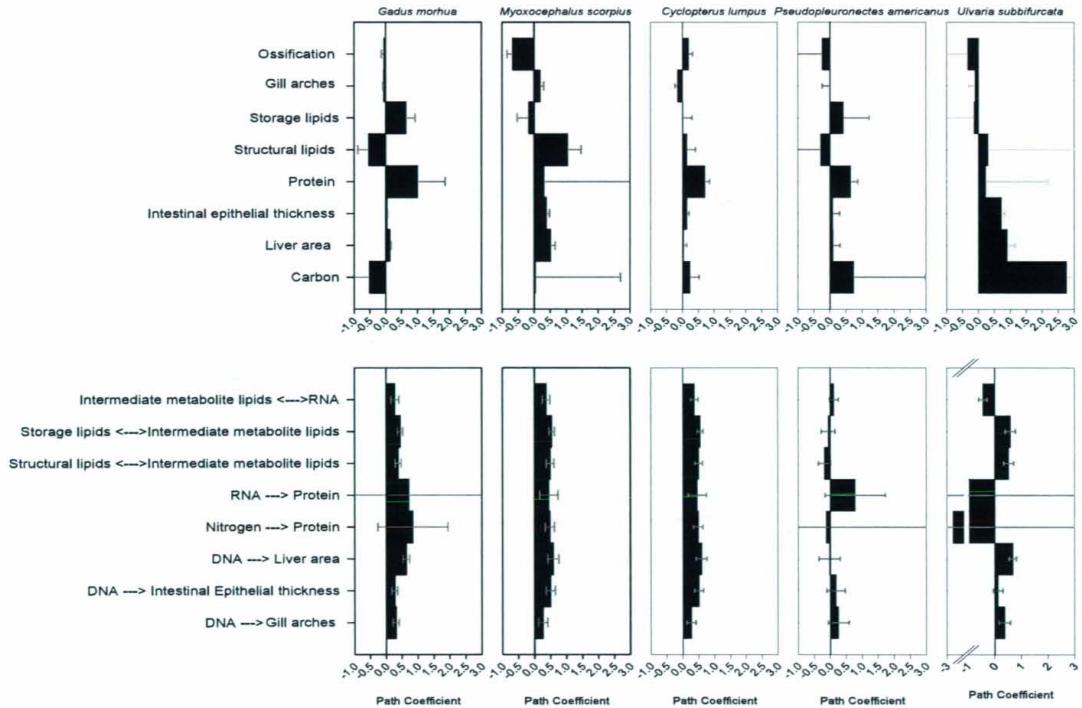

Figure 6.3 Path coefficients from the model diagrammed in Figure 6.1 when species were examined individually. The top panel shows all terms that directly affect dry mass, and the bottom panel shows all other terms. Error bars indicate jackknifed standard error corrected for multiple imputation. For error bars which exceed the figure, please see Appendix 6.1. Significant differences between species in pairwise analysis occurred in: Carbon → dry mass between every species and *U. subbifurcata*; and Structural lipids → Dry mass between *G.morhua, U. subbifurcata*, and *P. americanus*.

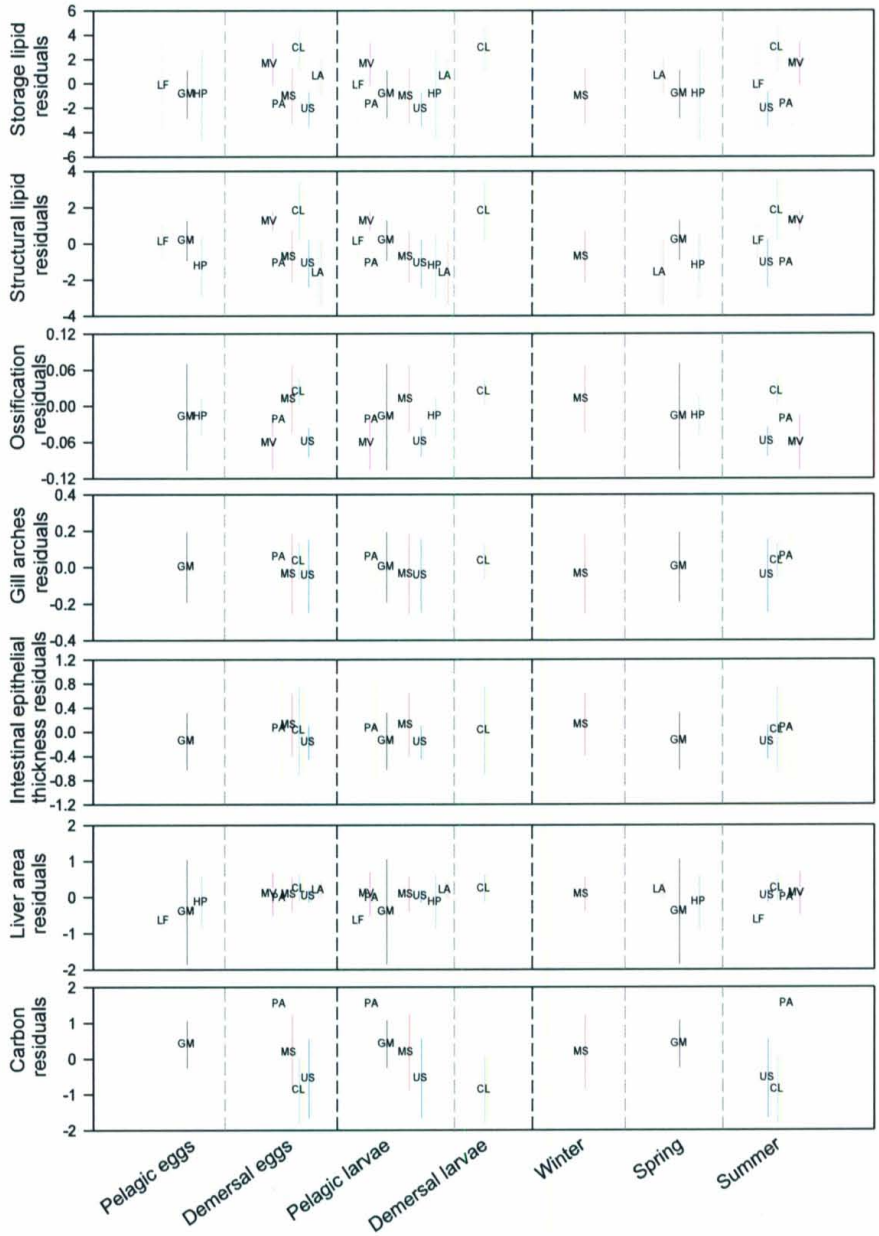

Figure 6.4 (Continuation and description on following page.)

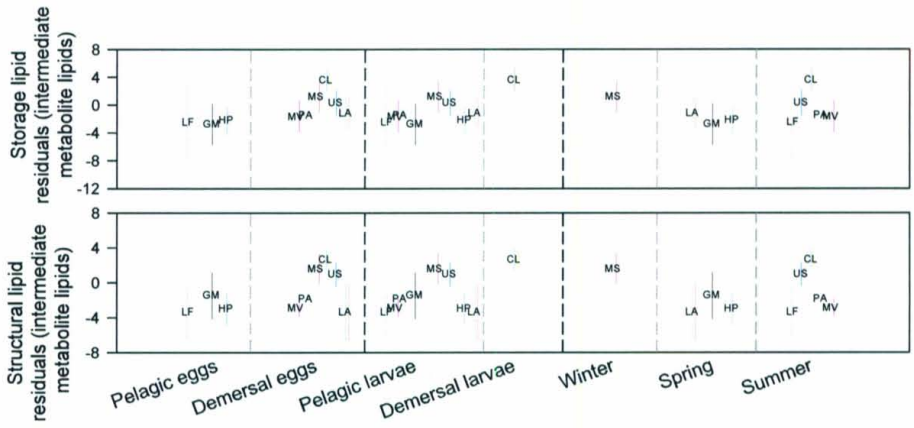

Figure 6.4 Average (± standard error, different colours = different species) of species values for egg development location, larval development location, and spawning season in the water column on storage lipid residuals, structural lipid residuals, ossification residuals, gill arch residuals, intestinal epithelial thickness residuals, liver area residuals, carbon residuals, storage lipid residuals (intermediate metabolite lipids), and structural lipid residuals (intermediate metabolite lipids). Residuals are a result from the species independent model regressing the variable of interest on dry mass, except for storage lipids and structural lipids, where the variables were regressed on intermediate metabolite lipids. GM = *Gadus morhua,* MS = *Myoxocephalus scorpius,* CL = *Cyclopterus lumpus,* PA = *Pseudopleuronectes americanus,* US = *Ulvaria subbifurcata,* MV = *Mallotus villosus,* HP = *Hippoglossus platessoides,* LF = *Limanda ferruginea* and LA = *Liparis atlanticus*.

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6.7 Appendix

Appendix 6.1 Path coefficients with standard error by jackknifing and corrected for multiple-imputation. Path coefficients significantly different from zero are shown in bold. Arrows indicate the directionality of the effect specified in the model. Degrees of freedom of the path models were 25.

		Multi-species model	*G. morhua*	*M. scorpius*	*C. lumpus*	*P. americanus*	*U. subbifurcata*
N		2226	522	549	495	209	90
Fit index		0.29	1.70	2.01	2.38	3.95	3.24
Goodness of fit index		0.99	0.94	0.91	0.90	0.80	0.86
Adjusted goodness of fit index		0.96	0.81	0.71	0.68	0.37	0.57
Dry mass ←	Ossification	-0.063 (0.02)	-0.052 (0.082)	-0.674 (0.167)	0.197 (0.126)	-0.241 (2.003)	-0.332 (27.449)
	Gill arches	-0.001 (0.028)	-0.053 (0.029)	0.186 (0.114)	-0.151 (0.084)	-0.006 (0.234)	-0.103 (0.208)
	Storage lipids	0.016 (0.113)	0.64 (0.286)	-0.163 (0.364)	0.012 (0.282)	0.416 (0.811)	-0.135 (47.91)
	Structural lipids	0.109 (0.121)	-0.534 (0.344)	1.046 (0.424)	0.140 (0.264)	-0.29 (2.486)	0.298 (37.068)
	Protein	**0.897 (0.04)**	1.003 (0.861)	0.301 (5.283)	**0.701 (0.153)**	**0.640 (0.224)**	0.224 (1.953)
	Intestinal epithelial thickness	**0.076 (0.026)**	0.039 (0.026)	**0.377 (0.106)**	0.134 (0.074)	0.087 (0.214)	**0.711 (0.11)**
	Liver area	**0.207 (0.023)**	**0.132 (0.04)**	**0.514 (0.143)**	0.031 (0.098)	0.093 (0.223)	**0.896 (0.241)**
	Carbon	0.396 (0.294)	-0.511 (0.728)	0.034 (2.67)	0.23 (0.287)	0.739 (2.247)	2.759 (14.25)
Intermediate metabolite lipids ↔	RNA	**0.458 (0.06)**	0.282 (0.12)	0.385 (0.171)	**0.365 (0.115)**	0.117 (0.142)	-0.440 (0.151)
	Storage lipids	**0.548 (0.034)**	**0.446 (0.083)**	**0.504 (0.091)**	**0.544 (0.089)**	-0.064 (0.214)	**0.589 (0.19)**
	Structural lipids	**0.558 (0.03)**	**0.381 (0.093)**	**0.47 (0.096)**	**0.493 (0.12)**	-0.186 (0.189)	**0.523 (0.187)**
Protein ←	RNA	0.115 (0.113)	0.715 (4.225)	-0.103 (7.11)	0.449 (0.285)	0.780 (0.941)	-1.333 (18)
	Nitrogen	0.334 (0.128)	0.842 (1.097)	-0.150 (4.883)	**0.48 (0.142)**	-0.126 (3.986)	-2.816 (15.431)
DNA →	Liver area	**0.416 (0.086)**	**0.638 (0.106)**	-0.292 (0.212)	**0.589 (0.171)**	-0.023 (0.33)	**0.682 (0.136)**
	Intestinal epithelial thickness	**0.341 (0.102)**	**0.275 (0.093)**	-0.154 (0.198)	**0.503 (0.14)**	0.178 (0.285)	0.126 (0.184)
	Gill arches	**0.295 (0.087)**	**0.313 (0.098)**	0.039 (0.215)	0.265 (0.144)	0.257 (0.325)	0.374 (0.217)

Appendix 6.2 Effect of ecological designations of egg development location, larval development location, and spawning season on original data. The nested ANOVA was performed on the residuals from the general species-independent model. Shown are sum of squares, Fisher's F-statistic and p-value. Species degrees of freedom = 8. Effects significant at $p<0.05$ are shown in bold.

			Eggs (df = 1)	Season (df =1)
Dry mass	Ossification N = 339	Effect	0, 0.15 0.70	0.084, 13.68 **<0.01**
		Species (nested effect)	0.202, 13.119 **<0.01**	0.193, 15.651 **<0.01**
	Gill arches N = 79	Effect	0, 0 0.99	0.041, 0.562 0.57
		Species (nested effect)	0.092, 0.837 0.48	0.049, 0.667 0.52
	Storage lipids N = 211	Effect	7.103, 1.534 0.22	33.858, 3.656 **0.03**
		Species (nested effect)	585.637, 18.068 **<0.01**	454.565, 16.362 **<0.01**
	Structural lipids N = 237	Effect	0.083, 0.04 0.84	33.691, 8.239 **<0.01**
		Species (nested effect)	295.846, 20.67 **<0.01**	247.507, 20.175 **<0.01**
	Intestinal epithelial thickness N = 88	Effect	0.38, 1.134 0.29	0.963, 1.438 0.24
		Species (nested effect)	0.472, 0.47 0.70	0.235, 0.35 0.71
	Liver area N = 74	Effect	1.378, 1.749 0.19	1.425, 0.904 0.41
		Species (nested effect)	18.867, 7.98 **<0.01**	10.614, 6.734 **<0.01**
	Carbon N = 372	Effect	1.378, 1.749 0.19	1.136, 0.769 0.46
		Species (nested effect)	18.867, 7.98 **<0.01**	6.167, 1.392 0.22
Intermediate metabolite lipids	Storage lipids N = 197	Effect	107.631 18.331 **<0.01**	173.746, 14.795 **<0.01**
		Species (nested effect)	536.102, 13.043 **<0.01**	562.456, 15.965 **<0.01**
	Structural lipids N = 204	Effect	84.628, 19.747 **<0.01**	320.609, 37.406 **<0.01**
		Species (nested effect)	639.114, 21.305 **<0.01**	590.294, 22.957 **<0.01**

Chapter 7 General Conclusion

In my dissertation I examined whether general multi-species, size-dependent models could be used to accurately predict the development of larval fish based on their ecology and phylogeny. Each chapter addressed a specific topic (developing an interspecies morphometric of state, understanding patterns of variations in lipid composition, examining developmental tradeoffs in larval fish, quantifying intrinsic selection, and building a general model of larval fish development) to investigate general ontogenetic development in marine fish. The contribution of this thesis in its entirety to the field of marine fish ecology is in three general areas: 1) the relevance of body size to larval fish ontogeny, 2) the potential of a multi-species model of larval fish development, and 3) sources of larval fish mortality.

7.1 Body size and larval fish

Body size is ecologically relevant for many animals, and particularly for larval fish undergoing their dramatic increase in size from hatching to metamorphosis (Chapter 1). Within this thesis, many of the analyses incorporated body size, and the relevance of body size to ontogeny was specifically addressed in Chapter 2. In doing so, two discussion areas arose: length *vs* mass metrics for interspecific analysis, and condition factors.

There is a debate surrounding the use of length or mass in larval fish research. Although many researchers avoid the debate by reporting both length and mass (e.g.

Conover et al. 1997, Pepin and Penney 1997, Fuiman et al. 1998, Otterlei et al. 1999), biochemical analysts tend to report mass (e.g. Jaworski and Kamler 2002) while field biologists tend to report length (e.g. Voss et al. 2006). Length is easier to measure in field-caught specimens stored in preservatives, whereas the mass of a very small larva can be difficult to obtain and subject to error depending on which preservative is used (e.g. Pepin et al. 1998 and references therein). The findings from my analyses favoured the use of mass, although to allow comparison among studies, reporting of both continues to be the most effective approach.

Little research has been undertaken to specifically address which metric to use for interspecific comparison, with the notable exception being the work by Fuiman et al. (1998). Fuiman et al. (1998) provided a relatively comprehensive analysis of interspecific metrics, which formed the basis of the body-size research in this thesis. I expanded upon their work by incorporating more morphometric elements, utilising continuous measures of the internal state of the larvae and including a greater variety of species. Results were inconclusive but body depth measured at the pectoral fin was the most versatile measure across all species. However, individual state variables (e.g. total lipids) were best described by different morphometrics. Future research on interspecific comparisons therefore requires consideration of the variables of interest before the most accurate morphometric can be selected.

The positive performance of body depth at the pectoral fin as a cross-species indicator (described above) raises questions about the use condition factors based on external measurements as proxies for the physiological status of the animal (for reviews, see Ferron and Leggett 1994, Shulman and Love 1999). In ichthyology and fisheries

ecology, body depth is often combined with another variable to describe the well-being of the fish. Although traditional condition factors are often dismissed as archaic because of the existence of more detailed information derived from analytical laboratory procedures, such as direct total lipid analysis, bioelectric impedance and microwave technology, this thesis suggests that morphological measurements of condition based on different elements of body size continue to offer substantive information about larval fish. Condition factors using morphometric methods do not require the amalgamation of samples, and this benefit recommends the continued investigation into and use of morphometric condition factors.

7.2 Ecology and phylogeny as frameworks for multi-species models of larval fish development

Previous attempts to analyse larval fish development from a multi-species perspective have produced mixed findings, often where one species behaved as an outlier. For example, despite an overall positive relationship between carbon and nitrogen in this thesis, *Pseudopleuronectes americanus* exhibited a negative relationship. Marked differences in ecological characteristics (e.g. Friedrich 1994, Hagen and Friedrich 2000, Kamler and Rakusa-Suszczewski 2001, Dantagnan et al. 2007), or different evolutionary histories (e.g. Houde 1989, Pepin 1991) are often alluded to as explanations for species-specific findings. Within this thesis, ecological or phylogenetic designations were examined explicitly to determine whether they contributed to similarities and differences among species (e.g. Sargent and Gross 1987, Moyle and Cech 1988). Phylogenetic

analysis did not provide significant insight into the different biochemical characteristics of larval fish but ecological analysis proved somewhat more useful.

Ecological designations included in this study were taken from common parlance about larval fish and formalised into designations of pelagic or demersal spawning location, development location, as well as the season of spawning. Using these broad categories to group larval fish resulted in similarities of larval development patterns in lipid allocation and in the number of developmental tradeoffs observed. However, when the energy allocation models were expanded in Chapter 6 to incorporate more variables, the ecological designations generally failed to categorise larval fish development. As such, ecological designations show limited promise, but further evaluation of other ecological criteria may show better results.

7.3 Sources of larval fish mortality

This thesis has provided valuable information to address the ongoing question of high mortality rates in larval fish. This has been done by highlighting the importance of variables that characterise developmental events during ontogeny (e.g. development progress variables, Chapters 4 and 6) and through the analysis of patterns of intrinsic selection (Chapter 5).

With regard to the use of developmental progress variables, measures of ossification and organ development are not normally included in energy allocation studies for a number of reasons (Van der Meer 2006), the most likely of which is the frequently qualitative nature of their assessment (e.g. Govoni et al. 1986, Baglole et al. 1997, Gisbert and Doroshov 2003). The field that studies this aspect of larval fish is often conceptually

separate from the field that quantifies patterns of energy allocation (Van der Meer 2006). In my research, I integrated information on developmental progression with quantitative indices of biochemical status, which led me to find an emergent pattern that suggested a tradeoff between changes in these two metrics: developmental progression and growth. I interpreted this as an indication of competition for energy within a larva between increasing size and increasing complexity. Energetic tradeoffs are well understood in adults in terms of the competing needs of reproduction and growth (Roff 1983), but have received little attention in the larval stage. The study of increasing complexity in larval fish could significantly improve our understanding about what prevents certain larvae from succeeding.

Further, the consistent treatment of larvae as individual samples throughout this thesis has allowed the quantification of 'intrinsic selection.' 'Intrinsic selection' (in the context of this thesis) refers to mortality arising from characteristics of the larvae themselves, independent of the external environment. My results indicate that some larvae are simply more likely to succeed than others, regardless of environment. Research to date has focused on growth rate as the primary determinant of intrinsic selection (e.g. Erzini 1994, Benoit and Pepin 1999); this thesis supports this view, but also expands it to other variables such as lipid accumulation.

7.4 Conclusions and future directions

The examination of larval fish development from a multi-species perspective has allowed an evaluation of a variety of existing organisational frameworks. My findings support the continuing use of condition factors and the evaluation of larvae from an

individual perspective to further understand intrinsic sources of loss. Perhaps the largest contribution to the understanding of how larval fish develop, and why species differ from each other, comes from evidence of the competing energetic demands between developmental progress, representing increased complexity, and those associated with increases in body mass during the early life history of fish (see Chapters 4 and 6).

The future direction from this thesis points to the need for more comprehensive analyses of the scale (e.g. individual, population, species, and ecological group) at which larval fish are similar and where they diverge. For example, do larvae of different populations of fish within the same species share the same patterns? Another aspect of scale is the question of at which point in development the larvae begin to diverge in energy allocation strategies? Finally, a paradigm shift for many larval fish biologists is required by including terms of increasing complexity, such as organogenesis, in larval development modelling, as it requires understanding not only what the energetic requirements are, but the how they change as ontogeny progresses.

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