FORM, FUNCTION AND ENERGETICS OF THE EARLY LIFE HISTORY OF ATLANTIC COD (Gadus morhua)

by

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by IONE HUNT VON HERBING

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ABSTRACT

The physiological energetics of the early life history of Atlantic cod, Gadus morhua were investigated in laboratory studies for two genetically discrete populations (Newfoundland and Scotian Shelf), by integrating information from the fields of ecology, physiology and functional morphology. Laboratory studies were designed to simulate temperatures that eggs and larvae of different stocks experience in the ocean. Functional morphological landmarks important to feeding and respiration were derived from specimens treated by clearing and staining, histology, and scanning electron microscopy. These landmarks defined 12 developmental stages at two temperatures, 5°C and 10°C, from hatching to metamorphosis, in larval cod. Results showed that staging is a more accurate way of determining developmental state than days post-hatch or degree days. Staging of specific structures such as the jaw and alimentary canal illustrated that variation in structural complexity may be influenced by intrinsic (genetic) factors and/or extrinsic (environmental) factors. Detailed anatomical and functional analysis revealed that while the yolk-sac serves as a food resource, primary skeletal structures and mechanisms for feeding are simple and non-integrated and respiration is cutaneous. As ontogeny progresses, requirements for exogenous food are met by the development of new skeletal elements, ligaments and muscles. Together these changes allow increases in jaw coordination and suction pressure generation necessary for prey capture and eventually for branchial respiration.

Landmarks and developmental stages were also used to mark major life history transitions in energy acquisition. At these transitions, changes occur in the allocation of finite energy resources between growth and activity metabolism. In addition, the energy allocated to growth and activity metabolism differed between populations. Larvae from the Newfoundland population were more cost-effective in conditions that reflected their native environment; these larvae appeared to be physiologically 'cold-adapted'. In contrast, larvae from the Scotian Shelf population appeared to be 'warm-adapted' but spent more energy on activity metabolism and less on growth at all temperatures. This suggests that the physio')gical energetics underlying the dynamics of production and survivorship are different between cod populations in Canada.

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
ATP	adenosine triphosphate
b	metabolic mass exponent
bc	blood cells
bh	basihyal
bl	body lengths
C	experimental chamber
°C	degrees Celsius
c	jaw length
cal	calories
cDNA	complementary deoxyribonucleic acid
Ch.	chapter
cm	centimetres
D	dentary
D°	degree days
do	dilitator operculi
E	pulsed electrode
G	absolute growth rate
g	geniohyoideus
gdwt	grams dry weight

HBQ	Hall and Brunts' quadruple stain
hrs	hours
hs	hyomandibular symplecticum
hy	hyoid
I	interoperculum
ih	interhyal
J	Joules
l	litres
lap	levator arcus palatini
ln	natural log
M	mass
m	maxilla
MC	Meckels' cartilage
md	mandible
mg	milligrams
mm	millimetres
ms	manuscript
NF	Newfoundland
NS	Nova Scotia
O	operculum
O_2	oxygen
oc	otic capsule

oc	opercular epithelium
P	preoperculum
p	pseudobranch
pm	premaxilla
PP	peristaltic pump
q	quadratal angle
qu	quadrate
R	activity metabolism
Re	Reynolds' number
RV	reservoir
R5T5	raised at 5 degrees, tested at 5 degrees
R5T10	raised at 5 degrees, tested at 10 degrees
R10T10	raised at 10 degrees, tested at 10 degrees
S	suboperculum
s	seconds
SEM	scanning electron microscopy
SG	specific daily growth rate
sp.	species
T	time interval
t	trabeculum cranii
ug	micrograms
um	micrometres (microns)

unpubl.	unpublished
v	ventral arches
vc	vertebral column
VO_2	rate of oxygen uptake
WT	dry weight at end of specified time interval
wt	dry weight at start of specified time interval

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GENERAL INTRODUCTION

Studies of the early life history of marine fish have progressed over the years from work done primarily for taxonomic purposes (Blaxter 1984, Cohen 1984) to studies which treat larval stages as a key component of the entire life history of the fish. Hence, embryonic and larval stages have been acknowledged as being integral in determining interannual variability in marine fish recruitment and adult population dynamics. Advances in our knowledge of embryonic and larval fish biology have resulted from better techniques for raising fish larvae and improvements in the microtechnology necessary to record physiological functions. Further advances will depend upon the integration of knowledge from a comprehensive interdisciplinary approach.

A comprehensive interdisciplinary perspective of early life history dynamics necessitates the integration of information from a number of sources: (1) data on the reproductive history of the spawning populations (i.e. size and fecundity of females and sex ratios of the spawning stock). (2) knowledge of the oceanographic conditions (e.g. temperature, salinity) that surround the eggs and larvae during development and during transport away from the spawning area, (3) detailed knowledge of the morphology and function of structures that form during development and their relative importance to survival, and (4) knowledge of early life energetics and how energy demands are met during growth to favour survival. To date, lack of the above basic knowledge for marine fish species has inhibited the formulation of comprehensive bioenergetic models which

may, in turn, aid in resolving the key mechanisms driving marine fish recruitment and survival.

(A)Reproduction and Early life of Atlantic Cod.

Atlantic cod spawn over a wide area of the continental shelf off the northeastern coast of North America. The northern (Labrador – eastern Newfoundland shelf) cod stocks usually spawn as early as March (Lear and Green, 1984). Time of spawning becomes progressively later southward, beginning in April and continuing into June on the Grand Bank. On the Scotian Shelf, most spawning occurs between October and April, with a peak in intensity in December. On the northern Scotian Shelf spawning begins in the autumn predominantly around Sable Island and along the Nova Scotian coast (personal observations). By April, spawning animals has moved south to Emerald, Western and Browns Bank areas (O'Boyle et al., 1984). Cod spawn at depths from less than 110 m to over 182 m, depending on the water temperature (Scott and Scott 1988). They are 'broadcast spawners', a large cod (140 cm) releasing some 12 million pelagic eggs (Powles 1958). The eggs are spherical, transparent, buoyant and measure 1.2 – 1.6 mm in diameter (Moser et al. 1984). There is no oil globule, usually present in other marine fish eggs (e.g. Thalassoma bifasciatum) to aid in buoyancy. Fertilized eggs float to the surface and remain there during incubation (Dahl et al. 1984). Newly hatched larvae are 3.5 – 5.7 mm long (standard length), and pelagic. Cod larvae remain pelagic until about 25 – 50 mm in length, or 2 or 3 months from hatching, when they transform to the pelagic juvenile stage (Fahay 1983, Bolz and Lough 1988). Transition from the

pelagic to demersal life occurs when the juveniles are about 4 to 6 cm (Lough et al. 1989, Potter et al., 1989).

During the egg and pelagic larval stage, individuals may be dispersed far from their spawning location and are likely to be subjected to extreme variations in environmental conditions. The eggs and larvae of northern cod, spawned between March and June, develop as they drift in the cold Labrador current to shallow nursery grounds 600 – 1000 km to the south (Lear and Green 1984). Indeed, they complete their development in the warmer water over the Grand Banks up to two months later. Eggs and larvae originating on the Scotian Shelf, however, seem to be retained over the shelf by gyres and fronts (Griffin and Lochmann 1993). This may reduce the likelihood of eggs and larvae being lost to the open ocean where food concentrations are lower and suitable settlement sites are absent. Studies on Georges Bank also show that larval transport is limited by gyres which seem to retain the larvae over the bank, thereby reducing larval dispersal (Frank 1984), but perhaps enhancing larval survival (Johannes 1978). Although these processes reduce dispersal off the Scotian Shelf and Georges Bank, eggs and larvae are also subject to sudden transient changes in temperature that occur due to the influence of warm – core rings from the Gulf–Stream that come onto the southwestern Scotian shelf (Scott and Scott 1988). In Norway, arcto–Norwegian cod eggs and larvae must also be able to tolerate changes in temperature and salinity when swept from the ocean into the fjords. Clearly, early life history stages of cod must be able to withstand a multitude of different types of changes in environmental parameters, and tolerance to stress may be partly genetically determined. Recently, Pogson (1993) used cDNA genomic clones to establish

for the first time that contiguous populations of cod along the Northeast coast of Newfoundland and along the coast of Nova Scotia are genetically discrete. Genetic differences may also partially explain the regional differences observed in the timing and location of spawning (Fletcher 1993). Together, these findings suggest that inter-population differences may have arisen by environmental selection and that genetically distinct groups of cod are adapted to their regional conditions.

(B) Morphology and Functional Morphology: the basis for life history stages.

Fish embryos and larvae are in a state of continuous dynamic change undergoing more rapid growth and development than will ever occur in their juvenile and adult stages. Even though this change is continuous, the rate of change is not constant, allowing stages of development to be defined for descriptive purposes. Commonly used general stages for the early life history of fish are as follows: the egg stage (spawning to hatching), the yolk-sac stage (reliant on endogenous food resources), the larval stage, obligate exogenous feeder (subdivided into preflexion, flexion, postflexion) and the transformation stage (between larva and juvenile; also referred to as metamorphosis) (Kendall et al., 1984). Other staging sequences have been suggested e.g. Balon's eleutheroembyro and protopterygiolarva (Balon 1981). These latter terms are based on the belief that developmental change only occurs at developmental thresholds and not continuously, i.e. hatching is not considered to be a major event in development as little morphological change occurs at this time (Balon 1980). However, these terms have not been generally adopted in general as no consensus has yet been reached as to their

derivation.

Embryonic and larval stages of Atlantic cod have been described most recently by Russell (1976), Hardy (1978), Fridgeirsson (1978), Thompson and Riley (1980), Fahay (1983), Fossum (1986) and Morrison (1993). In many of these studies, descriptions and stage definitions were based on different collections of larvae (from wild and raised specimens) and from varying environmental conditions, making clear identification of stages and any estimate of variation in morphology per stage impossible. Moreover, in some studies where conditions were controlled, larval fish were only described up to the end of the yolk-sac stage (e.g. Fridgeirsson (1978) and Fossum (1986)). Staging of the embryonic stage is more complete than that for the larval stage as raising cod to metamorphosis under controlled conditions to obtain a staging series is quite difficult. To date, studies comparing individuals raised under different conditions (e.g. temperature) have been made by using thermal summation methods (i.e. degree days (D°) = temperature°C x days post-hatch or fertilization; Lein and Homefjord, Harboe et al 1990, Budgey 1992, Kamler 1992, Suthers and Sundby 1993) or standard length or dry weight (Laurence 1978, Almatar 1984, Solberg and Tilseth 1984) to normalize development. These methods assume that developmental rates of all structures change linearly with temperature and that stages are size dependent. Herzig and Winkler (1986) did not support the above assumptions as they found a non-linear relationship between development rate and temperature in cichlids. Although, a complete descriptive series is invaluable to fisheries scientists for rapid identification of various stages in the early life of the species, some quantification of each stage is necessary to enable the observer to

document the relative variation of morphological traits with length and age in the field. Quantification of variation of developmental rates of essential structures may in turn elucidate developmental events that dictate survival.

In addition to qualitative and quantitative morphological stages, early life history stages should also be defined with reference to how the developing structures affect function and therefore possible mechanisms important to survival i.e. feeding, respiration and locomotion. Detailed functional morphological studies have been carried out on adult fishes, notably those by Liem (1970) and Lauder (1980, 1983) who were able to define the species–specific feeding niches for cichlids from close examination of jaw structure. Barel (1983), Gottfried (1986) and Otten (1983) went further, identifying specific jaw morphs in cichlids to specific feeding activities eg. 'biters and suckers'. Studies by Liem (1991) and his colleagues (Dilling, 1989 and Brainerd, 1985) on larval cichlids and pomacentrids showed that as head proportions change from a cylindrical to a truncated cone design the larva switches from ram feeding to a predominantly suction feeding strategy. The larval stages of the species studied by Liem et al. are quite morphologically advanced at hatch and are raised at high temperatures (27°C), thus accelerating development to the adult stage. However, little is known about the functional morphology of feeding in Atlantic cod. This species hatches at a less morphologically complex stage (Hunt von Herbing et al. 1992) and develops at a slower rate than cichlids (60–90 days to the juvenile stage vs 14 days) due to the colder temperatures (0–7°C) it inhabits. However, cod must still be able to feed exogenously 5 days after hatching even though they lack most of the structures that a larval cichlid possesses when it begins to

feed exogenously. Studies of functional morphology in larval fish, such as in this study, allow us to investigation of the relationships between form and function and help us understand that a structure or a complex of structures need not be completely developed to function. For example, the development of the lower jaw in cod larvae begins as a simple cartilaginous structure (Meckel's cartilage) and is involved in first feeding during the first week of larval life. Dentary teeth do not form until five to six weeks later, well into the obligate exogenous feeding stage. This supports the idea that ontogenetic change in morphology and function is finely tuned to meet the needs of larval fish throughout their development.

(C) Metabolic Energy Allocation During Early Life

Throughout early life, finite energy resources are distributed into metabolic compartments driving development and growth. How these resources are allocated has important consequences for the overall physiological fitness of an individual. Optimum patterns of resource allocation are those that lead to greater reproductive output, thereby transmitting more genes to future generations (Arnold 1988). Of all the factors that influence metabolism, the energetic cost of growth and activity and the effects of temperature on the balance between these two variables throughout development are considered to be the most important.

(i) Development and Growth

To determine how energy from food is allocated to growth (change in size with age)

as distinct from development (differentiation of cells, tissues and organs) changes in total metabolic rate need to be determined throughout the entire early life history. This should be done in conjunction with the developmental stages referred to earlier (see Section B). Attempts to link oxygen consumption (VO_2), expressed on an individual basis, to specific developmental events have proven confusing as a result of the effect of tissue mass on oxygen consumption. Therefore, oxygen consumption has been expressed on a mass specific basis. In most teleosts, including cod (Davenport and Lonning 1980), metabolic intensity (VO_2/M), increases from fertilization to hatch and continues to increase through the yolk-sac stage until midway through the period of exogenous feeding. Only a few studies (Holeton 1973, Forstner et al. 1983) have measured changes in metabolic intensity for fed larvae. Since it is during exogenous feeding that major organ systems (e.g. digestive and respiratory) complete their development, more work during this period is needed. Energy allocated to growth can be reported in two ways (1) as mass-specific oxygen consumption (also defined as metabolic intensity VO_2/M and (2) by using the allometric power function $VO_2=aM^b$ where a and b are constants. Expressing the cost of growth as metabolic intensity does not obscure important fluctuations in VO_2 by the overall trend as would occur by using the allometric relationship. However the allometric relationship is widely used, particularly for larvae (Rombough 1988a), and can be used in conjunction with other allometric relationships to study and compare the physiological ecology and energy budgets between life history stages. The most commonly observed value for the metabolic exponent (b) is 0.8 (Winberg 1956) which is the relationship between the metabolic rates of animals at rest or during routine activity with their body

weight (Schmidt-Nielson 1984). However, the value of 0.8 was for studies involving juvenile and adult fish and is not necessarily the same value one might expect for earlier life stages. In general, metabolic exponent values are high during the larval stage and decrease with development and growth (Kamler, 1976). However values seems to be highly variable and dependent on species (e.g. b = 0.65 for plaice, b = 0.82 for herring (DeSilva and Tytler, 1973) and development stage (i.e. prefeeding or feeding larvae) (Rombough 1988b). For larval cod mass exponents have been recorded to range from, b = 0.69 to b = 0.78 (Laurence 1978). However, as Rombough (1988) points out those values were based on data from all larval stages combined and may increase using data from a specific stage or size class within the larval stage. In addition, mass exponents close to unity have also been reported (Kamler 1972, Cetta and Capuzzo 1982, Quantz and Tandler 1984, Giguere et al. 1988). Giguere et al. (1988) advocate, based on their studies of larval mackerel and re-calculations of other studies' results, that metabolic rates scale linearly with body weight in all larval fish. Resolution of this issue will require careful investigation of the changes in stage specific mass exponents during the larval life.

Two major reasons have been proposed for the higher metabolic exponents found in fish larvae than in juvenile and adult fish (1) oxygen availability to metabolizing tissues is limited because the gas exchange surface i.e. cutaneous surface area or developing gill surface area expands at a slower rate than body mass and (2) a decrease in the amount of red muscle and associated oxidative enzymes with the onset of metamorphosis at which time there is an increase in white muscle and glycolysis (Forstner et al. 1983). However, Oikawa and Itazawa (1985) found no direct relationship between respiratory surface area

and resting metabolism and Hinterleitner et al. (1987) pointed out that glycolytic and oxidative enzymes develop differently in different species and therefore cannot be directly compared. Overall, these studies have shown that the mass exponents do shift from one life history stage to the next, decreasing as body size increases. This indicates that growth carries a high cost in early stages. However, genetically predisposed faster-growing larvae may pass rapidly from an energetically more expensive stage to one less energetically costly, thereby increasing their probability for survival. However, metabolic rates are greatly influenced by stage of development, temperature and activity. Therefore, in addition to the methodological differences, past studies did not control for all these factors and interspecific comparisons could only be made between species with the same metabolic exponent (Konstantinov 1980). Consequently, before general conclusions can be made about the changing cost of growth in larval fish, further, more rigorous studies, are necessary which normalize metabolic rates during ontogeny to developmental stage, temperature and activity.

(ii) Activity

Activity is thought to be the single most important factor affecting the metabolic cost of growth and development at every life history stage of fish, because activity VO_2 can be the major component of overall VO_2 at any stage. The relationship between activity and oxygen consumption has been investigated for juveniles and adults (Fry 1971, Brett and Groves 1979). However, only recently have the effects of activity been considered important to early stages undergoing rapid growth and development. The hydrodynamic

environment of a fish larva is very different to that of juvenile and adult fish (Webb and Weihs 1986). Larval fish undergo a shift in swimming behaviour which is related to the changes in their hydrodynamic environment. As a consequence of their small size fish larvae hatch into a viscous environment (Reynolds number (Re) < 20) and tend to be stationary between occasional periods of continuous swimming. As they grow they move into an intermediate hydrodynamic environments (20 < Re < 200) where inertial forces replace viscous forces and swimming is characterized by 'beat-and coast' patterns. However, at any stage during the larval life, fish larvae are capable of burst swimming into higher Re environments where inertial forces dominate (Webb and Weihs, 1986). These high bursts of speed occur during activities essential for survival, e.g. during predator avoidance (Webb 1981, Webb and Corolla 1981) and during prey capture (Hunter 1972, 1981, Rosenthal and Hempel 1977). In addition to changes in patterns of locomotion, development of muscle types (i.e. changes in the proportion and location of red and white muscle) and biochemical pathways (i.e. changes in the utilization of aerobic vs. anaerobic (glycolytic) pathways) also occur in-step with the changing needs of locomotion (Wieser et al. 1985, Hinterleitner et al. 1987). Therefore, swimming patterns along with morphological and physiological changes work in concert throughout larval life to reduce the net cost of transport and energy demand for locomotion (Weihs 1980, Kaufmann 1990).

Quantification of the energetic demand of locomotion throughout ontogeny is made by measuring metabolic rates at controlled activity levels for specific body weights or developmental stages; i.e. standard (sVO_2), routine (rVO_2) and active (aVO_2) metabolic

rates. Standard (or basal) metabolism (i.e. metabolism during minimal neuromuscular activity), has been estimated from anaesthetized animals (De Silva & Tytler, 1973; De Silva et al. 1986) or immediately before hatching (e.g. Gruber & Wieser, 1983). Most investigators attempt to measure routine metabolism, which can be defined as the average rate of aerobic metabolism under normal resting conditions. However, as Wieser (1985) pointed out, routine metabolism can vary as much as two–fold in response to intrinsic and extrinsic factors, making interspecific and intraspecific comparisons based on rVO_2 difficult. Active metabolism (aVO_2) in older fish is based on sustained activity – such activity is difficult if not impossible for most larval fish. Therefore most estimates of aVO_2 in fish larvae are based on spontaneous activity or burst swimming in response to electric shocks (Wieser 1985, Wieser et al. 1985).

The definitions of metabolic rates as outlined above were originally derived for respirometric measures of adult fish swimming against a current in a swim tunnel (Fry 1971, Brett 1964). Early – stage larval fish tend not to swim against currents and therefore it is difficult to obtain values for standard, routine and active metabolic rates and compare the resulting values against those of adults. However, Kaufman (1990) was able to derive relationships between swimming speed and VO_2 (equations which he termed 'power – performance' relationships) for a large range of sizes of cyprinid larvae, which are large at hatch (1 mg compared to < 60 μg for cod larvae), and able to swim against proportionately higher current speeds, unlike cod larvae (Hunt von Herbing, 1993). He determined, from these relationships, that the cost of transport decreased allometrically with body weight in cyprinids, optimizing energy utilization with changing hydrodynamic

regimes. Therefore, the ability to derive larval sVO_2, rVO_2 and aVO_2 values which can be compared to those of adults may depend on species-specific characteristics and capabilities at the larval stage. Consequently, the problem of isolating the energy demand of locomotion in species which have small larvae, such as cod, still remains. Moreover, difficulty of deriving relationships between swimming speed and metabolism for larval fish may mean that published indices such as 'absolute aerobic scope' (aVO_2–sVO_2) and 'absolute factorial scope' (aVO_2/sVO_2) may be inaccurate. These indices represent the amount of energy available for activities such as foraging and predator avoidance and are subject to changing abiotic factors such as temperature. However, temperature effects on aerobic scope are not clear and seem to vary between species. Wieser (1985) and Wieser et al. (1985) found that for young rainbow trout, between 4 – 12°C, active metabolism increased faster than routine metabolism thereby increasing aerobic scope. At temperatures greater than 12°C, the rate of active metabolism decreased compared to routine metabolism decreasing aerobic scope. In contrast, in cyprinids, relative factorial scope (aVO_2/rVO_2) seems to be independent of temperature (Wieser and Forstner, 1986). Accurate derivation of the above activity indices for many species of fish is important as they may represent fitness indices – differences which may occur in stocks subject to changing environmental pressures. Moreover, differences in indices of activity may also influence the energy available to other mechanisms such as growth and development in larval fish and hence affect the probability of survival.

(iii) Temperature

Of all the abiotic factors, temperature is thought to have the greatest influence on the rates of differentiation, growth, metabolism and activity (Rombough 1988a, Blaxter 1992). In general, metabolic rate increases with temperature, but temperature changes have a more profound effect during early life than in juveniles or adults. Values of Q_{10} (the factor by which metabolic rates change over a 10 degree temperature change) are around 2 for standard and active metabolism in juvenile and adult fish (Fry 1971). In contrast, values of Q_{10} in salmonid embryos and alevins range from 3.0 (Rombough 1988b) to 5.0 (Gruber and Wieser, 1983), respectively. Higher values in early life may indicate that embryos and larvae may be more sensitive to temperature fluctuations and potentially more stenothermal than juveniles and adults (Gruber and Wieser 1983, Rombough 1988a). However Wieser and Gruber (1983) also point out that Q_{10} values for larval fish are calculated largely from routine metabolic rates as standard and active metabolic rates are difficult to derive in larval fish (see section ii). Since, increases in temperature lead to higher activity and metabolic rates in fish (Fry 1971, Blaxter and Batty 1992), comparisons of Q_{10}'s between larvae, juveniles and adults must also consider the effects of temperature on resting and active metabolism at all life history stages.

Thermal tolerance and thermal growth optima may vary throughout ontogeny as well as between major life history stages. Adult fish show differential tolerance of thermal stress in the short term (acute) versus the long term (chronic) (Kelsch and Neill, 1990). Fish can compensate physiologically for extreme temperature changes in the short term e.g. by producing enzymes which are more efficient at the new temperature (Hochachka

1967) or by acclimating (adapting physiologically to function more efficiently at the new temperature) to the temperature change in the long term (Kelsch and Neill, 1990). The ability of an individual to expand its thermal tolerance through acclimation may be independent of its ability to withstand acute temperature change, and may also partly depend on its thermal history (Blaxter 1992). Results from the few studies conducted on larval fish indicate that they may not be capable of thermal acclimation (Clements and Ross 1977, Hinterleitner et al. 1987, Rombough 1988b). However, it is often difficult to isolate the effects of temperature on metabolic rate, due to changes in activity associated with temperature change (Batty and Blaxter 1992, Hunter 1981, Fukahara 1990). In most studies of metabolic response in larval fish to temperature, activity levels were not measured. Hettler (1976) did measure activity of larval menhaden and found that activity increased with temperature, but this was not reflected by a large increase in VO_2 (Q_{10} = 2.1). Metabolic adaptability of larval fish to thermal stress is also evident from changing upper and lower lethal temperature limits when examined through ontogeny and between latitudes. Brett (1970) showed that temperate species were that much more eurythermal than subtropical and tropical species, and embryonic (pre-hatching) stages had narrower temperature tolerances that post-embryonic stages. Lethal temperature limits and the extent of thermal tolerance are of particular importance to temperate species such as Atlantic cod which spawn over a wide range of temperatures. Differences in temperature during development may greatly influence the way in which energy from food is distributed to growth and metabolism. Temperature differences may also result in different adaptive strategies of energy allocation for thermal tolerance between genetically

different stocks (e.g. Newfoundland vs. Nova Scotian cod stocks) in response to different local thermal regimes.

In larval fish, growth rates (already high at this life stage) increase with increasing temperature (as does metabolic rate), unlike stage duration which decreases (Houde 1989). Stage duration is not a major factor in adults or juveniles, but it can have a critical effect on the probability for survival in early life history stages. For example, at lower temperatures where stage duration is longer, large eggs yielding larger larvae (Shirota 1970) generally survive better than small eggs yielding smaller larvae (Hunter 1981). However, duration of egg incubation and yolk-sac stage also increase with increases in egg diameter (Ware 1975). Therefore, although larger larvae are considered to be more successful at feeding and evading predators as a result of their higher swimming speeds (Hunter 1981), it is at the cost of decreasing fecundity of the parent stock and increasing the duration of stages most vulnerable to predation. Smaller eggs and larva hatch out with smaller endogenous reserves but higher growth rates move them through the more vulnerable stages, without any cost to fecundity. However, at higher temperatures metabolic rates increase and ingestion rates must also increase to provide the needed resources for growth. As a result, at high temperatures, such as those of low latitudes, high growth rates lead to higher mortality rates because oligotrophic oceans may not provide sufficient food resources. This has resulted in the evolution of continuous spawning fish which produce many large batches of eggs throughout the year insuring that some larval cohorts will survive during favourable windows of opportunity (Houde 1989, Sale 1980). At cooler temperatures (higher latitudes) spawning is temporally or spatially

constrained, and even though growth, mortality and ingestion rates are low, any slight changes in these factors can have major effects on recruitment (Houde 1989). Therefore, fine adjustments in these strategies may affect the way in which energy is allocated to development, growth and activity between cohorts and across latitudes to meet regional and seasonal differences in environment. Changes in egg sizes occur among seasonal cohorts of many temperate species of fish, e.g. herring (Blaxter and Hempel, 1963) and cod (Dahl et al., 1984), with the largest eggs produced in the spring at the coolest temperatures and egg size declining as the season progresses (Bagenal 1971, Ware 1975). It seems therefore, that selection has acted through many routes; variability in egg size, spawning frequency, growth rates, metabolism and mortality, to optimize the way in which energy is allocated in different thermal regimes for highest probability of survival of the early life history stages of marine fish.

Summary. To accurately assess the allocation of energy to development, growth and activity during the early life history stage of marine larval fish, more basic research into species specific biology, physiology and ecology is required. Specifically needed are measurements of the effects of temperature on larval fish development, growth and activity. Interaction of these variables will provide a foundation on which to build species – specific bioenergetic models of marine larvae. Development of bioenergetic models for cod early life history may aid in understanding how cod larvae balance their energy budgets as a function of environmental conditions. In turn, this may lead to identification of some of the factors that result in differential fitness and eventual recruitment to the adult stock.

The purpose of this thesis is to investigate the physiological energetics of two discrete populations of Atlantic cod, Gadus morhua, by integrating information from the fields of ecology, physiology and functional morphology. Specifically five objectives will be met. First, to describe and quantify, throughout development, those structures specific to vital functions such as feeding and respiration. and to determine the influence of temperature, size and age on the variation in developmental rates of the described structures. Second, to show that ontogenetic changes in feeding and respiratory systems are finely tuned to meet the needs of larval fish throughout development. Third, to determine how energy obtained through feeding and respiration is differentially allocated between activity, growth and metabolism within populations and between populations (ie. Newfoundland vs. Nova Scotia) throughout ontogeny. Fourth, to determine if energy allocation differs within or between populations under different thermal stresses. Finally, to use the morphological and physiological functions derived to construct energy budgets for the early life history of Atlantic cod from two discrete populations (Newfoundland and Nova Scotia).

Chapter 1

EFFECTS OF TEMPERATURE ON MORPHOLOGICAL LANDMARKS CRITICAL TO GROWTH AND SURVIVAL IN DEVELOPING LARVAL COD.

INTRODUCTION

Interannual fluctuations in mortality rates of early life history stages of cod are thought to greatly influence year class strength and adult population abundance. Although numerous workers have studied cod larval morphology (Neilson et al. 1986, Yin and Blaxter 1986, Kjorsvik et al. 1991, Van der Meeren 1991, Pedersen and Falk-Petersen, 1992), physiology (Laurence 1978, Davenport and Lonning 1980, Ellertsen et al. 1980, Solberg and Tilseth 1984, Serigstad 1988), and ecology (Frank 1984, O'Boyle et al. 1984, Bolz and Lough 1988, Lough et al. 1989, Lough and Potter 1993, Suthers and Sundby 1993), key mechanisms that dictate larval survival are little understood. My approach has been to consider simultaneously the developing structure and function of developing tissue and organ systems associated with respiration, feeding, growth, and activity metabolism. Such information should greatly enhance our ability to estimate survival from planktonic stages through to recruitment.

Morphological descriptions of embryonic and larval stages of Atlantic cod have been documented most recently by Russell (1976), Hardy (1978), Fridgeirsson (1978), Thompson and Riley (1981), Fahay (1983), Fossum (1986) and Morrison (1993). In many of these studies, definitions of discrete developmental 'stages' have been made on

collections of eggs and larvae complied from both wild and laboratory-raised specimens originating from quite different and variable environmental conditions. Moreover, in those studies where environmental conditions and source were controlled, fish larvae were described up to the end of the yolk-sac stage, but not beyond (e.g. Fridgeirsson 1978, Fossum 1986).

Studies comparing individuals raised under controlled conditions of temperature have used either the thermal summation method (i.e. degree days (D°) = temperature °C x days post-hatch or fertilization; Lein and Holmefjord 1990, Harboe et al. 1990, Budgey 1992, Kamler 1992, Suthers and Sundby 1993) or standard length or dry weight (Laurence 1978, Almatar 1984, Solberg and Tilseth 1984) to normalize development. These methods assume, however, that developmental rates of all structures change linearly with temperature, and that all structural changes are size/weight dependent. Such an approach may aid in the collection and compilation of a complete descriptive series of larval stages, but without concern for thermal history or interindividual variation.

In order to document the relative variation of morphological traits with length, age, and prevailing environmental conditions, staging of essential structures or 'landmarks' may be necessary. The term 'landmark' refers to specific structures or morphological characters which I consider crucial to the basic physiological processes necessary for survival, i.e. feeding, respiration and locomotion. For example, a larval fish must have a jaw which functions sufficiently well to capture prey, fins for propulsion, stability, prey capture and predator avoidance, and an alimentary tract to process food for growth. The morphological landmarks I describe should be viewed as dynamic, not static, that is, they

reflect the plastic nature of changes in morphology, physiology, and ecology, which occur during the larval period.

During the transformation to a pelagic juvenile, larval characteristics are gradually lost and juvenile body shape and features are attained. In Atlantic cod, there is no abrupt dynamic transition, morphological alteration, or 'metamorphosis' to the juvenile stage. Rather, it is during the larval period that the greatest morphological change takes place, and that quantification of structures is particularly important. For example, extensive quantification of gross morphology (O'Connell 1981, Sieg 1992 a,b), biochemistry (Buckley and Lough 1987) and ultrastructure (Theilacker 1978, O'Connell 1981) of the alimentary tract have been used to identify starvation as an index of larval 'condition' (i.e. likelihood of survival (Theilacker 1980, 1986)). Quantification of variation in developmental rates of essential structures may in turn elucidate developmental events that dictate survival.

The purpose of this study is to: 1) describe and quantify the morphological 'landmarks' crucial to feeding, respiration and locomotion in cod larvae, 2) utilize changes in selected landmarks to define descriptive stages of larval Atlantic cod raised under controlled conditions, and 3) determine the influence of temperature, size and age on the variation in developmental rates of described structures in larval Atlantic cod. My objective is to relate the morphology of various developing structures to their physiological competence throughout early life history of cod.

TERMINOLOGY

The following commonly used general stages will be referred to in this paper : the egg stage (spawning to hatching), the yolk–sac stage (reliant on endogenous food resources), the mixed feeding stage (some exogenous feeding supplements that of endogenous food supplies) the larval stage (obligate exogenous feeder) and the transformation stage (from larva to pelagic juvenile). Terms such as eleutheroembryo and protopterygiolarva (Balon 1975) were not considered appropriate for the present study, since this classification does not consider hatching to be a major event. Hatching is considered to be a major event in this study.

METHODS AND MATERIALS

Fertilized Atlantic cod eggs were obtained from two populations shown to be genetically distinct (Pogson et al. 1993): Newfoundland central (NF) and the Scotian shelf (NS). These were raised separately throughout the whole study.

Eggs and larvae were raised under two controlled temperatures, 5°C ± 0.5 and 10°C ± 0.5, in 80l closed–system glass aquaria. Eggs from both populations were acclimatized to the above temperatures at approximately the same stage in development (i.e 24 – 48 hrs post–fertilization). High concentrations of green algae, Isochrysis sp., were added to each aquarium and 'green' cultures were maintained throughout the experimental period. Rotifers and wild–caught zooplankton, sieved to appropriate sizes, were fed to the larvae throughout the experimental period. Prey density (as determined from counts of 10 x 1ml aliquots) was kept high (>20 prey/ml) to ensure good feeding conditions for all larvae.

From 5 to 8 animals were taken from each population every day for the first 10 days and then on every second or third day as the numbers of larvae declined. Most individuals at transformation stage were obtained from mesocosm studies run in parallel (Hunt von Herbing, unpublished data). These animals were used only for descriptions of developmental stage 12, as raising larval cod to pelagic juvenile stage was difficult in closed-system aquaria. Mesocosm studies were conducted in the Dalhousie Tower tank with a thermocline (gradient 3–5°C) set at the 5m depth. Late stage larvae and transformation stage individuals were observed at the thermocline feeding on zooplankton which aggregated there.

Animals were fixed in modified Karnovsky's fixative (Sire 1987) for light and scanning electron microscopy (SEM). Subsamples from each population were also taken for growth rate analysis. The latter were dried, without fixing, at 80°C for 24–48hrs, until no further weight loss was recorded. They were then weighed on a Cahn Gram electrobalance to the nearest 0.001mg.

General morphological landmarks were determined from observations made on a total of 280 individuals from both populations. Representative stages are illustrated primarily from cod larvae raised at 5°C, by using a camera-lucida equipped Wild dissecting microscope. Landmarks important to feeding and respiration were staged for both populations and temperatures, as follows (also described in detail in Table 1.2):

(i) Feeding

(a) yolk-sac – percent of yolk-sac utilized was estimated through observation of all fixed specimens.

(b) alimentary tract – the entire intestine was removed and a numerical value assigned to different intestinal stages, identified by significant changes in structure. Stage 1 is characterized by a simple straight tube, stage 5 is characterized by a highly coiled gut. In some cases, the alimentary tract changed only in diameter or length generally due to the differences either in the fullness of the gut or in the extent of peristaltic contraction. These changes were not designated as separate stages.

(ii) Respiration: whole animals fixed in modified Karnovsky's fixative were prepared for the SEM by dehydrating in a graded alcohol series and prepared with Peldri II (Peldri Inc.), as a sublimation dehydrant in place of critical point drying. Developmental stages of the gills, secondary lamellae and the development of the operculum were quantified by examining the samples under an SEM and assigning a numerical series to different stages (e.g. for gills: from 1 (bare gill arches) to 5 (double row of gill filaments with secondary lamellae); and for opercular development: from 1 (gill cavity) to 7 (full operculum with branchiostegal rays).

All numeric data were subjected to normal probability plots and Bartlett's Test for homogeneity of variance (SYSTAT, Wilkinson 1990) and were found to meet the assumptions of normality and homoscedasticity. ANOVA was used to determine differences in age-dependent and length-dependent variation between stages, populations and temperatures. Spearman Rank Correlations were used to determine the relationships

between intestinal stages, length (mm) and age (days post-hatch). Linear regressions were used to determine growth rates (μg dry weight day^{-1}) for each population at 5° and 10°C. Significant differences ($P<0.05$) in growth rate among populations and among temperatures were determined by t-tests between linear regression slopes (Zar 1984).

RESULTS

QUALITATIVE MORPHOLOGICAL LANDMARKS

Nine external diagnostic landmarks were selected, based on observation of 280 specimens (Table 1.1). These landmarks were easy to identify either with light or scanning electron microscopy. Criteria for their selection were that they should represent stages associated with the functional needs of feeding, respiration or locomotion. A major criterion was that the landmarks should not differ significantly in appearance between populations. Representative larval fish stages (primarily from larvae raised at 5°C) are shown in Figure 1.1 and are described in Table 1.2 with respect to each stage and landmark.

EFFECTS OF TEMPERATURE ON THE GROWTH AND DEVELOPMENT OF LARVAL STAGES.

Figure 1.2 illustrates the progression of developmental stages of larval cod over time, at 5°C and 10°C. Developmental rate of cod larvae is not linear over the whole larval period and is greater at 10°C than at 5°C for all stages. A high rate of structural change occurs during the early to mid yolk-sac stage (stage 1 to 5) at both temperatures (Figure 1.2). Larvae past stage 5, exhibited reduced rates of structural development at both 5°C

Table 1.1. Morphological landmarks and their functional relationships in developing Atlantic cod larvae from yolk-sac to transformation stage.

FUNCTIONAL MORPHOLOGICAL LANDMARKS

# LANDMARK	FUNCTIONAL RELATIONSHIP
(1) **Head**	– **feeding**– efficiency in prey capture relies upon head & mouth structure and position
(2) **Yolk-sac**	– **feeding** – endogenous food resource, facilitates transition to exogenous prey capture, provides buoyancy prior to swimbladder inflation
(3) **Alimentary Tract**	– **feeding** – increases in size and complexity enhance food digestion and assimilation
(4) **Gills**	– **respiration** –growth of filaments and lamellae facilitate the transition from cutaneous to branchial respiration
(5) **Gill Cover**	– **feeding and respiration** – growth may increase suction pressure for prey capture and enhance unidirectional currents over the gills
(6) **Swimbladder**	– **locomotion** – controls buoyancy and may aid in swimming activity & prey capture efficiency
(7) **Dorsal Finfold**	– **locomotion** – provides stability in the water and enhances forward thrust due to its large surface area
(8) **Paired Fins**	–**locomotion** – pectoral fins provide direction and stability during swimming, other fins form late in larval period and enhance swimming performance
(9) **Pigmentation**	– **general, taxonomic** – body and eye pigmentation (note that pigmentation is environment dependent and therefore cannot be used solely as a reliable landmark)

Figure 1.1. Representative drawings of general developmental stages for larval Atlantic cod Gadus morhua, based on nine functional morphological landmarks. ys = yolk-sac, at = alimentary tract, df = dorsal finfold, sb = swimbladder.

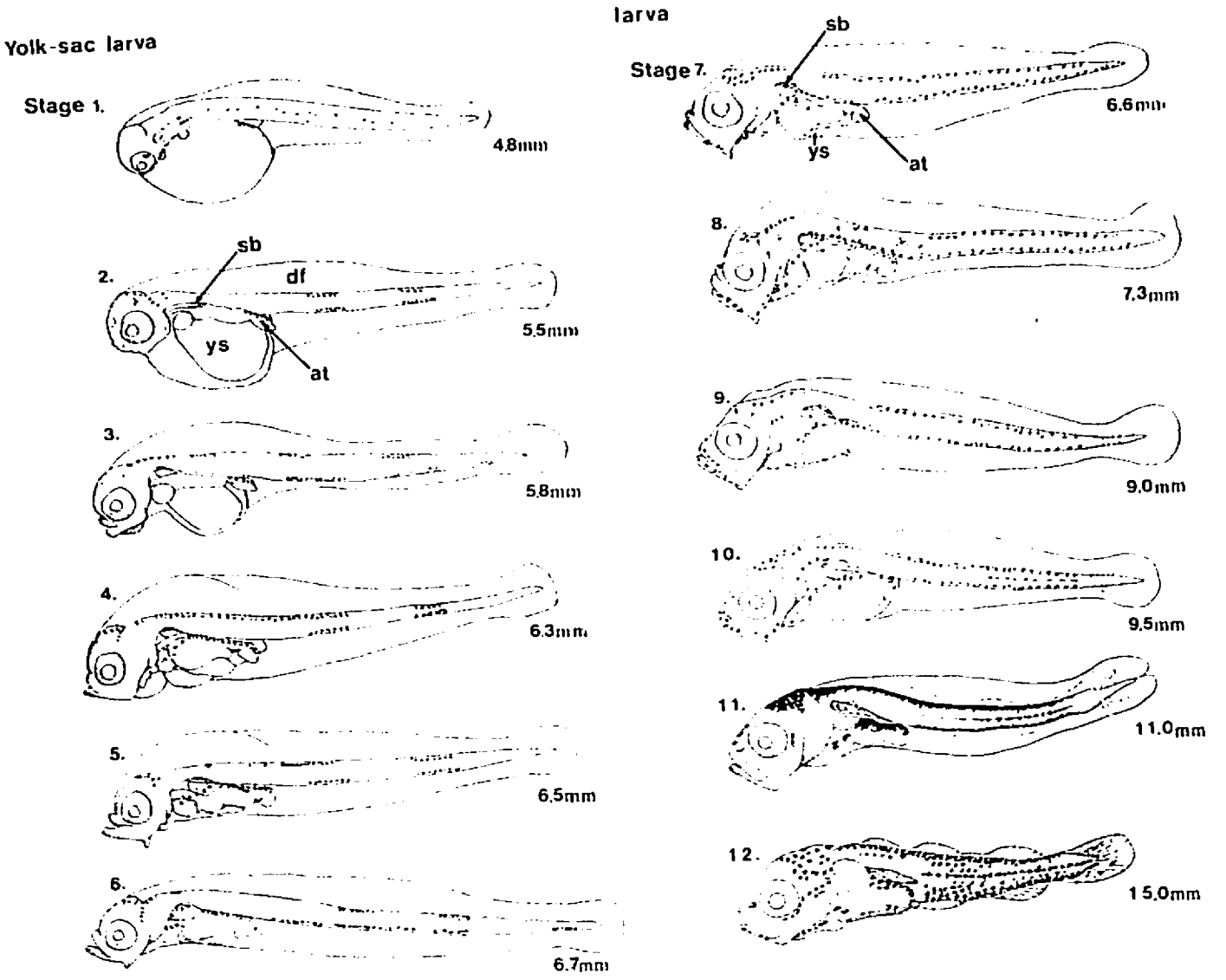

Yolk-sac larva
Stage 1.
4.8mm
2.
sb
df
ys
at
5.5mm
3.
5.8mm
4.
6.3mm
5.
6.5mm
6.
6.7mm
larva
Stage 7.
sb
ys
at
6.6mm
8.
7.3mm
9.
9.0mm
10.
9.5mm
11.
11.0mm
12.
15.0mm

Figure 1.1

Table 1.2. Description of developmental stages based on nine landmarks; H = head, YS = yolk-sac, AT = alimentary tract, GC = gill cover, G = gills, SB = swimbladder, DF = dorsal finfold, F = paired fins, P = body and eye pigment.

Stage 1: 0–1 days post-hatch

H: rounded, deflected downward;
mouth not open, oropharyngeal membrane intact;
hatching gland cells on top of head.

YS:large, spherical, full of yolk; >95% yolk remains

AT:straight simple tube, no differentiation;
anus not formed

GC:rounded vent or gill cavity at junction
of head and yolk-sac membrane

G: four gill arches; no gill filaments
or secondary lamellae

SB: connected to the intestine (Morrison 1992),
future position can be seen antero-dorsally to
intestine under a dense pigment cluster.

DF: surrounds the whole body including yolk-sac;
supracephalic sinus is small, not elevated.

F: pectoral fins are small, round and well formed.

P: transparent with a few pigment spots spread
along the trunk. Eyes are large in proportion
to the head, but only partially pigmented.

Stage 2: 2–3 days post-hatch

H: square shaped, detached ventrally
from yolk-sac membrane; upper jaw cushion
and lower jaw well defined covered by
oropharyngeal membrane which may be perforated.

YS: elliptical and reduced; 70%<ys<100% yolk left

AT: intestine has expanded; constriction
formed between mid-gut and hind-gut
(rectal valve, separates food
in foregut from hind gut); anus formed.

GC: gill cavity has grown ventrally

G : 4 gill arches; no filaments or lamellae

SB: small distinct sac, dorsal to intestine
and covered with pigment

DF: supracephalic sinus is elevated

F: simple, rounded pectoral fins

P: 2 post-anal bands on the trunk,
some cover the hindgut;
eye is heavily pigmented

Table 1.2 cont.

Stage 3: 3–4 days post-hatch

H: mouth open, subventral; jaw angle distinct.

YS: yolk-sac reduced; 70%<ys<50%

AT: liver enlarged; rectal valve present

GC: gill cavity has extended ventrally forming a gill slit, separating the head from the abdominal region.

G: gill arches covered by epithelial layer, which will later give rise to gill filaments. Arteries with pigmented blood cells present in each arch

SB: a distinct sac covered dorsally by chromatophores.

DF: supracephalic sinus of the dorsal finfold has reached is maximum elevation.

F: rounded pectoral fins

P: pigment bars extend further along the body, additional pigment covering the abdominal region; eyes are fully pigmented displaying deposits of guanine which make them appear golden in reflected light.

Stage 4: 6–7 days post-hatch

H: jaw angle of the head is distinct formed by posterior growth of the retroarticular cartilage; mouth is sub-ventral.

YS: still contains yolk; 50%<ys<25% yolk left

AT: foregut (oesophagus) is elongated and the mid-gut has expanded; intestine convoluted.

GC : thin epithelial border growing from the hyoid

G : 4 gill arches with epithelial covering

SB: enlarged rounded sac, dorsal pigmentation is dense; SB not yet functional.

DF: supracephalic sinus maximally elevated

F: large cartilaginous base to pectoral fin

P: Body pigmentation extended anteriorly covers most of the anterior trunk area and some of the intestine.

Table 1.2 cont.

Stage 5: 8–9 days post–hatch

H: Viscerocranial structures enlarge;
mouth sub–terminal position; sharp jaw angle.

YS: much reduced; 50%<ys<25% left

AT: mid–gut enlarged; intestine convoluted

GC: epithelial membrane growing posterio–laterally from the hyoid

G: the epithelial layer on the gill arches has thickened; no filaments

SB: bladder inflated, but inflation is not complete;
pigment clusters thicker over dorsal portion

DF: the supracephalic sinus remains elevated

F : larger rounded pectoral fins with cartilaginous base

P : more pigmentation generally, none between the
post–anal pigment bars.

Stage 6: 12–13 days post–hatch

H: increased girth of cranial elements

Y: small sac, still contains yolk; 25%<ys<10%

AT: intestine is more convoluted

GC: epithelial membrane covers 50% of gill cavity

G: first gill filaments appear on 2nd or 3rd gill arches

SB: inflated in most larvae; pigment spreading ventrally

DF: supracephalic sinus still elevated

F : large pectoral fins, cartilaginous base;
thickened cleithrum

P : pigments filling two post–anal bands,
more over intestine and liver

Table 1.2 cont.

Stage 7: 17–18 days post-hatch

H: jaw cartilage thicker; mouth terminal; jaw angle prominent

YS: no yolk left, only remnants of sac which surrounded yolk

AT: enlarged oesophagus, highly convoluted intestine

GC: >50% of gill cavity covered

G : gill filaments growing longer

SB: enlarged; dorsal portion covered by black/silvery pigment, fully functional

DF: supracephalic sinus elevated

F: caudal peduncle thickened

P: covering trunk, no pigment bars left, covers also head and dorsal portion of intestine

Stage 8: 25–26 days post-hatch

H: upper jaw more pronounced; lower jaw larger

YS: sac remnants

AT: one small loop between mid-gut and intestine; liver elongated

GC: >50% covering gill cavity

G: numerous gill filaments on 2nd and 3rd arches

SB: enlarged, pigment spreading over whole bladder

DF: supracephalic sinus beginning to collapse

F : caudal peduncle thickening, base of pectoral fin enlarged

P: more numerous and spreading over head, lower jaw, trunk and intestine

Table 1.2 cont.

Stage 9: 35 days post-hatch

H: cranial elements larger, thicker

YS: no remnants visible

AT: mid-gut large, intestine coiled

GC: gill covered entirely by opercular membrane

G : gill filaments numerous, first secondary lamellae

SB: very distinct, filled

DF: sinus almost collapsed off head

F : fin rays in pectoral fins, first caudal fin rays

P : plentiful around upper and lower jaws, intestine, head and trunk

Stage 10: 50 days post-hatch

H: large cranial structures; branchiostegal rays, small opercular bones; teeth on lower and upper jaws, large pharyngeal teeth

YS: no remnants

AT : Large oesophagus, highly coiled intestine, large liver

GC : dorsal growth of membrane containing opercular bones, 7 branchiostegal rays projecting from hyoid arch

G: two rows of gill filaments with secondary lamellae

DF: sinus completely collapsed

F: caudal fin rays, thickened pectoral fin

P: over upper and lower jaws, intestine, extending to end of trunk, lateral line pigment streak

Table 1.2 cont.

Stage 11 : 60 days post-hatch

H: head more elongate; jaws partly mineralized, larger teeth

YS: no remnants

AT: large stomach and intestine lumen, coils of intestine indistinct, as they are covered by pigment.

GC: well formed operculum with large opercular margin (valve).

G : two rows of gill filaments with numerous lamellae

SB: has become more elongate, extending posteriorly over the mid-gut and intestine

DF : sinus collapsed and finfold covering the trunk; narrower and scalloped at the extreme caudal end.

F: well formed caudal fin rays, precursors to the dorsal and ventral paired fins present

P: chromatophores are larger, thicker and more numerous

Stage 12: 70 day post-hatch

H: elongate head, mouth terminal

YS: none

AT: large and obscured by pigment

GC: well formed operculum, covers whole gill cavity

G: two rows of large gill filaments and large lamellae

SB: elongate and extends posteriorly above the anus, covered entirely by silver pigment

DF: not present

F: dorsal, anal and pelvic fins are formed but still small, caudal fin is well formed and large.

P: covers entire body

Figure 1.2. Progression of developmental stages of cod larvae from hatching to transformation stage at 5° and 10°C. Symbols represent modal stage at a given age (days post-hatch).

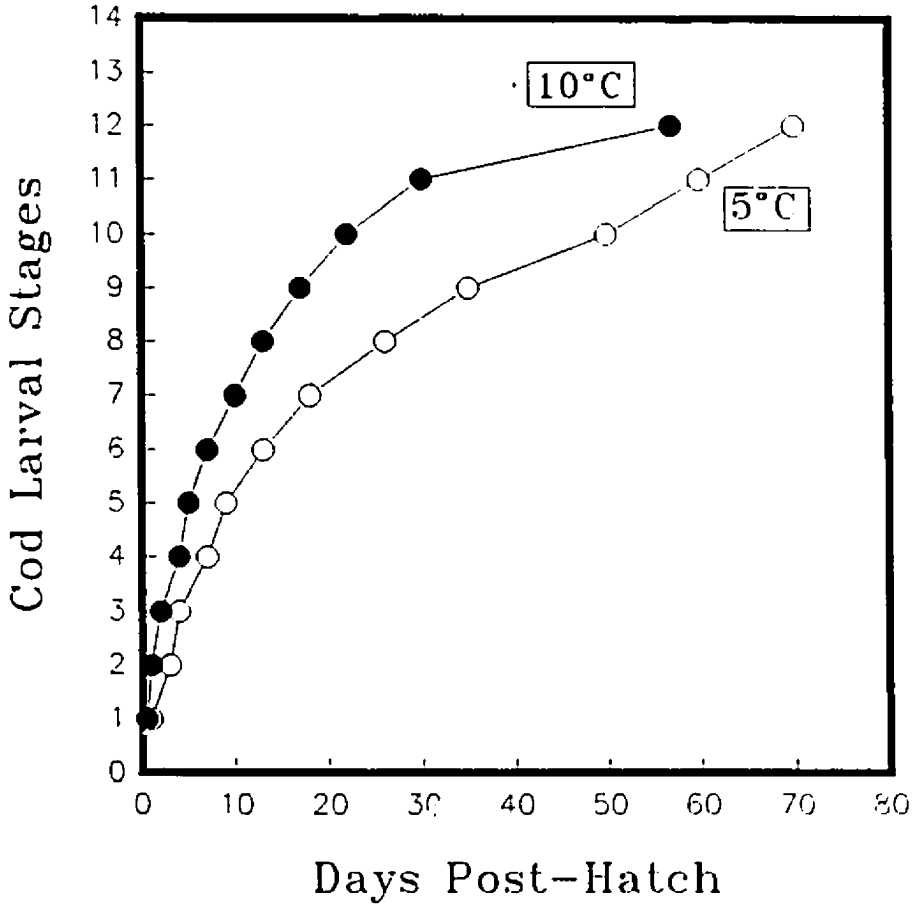

Cod Larval Stages
Days Post-Hatch
10°C
5°C
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0
10
20
30
40
50
60
70
80

Figure 1.2

and 10°C, but late in development, the rate of development accelerated in the 5°C animals, while it was maintained or reduced in the 10°C animals.

Effects of temperature on growth

Growth, measured as an increase in dry weight over time, correlated significantly with days post-hatch; for 10°C ($r^2 = 0.85$, $p < 0.001$ and $r^2 = 0.44$, $p < 0.001$; for NF and NS respectively): and for 5°C ($r^2 = 0.72$, $p < 0.0001$ and $r^2 = 0.50$, $p < 0.0001$; for NF and NS respectively). Growth rates were significantly higher in the Newfoundland population than in the Scotian Shelf population at 5°C (t-test; $t = 3.99$, $P < 0.05$) and at 10°C (t-test; $t = 5.14$, $p < 0.05$) (Figure 1.3 a,b).

Effects of temperature on structures associated with feeding.

(a) Alimentary Tract Development

Stages in the development of the alimentary tract (general landmark #3, see Table 1.1) are shown in Table 1.3. Five distinct intestinal (alimentary tract) stages were identified by clear differences in structure. Stage 2 intestine exhibited changes in degree of expansion and size, either due to fullness of the gut or degree of peristaltic contraction. Numerous variations in shape of stage 2 intestines are illustrated in Table 1.3, in order to display the corresponding changes in yolk-sac utilization over time.

In the NF population all five intestinal stages were present at both temperatures (Figure 1.4a-b) and intestinal stage increased with larval age at both temperatures (Spearman Rank Correlation, $r_s = 0.887$ and 0.846, $p < 0.0001$; for 5°C and 10°C

Figure 1.3. Growth rates (μg dry wt/day) of larval cod in two populations, Newfoundland (NF) and Scotian Shelf (NS) at two temperatures, 5°C and 10°C.

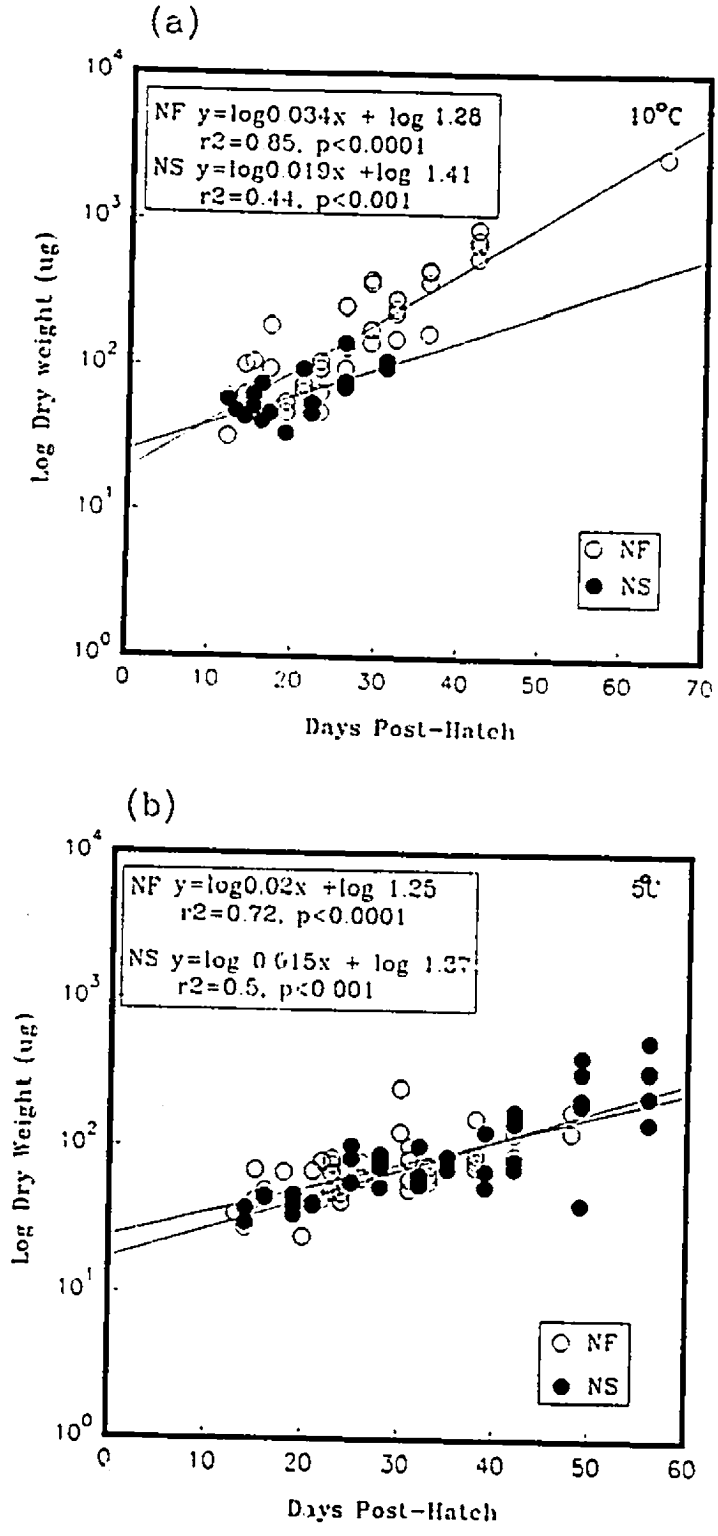

(a)
NF y=log0 034x + log 1.28
r2=0 85, p<0.0001
NS y=log0.019x +log 1.41
r2=0.44, p<0.001
10°C
NF
NS
Log Dry weight (ug)
Days Post-Hatch
(b)
NF y=log0.02x +log 1.25
r2=0.72, p<0.0001
NS y=log 0 615x + log 1.37
r2=0.5, p<0 001
5°C
NF
NS
Log Dry Weight (ug)
Days Post-Hatch

Figure 1.3

Table 1.3. Description and staging of feeding landmarks.
▨ yolk-sac, ■ liver, □ intestine, ▥ swimbladder.

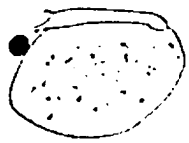
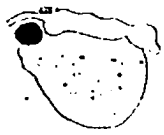
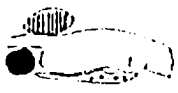
General Larval Stage	Intestinal Stage	Description of intestinal structure and % yolk-sac utilized.	Appearance
1	1	simple straight gut, no differentiation, no anus; < 5% yolk utilized.	
2	2	intestine expanded, rectal valve formed, anus formed; 25% yolk utilized	
3	2	three part gut present; oesophagus, mid-gut and hind-gut; 50% yolk utilized	
4	2	foregut (oesophagus) and mid-gut expanded, intestine convoluted; 65-75% yolk utilized	
5	2	mid-gut enlarged, large peristaltic waves, intestine convoluted; 85% yolk utilized	
6	2	intestine becoming increasingly convoluted; >90% yolk utilized, only very small remnants left.	
7	2	large oesophagus, highly convoluted intestine; no yolk left in sac.	
8	3	intestine contains one small lateral loop, posterior to mid-gut; no yolk, only sac remnants.	
9	4	mid-gut (stomach) large, intestine has two coils; no sac remnants.	
10	5	large oesophagus, stomach, highly coiled intestine; no sac remnants.	

Figure 1.4. Intestinal stages of cod larvae vs days post-hatch in two populations, Newfoundland (NF) and Scotian Shelf (NS) at two temperatures, 5°C and 10°C. Symbols represent individual larvae.

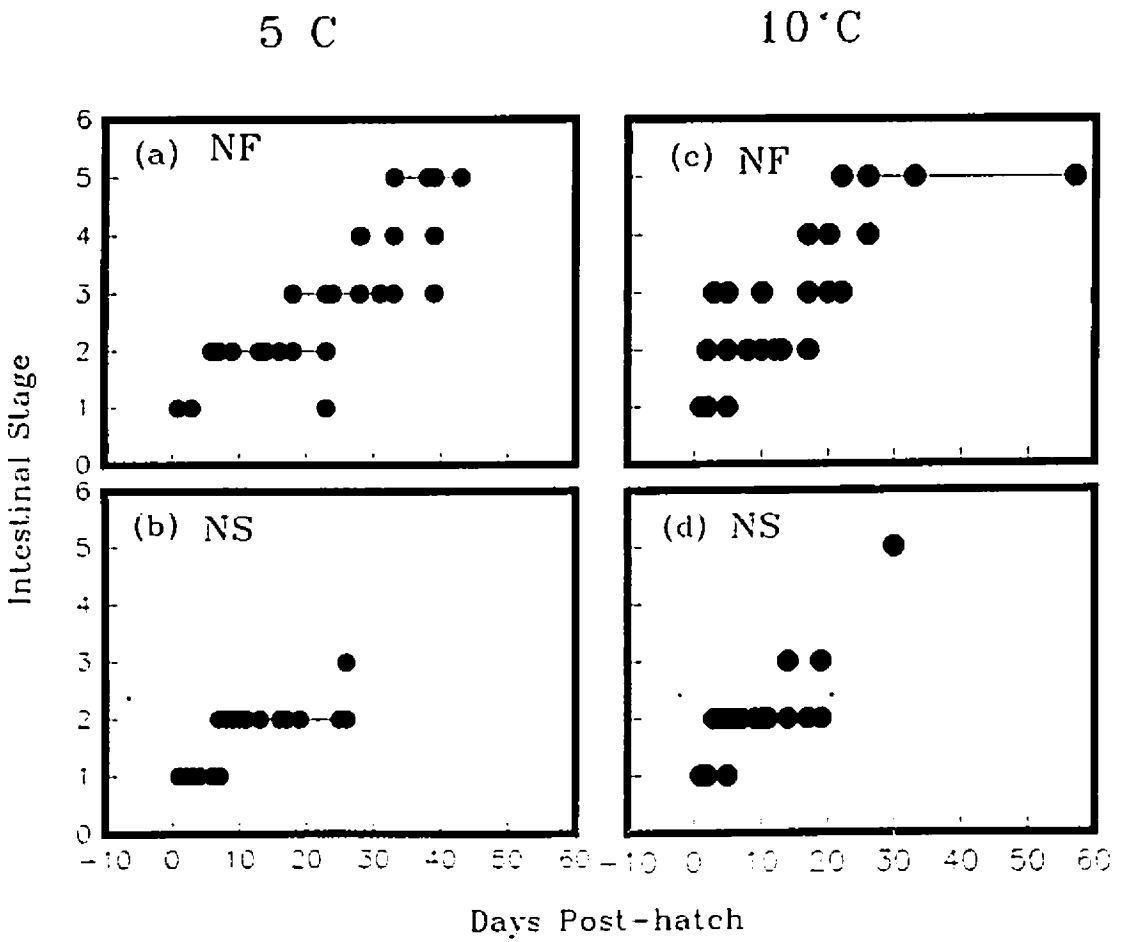

5 C
10 C
(a) NF
(c) NF
(b) NS
(d) NS
Intestinal Stage
Days Post-hatch
0
1
2
3
4
5
6
-10
0
10
20
30
40
50
60

Figure 1.4

respectively) (Figure 1.4a). A simple straight-through intestine (stage 1, Table 1.3) was present from hatch to 5 days at 5°C and from hatch to 3 days post-hatch (dph) at 10°C. The tripartite gut (Stage 2, Table 1.3), appeared at first feeding for both temperatures (at 6 dph and 2–3 dph; at 5°C and 10°C respectively). At the time of complete yolk-sac absorption (10 dph for 10°C and 16–18 dph for 5°C) larvae had either a tripartite gut or simple looped guts (stage 2 & stage 3, Table 1.3). At older ages (17–26 dph at 10°C & 28–39 dph at 5°C), the alimentary tract of larvae at both temperatures ranged in development from a simple looped gut to highly coiled intestines (stage 4 & 5, Table 1.3). Higher rates of intestinal development occurred at 10°C, because the mean larval age at which a particular stage appeared (with the exception of stages 1 and 5) was significantly lower than at 5°C (ANOVA, $F = 50.2$, $p < 0.0001$). However, the large range in larval age over which stages 2 and 3 persist at both temperatures indicated that these two stages often occur together in the population.

Intestinal stage also increased with larval length at both temperatures (Spearman Rank $r_s = 0.71$ and 0.70, $p < 0.0001$; for 5°C and 10°C respectively, Figure 1.5a–b). The mean length at all stages (with the exception of the stage 5) did not differ between temperatures (ANOVA, $F = 1.07$, $p > 0.05$). At both temperatures; the smallest larvae (4.0–4.5 mm) had only stage 1 intestines, larvae that ranged in standard length from 5–7 mm ranged in intestinal stage from 2 to 4. The most complex stage 5 intestines were always found in larvae > 7.0 mm (Figure 1.5a–b).

The NS population, in contrast to the NF population, displayed only the 3 earliest intestinal stages at both temperatures (Figure 1.4c–d). However, intestinal stage in the

Figure 1.5. Intestinal stages of cod larvae vs standard length in two populations, Newfoundland (NF) (a & c) and Scotian Shelf (NS) (b & d) at two temperatures, 5° (a & b) and 10°C (c & d). Symbols represents individual larvae.

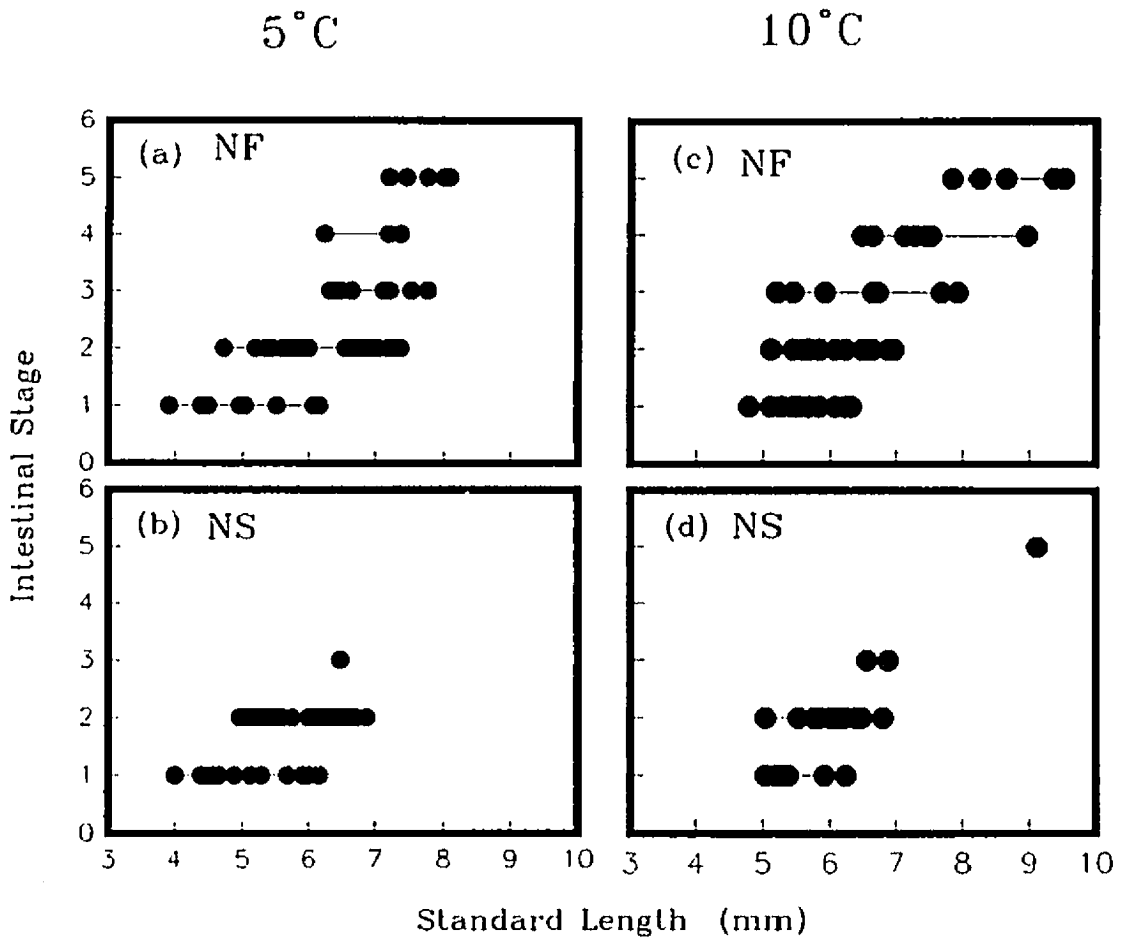

5°C
10°C
(a) NF
(c) NF
(b) NS
(d) NS
Intestinal Stage
Standard Length (mm)
0
1
2
3
4
5
6
3
4
5
6
7
8
9
10

Figure 1.5

NS population increase with age at either temperature (Spearman Rank Correlation, r_s =0.70 and 0.75, $p < 0.05$; for 5°C and 10°C respectively). Stage 2 was the dominant stage at both temperatures, but mean age at stage 2 was significantly lower for 5°C than for 10°C (t-test, $t = 7.76$, $p < 0.01$). Intestinal stage also increased with increasing length at both temperatures (Spearman Rank, $r_s = 0.4$ and 0.7, $p < 0.05$; for 5°C and 10°C respectively; Figure 1.5 (c-d) and mean length of larvae with intestinal stage 2 was not significantly less at 5°C than at 10°C (t-test, $t = 0.18$, $p > 0.05$).

Between-population comparisons of intestinal stage development showed that with the exception of stage 2 (t-test, $t = 13.56$, $p < 0.005$) there were no significant differences in mean ages at 5°C (ANOVA, $F = 0.38$, $p > 0.05$) or at 10°C (ANOVA, $F = 0.27$, $p > 0.05$). Intestinal stage 2 formed before first feeding in both populations and at both temperatures (Figure 1.4a-d). However, in the NS population, intestinal stage 2 also occurred in the oldest and largest larvae at both temperatures (Figures 1.4c-d & 1.5c-d), while a higher proportion of older/larger larvae of the NF population possessed more developed intestines (i.e. > stage 2) (Figures 1.4a-b & 1.5a-b). In addition; the NF population at 10°C exhibited intestinal stages 1-3 by first feeding (2-3 days post-hatch) (Figures 1.4 a-b), while only stages 1-2 were present in the NS population (Figures 1.4c-d). Relative frequency of larval intestinal stages in both populations is shown in Figures 1.6a-b). In the NS population, stage 2 was the dominant stage for both 5°C and 10°C (the NS population), while in the NF population the relative frequencies were evenly distributed between all stages for both 5°C and 10°C (Figure 1.6a & b). This suggests that larvae in the NF population progressed from a less to a more complex intestinal

Figure 1.6. Relative frequency (%) vs intestinal stages of cod larvae for two populations Newfoundland (NF) and Scotian Shelf (NS) at 5°C (Figure 6a) and at 10°C (Figure 6b).

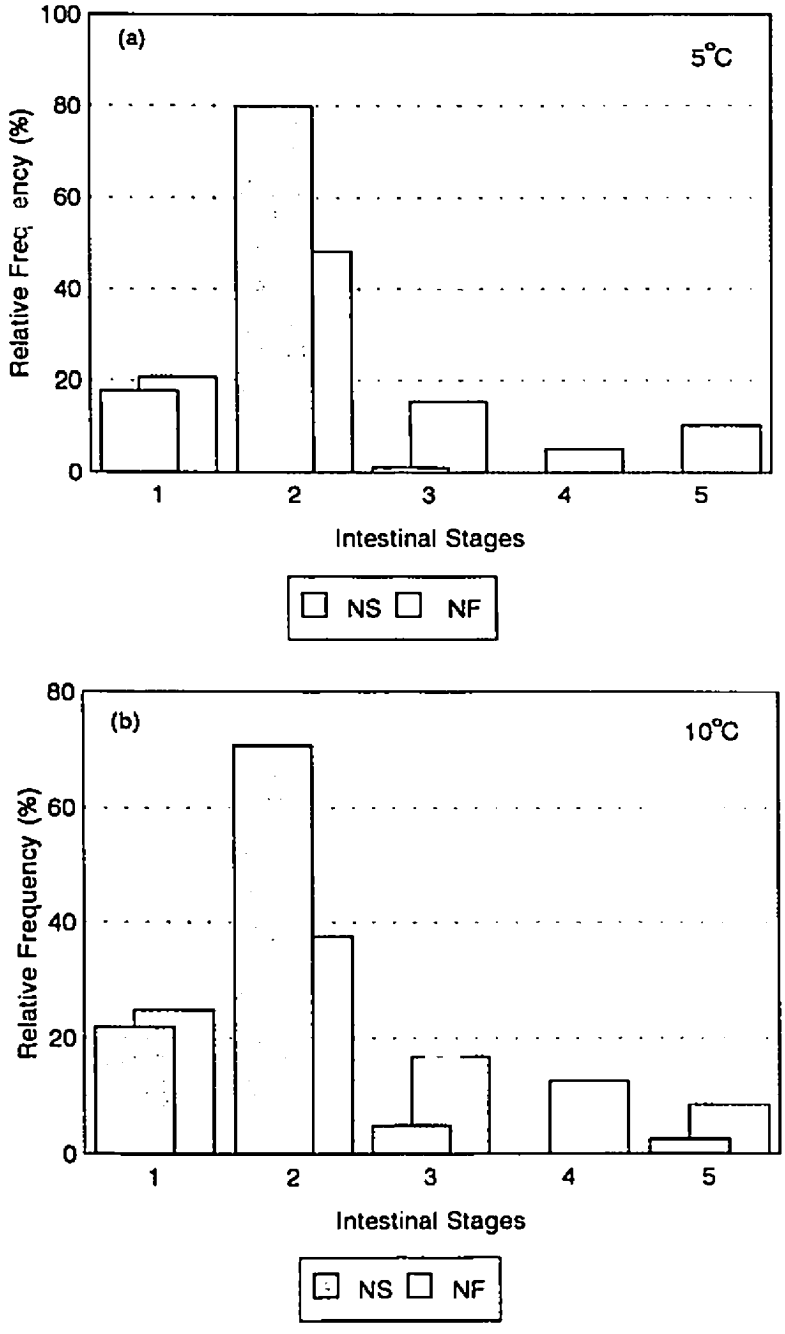

(a)
5°C
Relative Frequency (%)
100
80
60
40
20
0
1
2
3
4
5
Intestinal Stages
NS
NF
(b)
10°C
Relative Frequency (%)
80
60
40
20
0
1
2
3
4
5
Intestinal Stages
NS
NF

Figure 1.6

stage, while most individuals in the NS population did not progress past the relatively primitive intestinal stage 2.

b) Yolk–Sac Utilization

The rate of yolk–sac utilization was quantified by estimating the percentage utilized over time (Table 1.3). Yolk–sac utilization rates were high for the first few days after hatching at both temperatures and in both populations (Figures 1.7a–b). For the NF population, 50% of the yolk–sac was utilized by 6 dph at 5°C and by 3 dph at 10°C (Figure 1.7a). For both temperatures this coincided with first feeding and the appearance of intestinal stage 2 (see Figure 1.4a). Greater than 75% of the yolk–sac was utilized by 13 dph at 5°C and by 5 dph at 10°C, coinciding with the appearance of intestinal stage 3 at both temperatures (Figure 1.4a–b). At 5°C, complete utilization of the yolk–sac was first observed at 9 days, but generally occurred between 14–18 dph. At 10°C, complete absorption generally occurred between 10 and 17 dph. Any remains of the yolk–sac disappeared between 24–43 days at 5°C and between 10 and 57 days at 10°C.

In the NF population, yolk–sac utilization increased with age at both temperatures (r^2 = 0.766 and 0.702, $p < 0.05$; for 5°C and 10°C respectively, Figure 1.7a). Utilization rates were significantiy higher at 10°C than at 5°C (t–test, $t = 3.6$, $p < 0.001$). Yolk–sac utilization increased with length at 5°C ($r^2 = 0.40$, $p<0.001$), but was independent of length at 10°C ($r^2 = 0.056$, $p = 0.156$; Figure 1.7b). In the NS population, yolk–sac utilization increased with age at both temperatures (r^2 = 0.652 and 0.858, $p < 0.0001$; for 5°C and 10°C respectively, Figure 1.7c) and rates were significantly higher at 10°C than

Figure 1.7. Percent yolk–sac utilization in cod larvae vs days post hatch (a–b) and standard length (mm) (c–d) for two populations, Newfoundland (NF) and Scotian Shelf (NS) at 5° and 10°C. Symbols represent individual larvae.

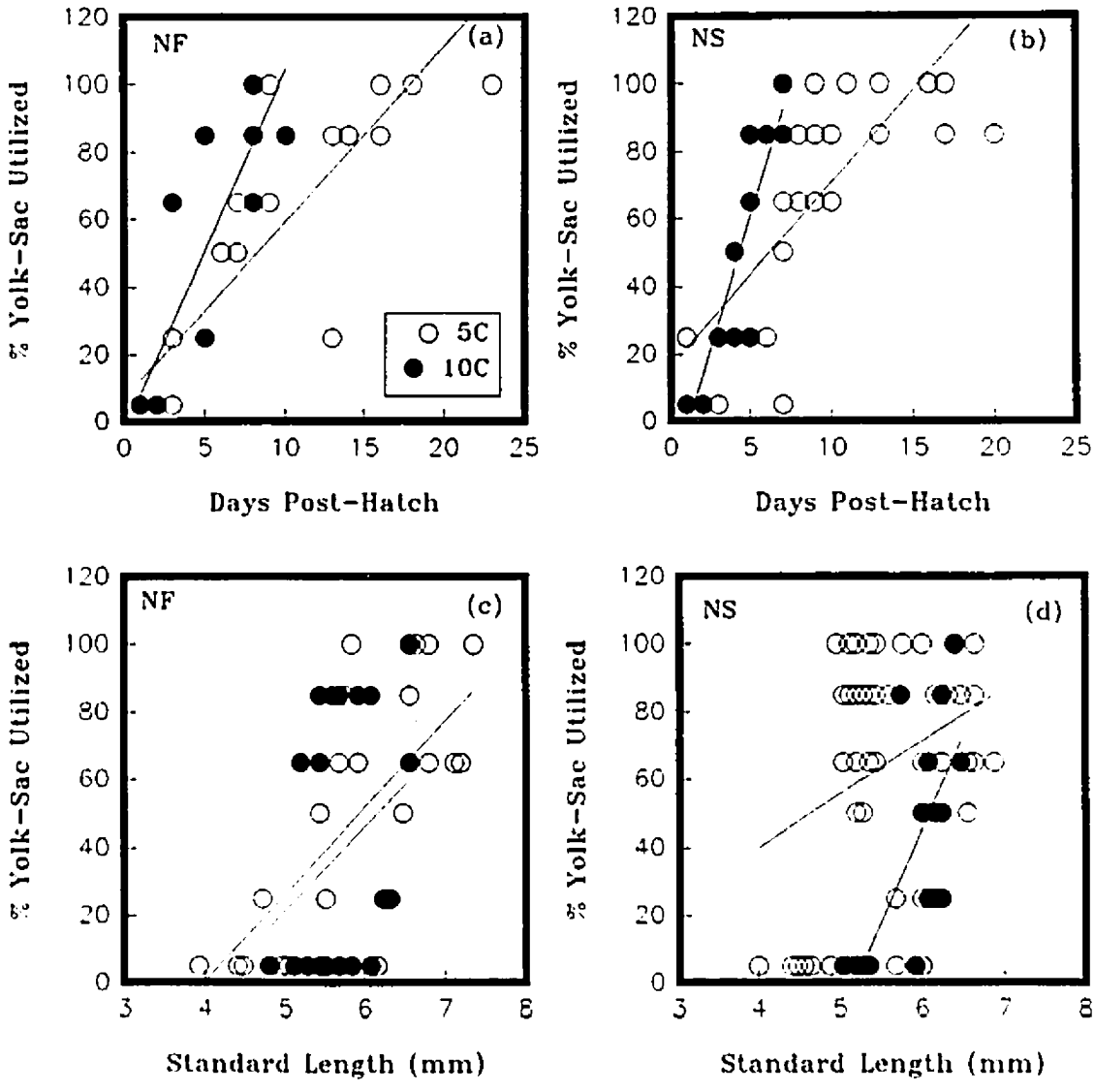

NF
(a)
5C
10C
% Yolk-Sac Utilized
Days Post-Hatch
NS
(b)
% Yolk-Sac Utilized
Days Post-Hatch
NF
(c)
% Yolk-Sac Utilized
Standard Length (mm)
NS
(d)
% Yolk-Sac Utilized
Standard Length (mm)

Figure 1.7

at 5°C (t–test, $t = 4.08$, $p < 0.001$). Yolk–sac utilization was poorly correlated with size at 5°C ($r_2 = 0.070$, $p < 0.05$) and 10°C ($r^2 = 0.44$, $p < 0.01$). Between – population comparisons showed no significant differences in yolk–sac utilization rates at 5°C or 10°C (NS vs NF; t–test; $t = 1.92$, and $t = 0.31$, $p > 0.05$ for 5°C and 10°C respectively.).

EFFECTS OF TEMPERATURE ON LANDMARKS ASSOCIATED WITH RESPIRATION.

Morphological changes in structures associated with respiration were staged and are shown in Table 1.4. Due to the difficult nature of SEM preparation and observation, the sample size for each respiratory landmark was small ($n = 3$), as was the number for estimates of variation with age and size.

a) Gills and Gill Cover Development

The timing of initial appearance of respiratory structures did not vary between the two populations (NS and NF), and therefore the two populations will be treated together.

All four gill arches were present at hatch and remained devoid of gill filaments for the first two weeks of larval life at 5°C (Stage 1, Figure 1.8) and for the first week of larval life at 10°C. Gill filaments first appeared (usually on the 2nd or 3rd arch) at 12–14 dph at 5°C and 6 – 8 dph at 10°C. Gill filaments grew longer and became more numerous in the third week (22 – 23 dph) at 5°C and in the second week (10 – 11 dph) for 10°C. Secondary lamellae first appeared 35 – 37 dph at 5°C and 20 – 25 dph at 10°C becoming numerous by 50 dph at 5°C and by 27 dph at 10°C. Total length of the larvae at each

Table 1.4. Description and staging of respiratory landmarks.

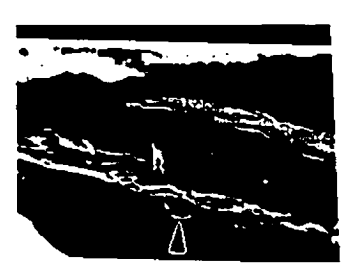
General Larval Stage	Gill Stage	Gill Cover Stage	Description of gill and gill cover development	
1&2	1	1	four gill arches, no filaments; rounded vent or gill cavity (arrowhead)	
3	1	2	vent has grown ventrally forming a gill slit (arrowhead) which separates the head from the trunk region.	
4&5	1	3	thin gill cover growing posterior–laterally from the hyoid (arrowhead)	
6	2	4	gill filaments (arrowhead) appear on the 2nd and 3rd gill arches; gill cover extends over 50 % of gill cavity	

Table 1.4. cont.

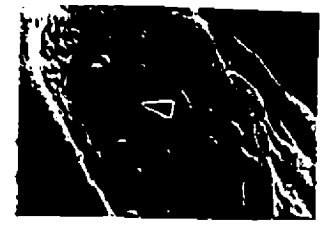
7&8	3	5	filaments are more numerous; gill cover (arrowhead) extends over 75% of gill cavity.	
9	4	6	some secondary lamellae (arrowhead) have formed on gill filaments; gill cover extends over entire gill cavity and is forming the operculum	
10	5	7	two rows of gill filaments with secondary lamellae; gill membrane contains opercular bones and branchiostegal rays (arrowhead)	

Figure 1.8. Development of respiratory landmarks in cod larvae from hatch to transformation. ○ gill 5°C, ● gill 10°C, □ gill cover 5°C, ■ gill cover 10°C. Symbols represent modal stage at a given days post-hatch.

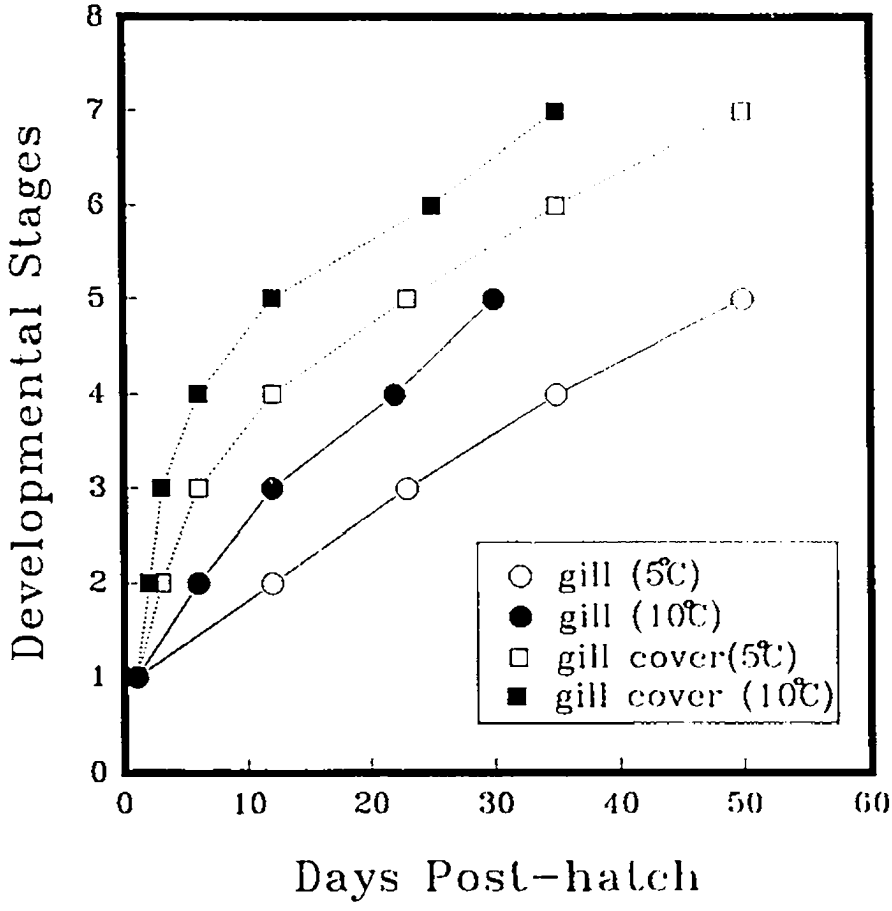

Developmental Stages
Days Post-hatch
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
gill (5℃)
gill (10℃)
gill cover(5℃)
gill cover (10℃)

Figure 1.8

of the respiratory stages could only be approximated, due to the large amount of shrinkage which occurs during preparation for SEM examination. Estimated mean sizes are as follows for NS and NF population respectively: first gill filaments at 5°C, 6.3 ± 0.3 mm and 6.4 ± 0.8; at 10°C, 6.3 ± 0.6 mm and 6.2 ± 0.4 mm ; and first secondary lamellae at 5°C, 6.72 ± 0.3 mm and 7.42 ± 0.3 mm; at 10°C, 7.00 ± 0.4 mm and 7.68 ± 1.4 mm.

Formation and growth of the gill cover, including growth of the branchiostegal rays (see Ch. 2), is closely associated with development of the gills (Figure 1.8). Timing of gill cover stages did not differ between populations (NS & NF), thus both populations will be treated together. The gill 'pit' (stage 1) was present at hatch in animals raised at both 5°C and 10°C. This cavity grew ventrally to form a gill slit (stage 2) at 3 – 4 dph at 5°C and at 1 dph at 10°C. This slit separated the head from the membrane surrounding the yolk-sac (see general stage 2, Table 1.2). Growth of the gill cover began at 6 – 7 dph at 5°C and 4–5 dph at 10°C (stage 3). With growth, the gill cover extended posterio-dorsally and covered 50% of the gill cavity by 12 – 13 dph at 5°C and 7 – 8 dph at 10°C (stage 4), coincident with first gill filament formation. As gill filaments proliferated, growth of the gill cover continued posteriorly, covering about 75% of the gill cavity by 21 dph at 5°C and 11 dph at 10°C (stage 5). The gill cavity is almost entirely covered by the gill cover by 35 dph at 5°C, and by 20 dph at 10°C (stage 6). At this time, secondary lamellae begin to form on the primary gill filaments. By 50 dph at 5°C (30 dph at 10°C), opercular bony complex and branchiostegal rays had begun to form within the gill cover connective tissue (see Ch. 2). At this time, double rows of gill

filaments and secondary lamellae appeared on the gill arches (Figure 1.8). Overall, the greatest developmental changes in the gills and gill cover occurred during the yolk – sac stage, after which growth in number and size of the new structures continued.

DISCUSSION

Development of functional morphological structures may be crucial to survival during specific ontogenetic periods such as hatching, the yolk–sac period, and the obligate exogenous feeding period (Moser 1981). The efficiency with which changes in form and function meet the demands of growth and development may in part regulate survival to the juvenile stage. Variation in the timing of development of these structures and functions will be determined by the interaction of intrinsic (genetic) factors and extrinsic (environmental) factors (see Via and Lande, 1985).

CRITICAL PERIODS DURING LARVAL DEVELOPMENT

Hatching

Hatching from egg to free–swimming larva is the first event in larval life marking a significant change in function and behaviour. Structures such as the gill 'pit' and hatching gland, perform specific functions that are designed to facilitate transition of the embryo into the oceanic environment (Yamagami 1988). The gill 'pit' develops in the embryo before hatching (Miyake et al. 1992a). At hatching, the gill 'pit' acts as a conduit into the pharyngeal cavity, absorbing water to prevent dehydration as the larva moves rapidly from a hyposmotic environment in the egg into one of full strength sea–water (Major–Jensen

1987). Within this period, larvae must absorb water and eliminate accumulated salts to prevent dehydration (Major-Jensen 1987). The epithelium of cod larvae is relatively impermeable to water (Tytler and Bell 1989), and the mouth is sealed with the oropharyngeal membrane (Table 1.1). Therefore, the gill 'pit' appears to be the only possible conduit for water uptake. Moreover, the 'gill pit' opens into a pharynx which is lined by chloride cells which may represent a site for ionoregulation (Holliday 1963). A gill cavity or 'pit' is also present in killifish (Guggino 1980), anchovy (O'Connell 1981), and halibut (Kjorsvik and Reiersen 1992), in which similar functions of water intake have been suggested. Water could also be derived from the yolk but this is almost certainly a slower process (Ronnestad et al. 1993). At later stages, larvae have been observed to actively drink water to maintain osmolarity (Tytler and Blaxter 1988).

Balon (1981, 1984) has suggested that hatching should not be considered a major morphological event, as little structural change occurs at this time. His 'eleutheroembryo stage' refers to the discrete stage which extends from within the egg and ends with yolk-sac utilization. Although little morphological change occurs at hatching for cod and other species (e.g. killifish (Guggino 1980), haddock (Fridgeirsson 1978), halibut (Kjorsvik and Reiersen 1992)), large changes in function do occur. For example, the very high mortality that occurs at hatching (Hewitt et al. 1985) may represent developmental mismatch between functional events, such as expression of hatching gland enzymes targeted at the chorion (DiMichele and Taylor 1980, Pittman et al. 1990) and the timely emergence of the larva. If changes in structures crucial to function (i.e. specifically feding , repiration and locomotion) and not developmental/morphological changes alone

are used as the basis for major developmental stages, (Balon 1981, 1984), then the *processes* on which survival depends may become more clear.

Yolk-sac period

Dependence on the yolk-sac for energy during the first few days of life allows structures such as the jaws, eyes and alimentary tract to develop and differentiate so as to become functional in time to meet the needs of exogenous feeding (Blaxter 1988, Kjorsvik 1991, Hunt von Herbing et al., 1992). Pigmented eyes and a degree of mobility provided by the dorsal finfold and pectoral fins should allow larval cod to orient and focus on food particles a few days before they begin feeding exogenously.

Early development of these morphological features enable cod larvae to 'filter-feed' on phytoplankton during the first days of life, using the four rudimentary gill arches as a sieve (Van der Meeran 1991). Fish larvae ingest algae at the very early stages of exogenous feeding and to cope with variations in feeding conditions (Van der Meeran and Naess 1993). However, in addition to being a supplemental food source, algae are thought to trigger development of the intestine of halibut larvae (Kjorvsik and Reierson 1992), priming the alimentary canal for the digestion of copepod nauplii and other zooplankton during first feeding. Therefore, it may not be coincidence that larvae are found with 'green guts' only prior to first feeding (algae can be ingested through the perforated oropharyngeal membrane, see Ch. 2), or that algae are rarely found in the gut after the jaws have become functional (Ellertsen et al. 1981).

The developmental changes of the jaws and alimentary tract at first-feeding and after

are functionally geared for prey capture and nutrient assimilation for growth. First – feeding in cod is initiated when the yolk–sac is approximately 50% utilized (Figure 1.7) marking the transition between endogenous and exogenous feeding. The transition period in cod larvae is accompanied by the following developmental changes; formation of a true gill slit, a functional lower jaw, and differentiation of the alimentary tract by the formation of the rectal valve (Table 1.2). Initiation of exogenous feeding with some yolk–sac still present presumably enables fish larvae to develop effectiveness as predators while still carrying a food reserve. After yolk–sac extinction cod larvae move beyond the landmarks seen at first feeding, or show signs of starvation, e.g. gut shrinkage (Theilacker 1978, I. Hunt von Herbing, personal observations).

Exogenous Feeding Period

During this period, changes in the structure and function of the feeding, respiratory and locomotory apparatus may serve to coordinate foraging and prey capture efficiency, and the efficiency of digestion. The exogenous feeding stage extends throughout larval life and involves the growth in size of pre–existing structures. This is in contrast to the differentiation of new structures that occurred at earlier endogenous feeding stages. Cartilaginous elements in the head become larger and more robust (see Ch. 2), the intestine convolutes and coils, providing more surface area for digestion (Table 1.3), and gastric enzymes are secreted by the stomach and intestinal wall improving nutrient assimilation for growth (Govini et al. 1986). In addition, the swimbladder inflates (Table 1.2), counteracting the tendency for larvae to sink as they become larger and heavier (I.

Hunt von Herbing, personal observations). Partial inflation may occur during the endogenous feeding stage but the swim bladder is not fully functional until the end of the transition to exogenous feeding (see Ch. 4). Many studies have reported that lack of swimbladder inflation after yolk-sac absorption can lead to high mortalities (Dannevig and Dannevig 1950, Riley 1966 and Thompson and Riley 1981). One possible explanation for such mortalities may be reduced prey capture efficiency (eventually leading to starvation), resulting from inefficient buoyancy regulation and ineffective foraging.

FACTORS AFFECTING THE DEVELOPMENT OF FEEDING AND RESPIRATORY STRUCTURES.

Intestinal development

(i) Age-dependent factors

For both Scotian Shelf and Newfoundland populations, age at a given intestinal stage was highly variable (Figure 1.4). However, the faster growing Newfoundland larvae displayed a higher degree of intestinal complexity at a given age than did Scotian Shelf larvae. Note that growth rate and intestinal complexity covary; increasing intestinal complexity presumably enhances growth rate through enhanced nutrient assimilation (see previous section). Since Scotian Shelf cod were spawned and raised in late winter and early spring, while those from Newfoundland were spawned and raised in summer, differences in the composition of wild zooplankton fed to the larvae could possibly contribute to the between-population differences in growth rate and intestinal complexity.

Thus, co-occurrence of larval fish with high concentrations of suitable prey types ('match-mismatch' hypothesis, Cushing 1975) might affect the degree of intestinal development and somatic growth rates.

Temperature is another extrinsic factor which affects age-dependent variation in the rate of intestinal development. To date, investigation of the effects of temperature has been confounded by the use of thermal summation or 'degree days'. Thermal summation assumes that all morphological change is age-dependent, with little age-dependent variation in stage. This concept has generally been used when comparing larvae raised at different temperatures (Thompson and Riley 1981, Pitman et al. 1989). The present study shows that the rate of development is accelerated in the 5°C animals late in development (Figure 1.2). Utilization of 'degree days' for between-temperature comparisons would contribute to the erroneous perception that development rate varies linearly with temperature.

The concept of 'degree days' does not satisfactorily address the large observed variation in age at each intestinal stage. Mean age increased with intestinal stage for both populations (Figure 1.4), but a given intestinal stage at 10°C is more variable than at 5°C (Figure 1.4). Moreover, using degree days would ignore the variation in stage at a given larval age, e.g. 50 degree days at 5°C (10 days post-hatch) implies that stage two is the dominant stage. However, as shown in this study, at 50 degree days at 10°C (5 days post-hatch) three types of alimentary tract are present (Figure 1.4). This suggests that the degree and rate of intestinal development is age-dependent (i.e only determined by intrinsic (presumably genetic) components), but may also depend on both feeding success

and environmental perturbations (e.g. temperature). Intestinal development thus may depend on a combination of extrinsic and intrinsic components. Figure 1.9 shows an interpretive, conceptual flowchart depicting the changes in structures associated with feeding and respiration from hatching to juvenile stage in Atlantic cod. At the onset of first feeding, variation in intestine stage may be influenced by a combination of intrinsic (genetic) and extrinsic (environmental) factors. Following the yolk–sac stage, one might expect variation in intestinal stage to be dominated by extrinsic factors.

(ii) Size–dependent factors

Marine fish larvae are known to exhibit variation in developmental state at a given size (Sieg 1992b). In the present study, larval size was a poor predictor of intestinal development (Figure 1.5). Intestinal and yolk–sac stages varied widely at a given size throughout the larval period, with the greatest variation occurring at the higher temperature (10°C). Intestinal development did not progress past stage 2 in the Scotian Shelf population (Figure 1.6a), while many Newfoundland larvae developed more complex alimentary tracts (Figure 1.6 a,b) which are presumably more efficient at converting food into energy for growth (Govoni et al. 1986). If the faster intestinal development and higher growth rates of Newfoundland larvae result from more efficient exogenous feeding, one might expect their yolk–sac utilization rate to decrease. A decrease in yolk–sac utilization rate may be expected as exogenous food may provide the nutrients the larva utilize in growth and metabolism as an alternative to catabolism of the yolk–sac. However, yolk–sac utilization rate depends on egg diameter (i.e. is genetically

Figure 1.9. Flowchart for Atlantic cod from the egg to the juvenile stage showing the interaction of intrinsic factors and extrinsic factors on morphological structures of feeding and respiration.

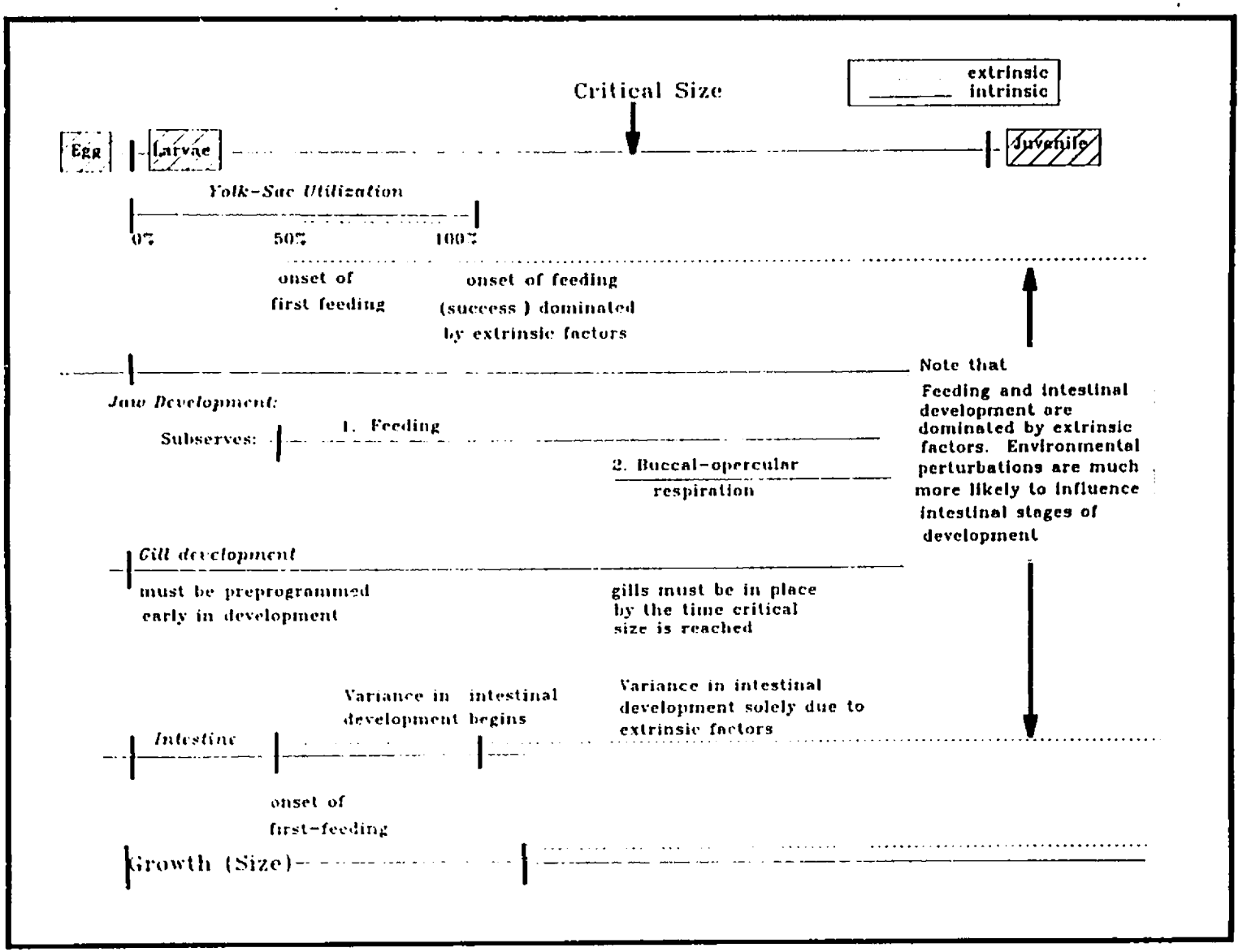

Critical Size
extrinsic
intrinsic
Egg
Larvae
Juvenile
Yolk-Sac Utilization
0%
50%
100%
onset of first feeding
onset of feeding (success) dominated by extrinsic factors
Note that Feeding and intestinal development are dominated by extrinsic factors. Environmental perturbations are much more likely to influence intestinal stages of development
Jaw Development:
Subserves:
1. Feeding
2. Buccal-opercular respiration
Gill development
must be preprogrammed early in development
gills must be in place by the time critical size is reached
Variance in intestinal development begins
Variance in intestinal development solely due to extrinsic factors
Intestine
onset of first-feeding
Growth (Size)

Figure 19

determined, Shirota 1970), and can be altered by changes in temperature, whereas intestinal development may be much more plastic (this study) and subject to variations in extrinsic factors such as

prey density and temperature.

Development of Respiratory Structures

Respiratory gas exchange is almost certainly cutaneous until later larval stages (Rombough 1988a). However, the branchial respiratory system must be fully in place by the time cod larvae reach a critical size (see Figure 1.9). The critical size is dictated by the cutaneous surface area to volume ratio, which decreases with growth (Blaxter 1988). Timely development of a functional branchial system is required to ensure that gas exchange can match the metabolic needs of the growing larvae (Blaxter 1988). Development of gill respiratory machinery is a slow process (Figure 1.8) beginning early in development and predetermined to some extent by the necessity for completion by a certain size but not necessarily a certain age (i.e. size at age varies). Thus, branchial gill development must be closely related to growth and ultimately size. However, once the branchial apparatus is functional, extrinsic factors become much more important in determining the morphological development. For example, the number of filaments and secondary lamellae, and degree of vascularization, are known to vary with oxygen partial pressures after the gills have become functional (McDonald and McMahon 1977).

SUMMARY.

During the endogenous feeding period, the development of structures associated with feeding and respiration is dictated largely by the genetic component. For example, jaw and gill cover development, which are important both for feeding and respiration, appear to be influenced much less by extrinsic factors such as temperature or prey density than are other systems such as gut development.

As exogenous feeding predominates, extrinsic factors play an increasing role in affecting larval development. This is exemplified in the present study by the size- and age-dependent variation in intestinal development of larval Atlantic cod raised at different temperatures. Environmental influences on phenotypes can potentially enhance variability which could, in itself, be adaptive for the population.(Waddington 1957, Hall 1992). Therefore, it is presumably during the obligate exogenous feeding period, the longest period of larval life, that the effects of extrinsic factors should be most strongly felt, and the highest phenotypic variability produced.

Chapter 2

THE ONTOGENY OF FEEDING AND RESPIRATION IN LARVAL ATLANTIC COD: (I) MORPHOLOGY.

INTRODUCTION

The adult teleost skull is a complex, highly integrated, kinetic structure which is well adapted to feeding and respiration in the aquatic environment. Kinesis occurs when interconnected cavities expand and contract through muscles acting upon a complex network of skeletal elements (Liem 1985). Extensive work has revealed numerous structures in the adult teleost skull and their adaptive importance (e.g. Aerts et al. 1987, Lauder 1980, 1983, Liem 1978, 1980, Wainwright 1991). However, only a few studies have investigated cranial structural development of early life history stages (Otten 1982, 1983, Langille and Hall 1987, Dilling 1989, Liem 1991).

Changes in skull organization occur throughout larval life, not only as a result of differences in size-related requirements, but also as flow velocity increases during growth. As larval fish size increases so does the Reynolds number, a dimensionless number that characterizes the ratio of viscous to inertial forces in fluid flow. Reynolds numbers are much lower in the larval fish environment than in the adult (Webb and Weihs 1986). Therefore, critical processes such as feeding and respiration may rely upon skull development keeping pace with fish larval requirements in a changing environment. In addition, structural organization in larval fish skulls must allow key processes, such as

feeding and respiration, to operate cooperatively in environmental conditions very different from those encountered by adult fish.

To date, only a few studies have attempted to relate cranial development to feeding and respiration in early life history stages (Tanaka 1973, Otten 1982 1983, Dilling 1989, Kjorvsik 1991, Liem 1991). Comparisons among these studies show that while the extent of development at hatching varies between species, key structures develop in an orderly pattern to facilitate feeding and respiration. Branchial arches without gill filaments are generally present at hatch, while gill filaments develop after first-feeding. The early presence of branchial arches may be primarily to fulfill the requirements of exogenous feeding (Van der Meeran 1992, Kjorvsik et al. 1991) while respiratory needs are probably met exclusively by cutaneous gas exchange (Rombough 1988a, Osse 1990). McDonald and McMahon (1977) noted, however, that buccal and opercular movements of newly hatched Arctic char were weak and poorly coordinated in normoxic water, but became stronger and fully coordinated in hypoxic water. Thus, they concluded that whilst the larvae possessed the necessary neural and musculoskeletal mechanisms to irrigate their gills, they did not do so unless oxygen was limiting.

In the marine environment, where less than 1% of pelagic larvae survive to recruitment (Hewitt et al. 1985), the importance of structural design to feeding and respiration has received little attention. Atlantic cod larvae (4.0-5.0 mm) hatch from small transparent pelagic eggs (1.1-1.9 mm in diameter; Colton and Marak 1969) and spend approximately 60-90 days in the planktonic environment, when they must progress from endogenous to exogenous feeding and from cutaneous to branchial respiration.

Thereafter, larvae transform to pelagic juveniles, (characterized by a gradual transformation to juvenile body form, squamation and juvenile pigmentation) and 1–2 months later, settle on the substrate as demersal juveniles (Lough et al. 1989).

Numerous general descriptions of larval Atlantic cod are available (Hardy 1978, Fridgeirsson 1978, Thompson and Riley 1981, Fahay 1983, Fossum 1986 and Ch. 1; Table 2.1), but none of these studies describe specific changes in internal and external cranial morphology important to feeding and respiration. To date, only two studies have examined changes in viscerocranial morphology of larval cod: Kjvorsik et al. (1991) and Morrison (1993). The former described changes only to first feeding (5 days at 5°C) and the latter did not address links between form and function and their importance to larval fish survival. Neither addressed whether the rate of development in viscerocranial structures varies throughout the larval life, or whether developmental rate varies with changing survival needs of fish larvae.

The objectives of this paper are: (1) to describe the changes in viscerocranial structure associated with feeding and respiration in larval Atlantic cod and (2) to determine if the rate of structural change varies throughout the larval life and whether such change could be related to larval fish survival.

METHODS AND MATERIALS

Animals

Cod eggs and sperm were obtained by stripping adults from captive populations at St. Andrews Biological Station and from wild Sable Bank populations. Eggs were fertilized

in petri dishes, transferred into 80 l closed-system glass aquaria and incubated at 5–7°C. Fertilized cod eggs are transparent and float on the surface. Gentle of aeration kept the eggs suspended in the water column; and reduced the build up of fungi. Low concentrations (0.02 g/l) of antibiotics (Penicillin-G and Streptomycin sulphate, SIGMA) were also added to the water in the aquaria to inhibit bacteria. In addition, high concentrations of the green alga, Isochrysis sp, were added to the aquaria and 'green' cultures were maintained throughout the experimental period.

Upon hatching, larval cod floated upside-down with their yolk-sac touching the surface of the water. At this time, aeration was reduced as larvae cannot effectively swim against any currents. At about 1 day post-hatch, newly hatched larvae righted themselves and began to swim more actively. At this time aeration was again increased. Rotifers were added when the larvae began to orient toward particles in the water column, but before they actually started to feed (about 2 days post-hatch at 5–7°C). At 50% yolk-sac utilization (approximately 10 days post-hatch), wild zooplankton, sieved to the appropriate sizes, were introduced to the aquaria in addition to the rotifers. Prey concentrations were kept high (>20 prey/ml) to ensure good feeding conditions for all larvae.

Larvae were sampled every day for the first 10 days after hatching and then less frequently as the numbers declined. Some individuals at transformation stage (i.e. larvae-juvenile transition) were obtained from mesocosm studies run in parallel (Hunt von Herbing, unpublished data). These animals were used only for descriptions of the oldest developmental stages, as raising larval cod to pelagic juvenile stage was difficult in

closed-system aquaria. Larvae were staged according to the general criteria outlined in Chapter 1. Some stages were combined on the basis of growth or development of various skeletal elements in the head.

Skeletal preparation, histology and scanning electron microscopy.

Larvae from hatch to 70 days old were fixed in modified Karnovsky's fixitive (Sire 1987). Animals processed for whole skeletal staining were first washed in water for 2 days before being stained with Alcian blue and Alizarin red S to visualize cartilage and bone. Specimens for light microscopy were processed for embedding in paraffin. Serial sections (5µm thick) of the head were cut to the level of the first or second vertebrae, placed on slides and stained using either Hall and Brunt's quadruple stain (HBQ) (Hall 1986), Mallory's stain, or Masson's Trichrome stain (Pantin 1960). For scanning electron microscopy, fixed samples were dehydrated in a graded alcohol series and prepared with Peldri II (Peldri Inc.), a sublimation dehydrant (in place of critical point drying). After coating with silver, samples were viewed by a Nanolab 2000 scanning electron microscope (SEM) (Bausch and Lomb).

Skeletal Analysis

Cartilage and bone were first identified in histological sections. Cartilage was stained dark blue in HBQ or light blue in Mallory's stain. Bony elements, including membrane and perichondral bones were found to be acellular throughout the visceral skeleton and were stained red in HBQ or bright blue in Mallory's stain. Results from histological

sections were compared with Alcian blue and Alizarin red stained elements in cleared larvae. Some membrane bones e.g. the premaxillary and the maxilla, did not lose their alcian blue staining during destaining and clearing and thus appeared cartilaginous. A possible reason for this may be the high content of muccopolysaccharides in bony elements (B.K. Hall, pers. comm.). In addition, some bony elements e.g. perichondral bones around cartilages such as the hyomandibular and membranous opercular bones, were not stained with alizarin red and remained opaque. Such a reaction is typical of unmineralized bony (or osteoid) structures. These observations on Alcian blue and Alizarin Red stained specimens thus illustrate the importance of using histological sections to confirm the identity of skeletal elements (see Hall 1987, Hanken and Hall 1988).

The nomenclature of bony and cartilaginous elements was based on general teleost skeletal terminologies of Harder (1975), those of adult Atlantic cod (Mujib 1967, Howes 1988, 1989) and those of fish larvae and embryos (Bertman 1959, Otten 1982, de Beer 1985, Langille and Hall 1987, Dilling 1989, Liem 1991). Teleost muscle terminologies were take from Winterbottom (1974) and Miyake et al. (1992). Skeletal elements were grouped by regions according to Mujib (1967) and Langille and Hall (1987) (Table 2.1). In addition elements were grouped into mechanical units (after Lauder 1985) (Table 2.1). The musculoskeletal couplings between mechanical units define the structures proposed to be necessary for respiration and feeding.

Table 2.1. Skeletal elements of the viscerocranium of Gadus morhua, grouped by region and mechanical unit (after Lauder 1985).

Region	Mechanical Unit	Elements
Maxilla	Upper Jaw	Premaxilla
		Maxilla
Mandibular Arch	Lower Jaw Apparatus	Meckel's cartilage
		Dentary
		Quadrate
Hyoid Arch	Hyoid Apparatus	Basihyal
		Hypohyal
		Ceratohyal
		Epihyal
		Interhyal
	Suspensory Apparatus	Hyomandibular
		Symplectic
Branchial	Branchial Apparatus	Basibranchial
		Hypobranchial
		Ceratobrachial
		Pharengeal jaws
Opercular	Opercular Apparatus	Opercular
		Preopercular
		Interopercular
		Subopercular
		Branchiostegal

RESULTS

The following descriptions of morphology provide a foundation for subsequent functional analysis (see Ch. 3). Developmental descriptions will only be given for those skeletal structures and muscles thought to be of major kinematic importance to feeding and respiration. The morphological groupings of stages given below reflect changes in rate of development of skeletal structures. The stages listed beside each major grouping are the general stages described in Ch. 1 and are added for clarification; there are other staging sequences available for cod larvae (e.g. Fossum 1986).

(a) 1st Period of development: (stages 1–4)

At hatch larval cod are about 4.0 to 5.0 mm long and entirely reliant on their yolk–sac. The mouth is closed and swimming movements appear random and not well coordinated. Skeletal changes occur rapidly during the first 5 to 6 days after hatching, the first jaw movements occur and a transition from obligate endogenous feeding (from the yolk–sac) to facultative exogenous feeding begins. During this period, the mouth is sub–ventral (Figures 2.1a–c).

Hatched Cod larvae (0 – 3 days old): (stages 1–2; 4.2–5.5 mm)

In newly hatched yolk–sac larvae (stage 1), the mouth is closed and covered by the oropharyngeal membrane. The eyes are large and unpigmented. In the buccal region, the lower jaw is composed of Meckel's cartilage but no associated dentary bone. No maxillary or premaxillary bones are present in the upper jaw (Figure 2.1a). However, the structure

Figure 2.1. Camera lucida drawings of Alcian Blue and Alizarin Red–stained Atlantic cod larvae, in lateral view, illustrating cranial skeletal elements throughout ontogeny. (a–c) yolk–sac stage (0–4 days post–hatch), (d–f) mixed feeding stage (6–17 days post–hatch), (g–i) exogenous feeding stage (23–50 days post–hatch).

Abbreviations: basihyal or tongue (bh), hyoid (hy), (hyomandibulo–symplectic(hs), interhyal (ih), maxilla (m), Meckel's cartilage(mc), otic capsule (oc), premaxilla (pm), quadrate (qu), trabeculum cranii (t). Arrow shows the anterior insertion point of the adductor mandibulae muscle (i.e Meckel's cartilage process). Stippled elements represent cartilage while solid elements represent bone.

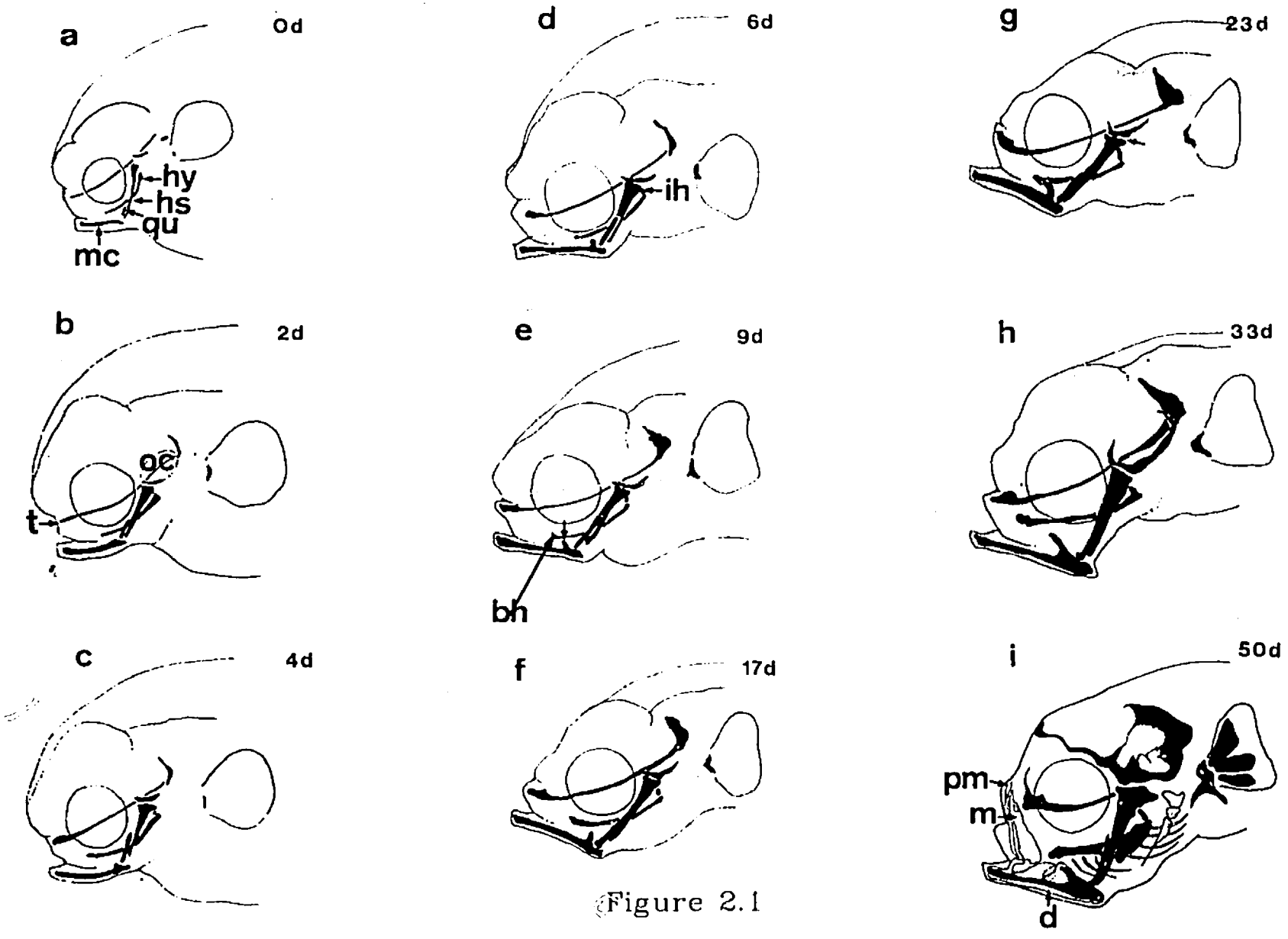

a
0d
hy
hs
qu
mc
b
2d
oe
t
c
4d
d
6d
ih
e
9d
bh
f
17d
g
23d
h
33d
i
50d
pm
m
d

Figure 2.1

of the upper arch or lip is evident, being mostly composed of connective tissue with associated mesenchymal cells. In the pharyngeal region, (hyomandibular, symplecticum and quadrate) elements of the suspensory apparatus are present. However, the symplecticum is not distinct from the hyomandibular (hyomandibulo-symplectic cartilage (hs) (Figure 2.1a). The hyoid arch is composed of the ceratohyal cartilage, and the basihyal. Four distinct ventral arches (ceratobranchia) display early matrix formation (Figure 2.2a). The internal branchial cavity has not yet expanded, but an external cavity or pit can be seen in the region of the future gill slit (see Miyake et al. 1992) (Figure 2.2b). No opercular structures or gill cover (operculum) are associated with the 'pit'. Hence all cranial cavities present at this stage are open to the environment via the external gill cavity or pit.

Within the first 24 hrs after hatching, no articulation exists between the mandibular arch and quadrate or the hyomandibulo-symplectic cartilage and cranial skeleton. However, the large muscles primarily responsible for early jaw movement and mouth opening, e.g. epaxial, geniohyoideus, sternohyoideus and hypaxial muscles, are already formed and are composed of large muscle fibres. The membranous cleithrum in the pectoral girdle is also well formed and acts as an anchor for the insertion of both the hypaxial and sternohyoideus muscles. Caudal to the large eyes, the earliest formation of both the levator arcus palatini and the dilatator operculi was observed (Figure 2.2a). However, at this early stage it was difficult to discriminate between these muscles, especially at their dorsal end.

Two to three days after hatching (stage 2), the oropharyngeal membrane is disrupted

Figure 2.2 (a)Frontal section of a hatched cod larva, illustrating four ventral arches (v) and differentiating levator arcus palatini or dilitator operculi muscles (see text). Mag.= 420x (b) Scanning electron micrograph of just hatched cod larva, showing external gill pit (arrowhead). (c) Frontal section of 2–3 day old cod larva, showing four separated ventral gill arches (v). Mag = 420x.

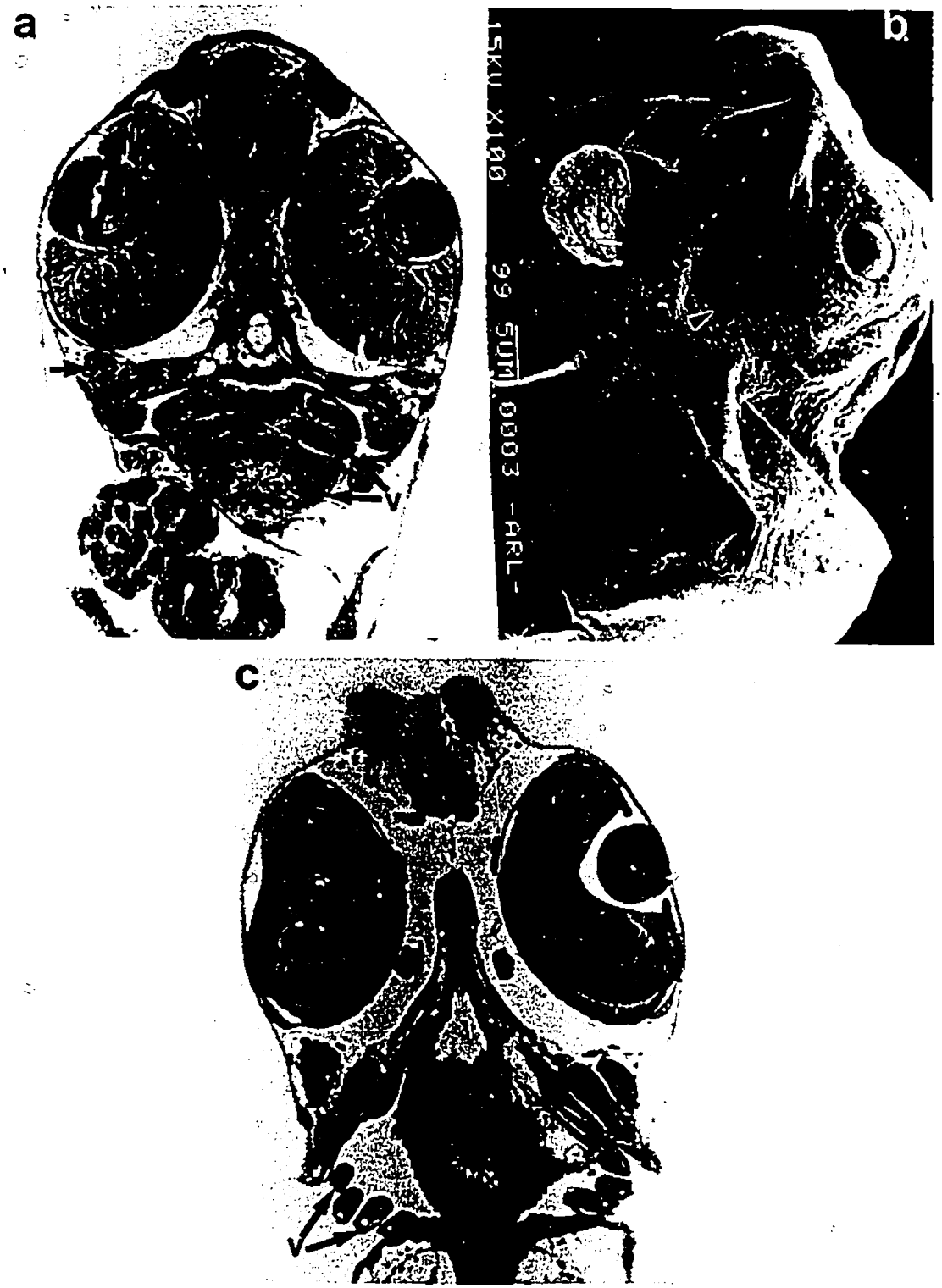


Figure 2.2

and the mouth opens. Tiny unmineralized (membranous) premaxillae and maxillae, appear in the upper jaw. In the lower jaw, Meckel's cartilage and the quadrate are in close proximity (Figure 2.1b). In the suspensory apparatus, the posterior end of the hyomandibulo-symplectic cartilage is in close proximity to the posterio-lateral region of the otic capsule (Figure 2.1b). However, no distinct articulations occur and no jaw movements were observed. The branchial cavity has expanded relative to that in the first day after hatching and the gill arches are distinct and separate the branchial from the buccal cavity (Figure 2.2c). Each gill arch has developed a vascular system, but the arteries lack pigmented blood cells (Figure 2.2c). In addition, muscles have developed, such as the adductor mandibular complex, important in closing the mouth, and the tranversi ventralis (muscles running laterally between the gill arches), in preparation for future jaw movements associated with exogenous feeding.

Yolk-sac Larvae (4 – 6 days old): (stages 3-4; 5.6-6.3 mm)

All of the cranial structures that were present during the first few days after hatch have increased in length and diameter. As a result of this growth, two major articulations have formed: (1) between Meckel's cartilage and the quadrate and, (2) between the hyomandibulo-symplectic and the cranium (otic capsule) (Figure 2.1c). The interhyal cartilage, which first developed at 4 days post-hatch, has enlarged and is distinct by 6 days post-hatch (Figure 2.1d). It is the pivotal point between two skeletal mechanical units, the suspensory and the hyoid apparatus. In addition to skeletal growth, cranial muscles, such as the levator arcus palatini, have also increased in size (Figure 2.3a). Both

Figure 2.3. (a) Frontal section of a 6 day old cod larva, showing a large, distinct levator arcus palatini (lap), the site of the future pseudobranch (p) and pigmented blood cells (bc) in the gill arch arteries and in the sinus venosa. Mag = 670x. (b) Scanning electron micrograph of a six day old larva, showing the gill slit (arrowhead).

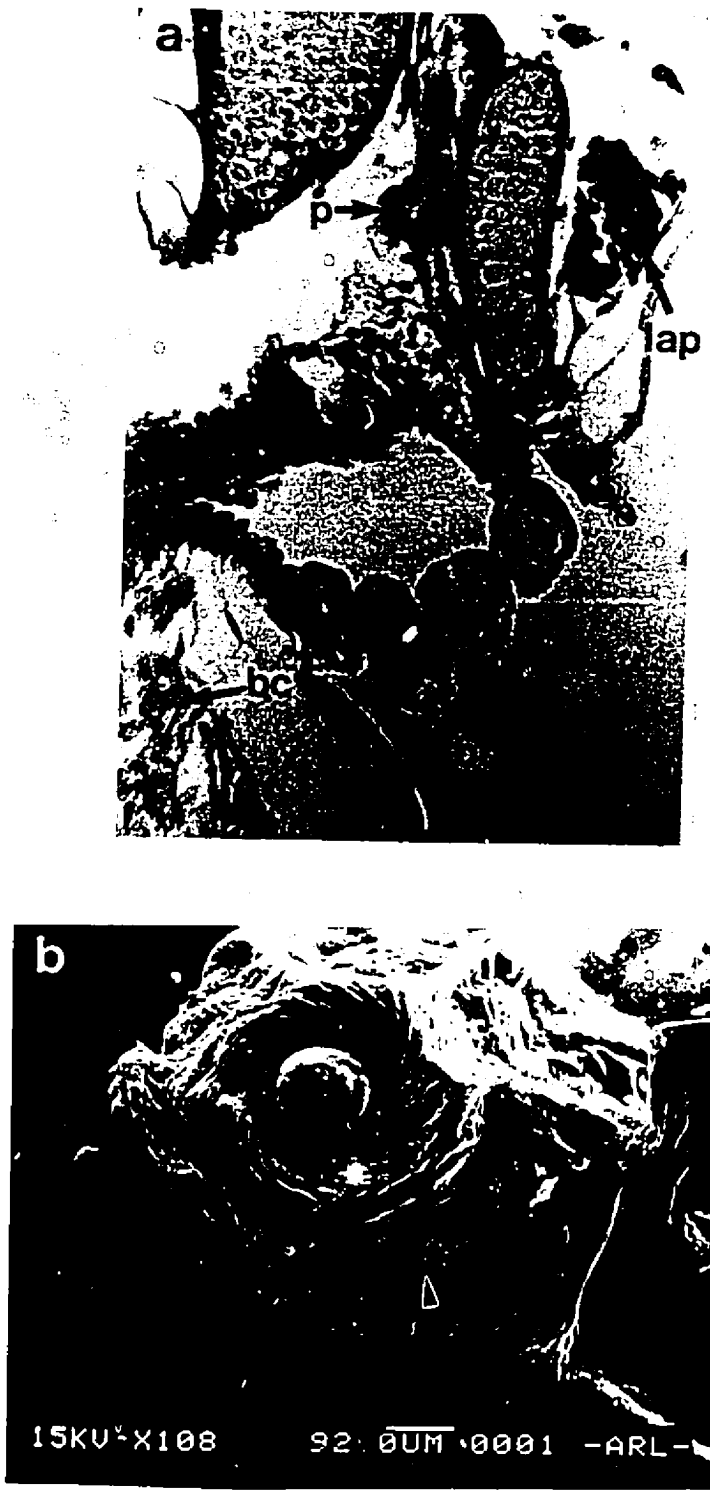


Figure 2.3

the development of the articulation points and the enlargement of the levator arcus palatini muscle facilitate jaw movement, which is infrequent and uncoordinated at this stage.

Externally, the gill pit or cavity, present at hatch, has extended ventrally and formed an external gill slit (Figure 2.3b). Internally, a small protuberance in the dorsal wall of the pharynx antero-lateral to the gill arches is the future site of a pseudobranch (Figure 2.3a). Neither gill filaments nor gill rakers are present on the gill arches at this time. Pigmented blood cells first appear at 3-4 days post-hatch and can be readily observed in the arteries of the gill arches at 6 days post-hatch (Figure 2.3a) as well as in the early pseudobranch. Large numbers of pigmented blood cells are also present in the sinus venosus (Figure 2.3a). However, the density of blood cells is not sufficiently high for blood pigmentation to be visible in live specimens. At the end of this first period of development : three mechanical units of the viscerocranium are linked together: the lower jaw, the hyoid and the suspensory apparati.

(b) **Second Period of Development:** (stages 5-6)

After the initial rapid structural and functional developments during the first week after hatching, elements in the head slow in their developmental rate. However, pre-existing internal skeletal structures (muscles and ligaments) thicken and strengthen. During this period the mouth moves from a sub-ventral position to a sub-terminal/terminal position (Figures 2.1d-f).

Yolk–sac Larva (1–2 weeks post–hatch): (stages 5–6; 6.5–6.8 mm)

At about ten days post–hatch (stage 5), the yolk–sac is still present but much reduced, and the intestine has expanded (see Ch. 1) for detailed description of intestinal development). At this stage, prey capture was observed to occur and food is seen in the intestine from this stage onward. Larvae locate the prey and advance toward it. The larva stops and curls its tail into a C–shape (C–start), opens its mouth and drives the tail posteriorly, lunging at the prey.

In the buccal cavity, Meckel's cartilage in the lower jaw has thickened and a mandibular process (first observed as a small process on Meckel's cartilage at 4–6 days post–hatch) has grown in size (Figure 2.1e). This process forms an insertion point for the adductor mandibulae muscle. The basihyal has enlarged anteriorly and will continue to enlarge in older larval stages, eventually forming the 'tongue' (Figure 2.1e).

Two weeks after hatching (stage 6), only a small remnant of the yolk–sac remains. Rapid growth has increased the size and thickness of all cartilages (Figure 2.1f) and muscles (Figure 2.4a). Only a few new skeletal structures have developed. In the upper jaw, the premaxilla and maxilla have continued to enlarge. A flap of tissue extends down from the upper jaw to either side of the lower jaw. In the hyoid and branchial arches, all cartilaginous elements have grown, and in most specimens, the first gill filaments have begun to appear on the second/third ventral arches (Figure 2.4b). In addition, in some specimens, one small pharyngeal tooth has appeared on the fifth gill arch and a thin epithelial border (or opercular epithelium) has also begun to grow dorsally from around the ceratohyal cartilage. The epithelial border will eventually cover the gill cavity, and

Figure 2.4. (a) Frontal section of a 12 day old cod larva, showing a distinct levator arcus palatini (lap) and dilator operculi (do) and a developing opercular epithelium (oe). Mag = 420x . (b) Scanning electron micrograph of a 13 day old cod larva showing initial gill filaments forming on the gill arches.

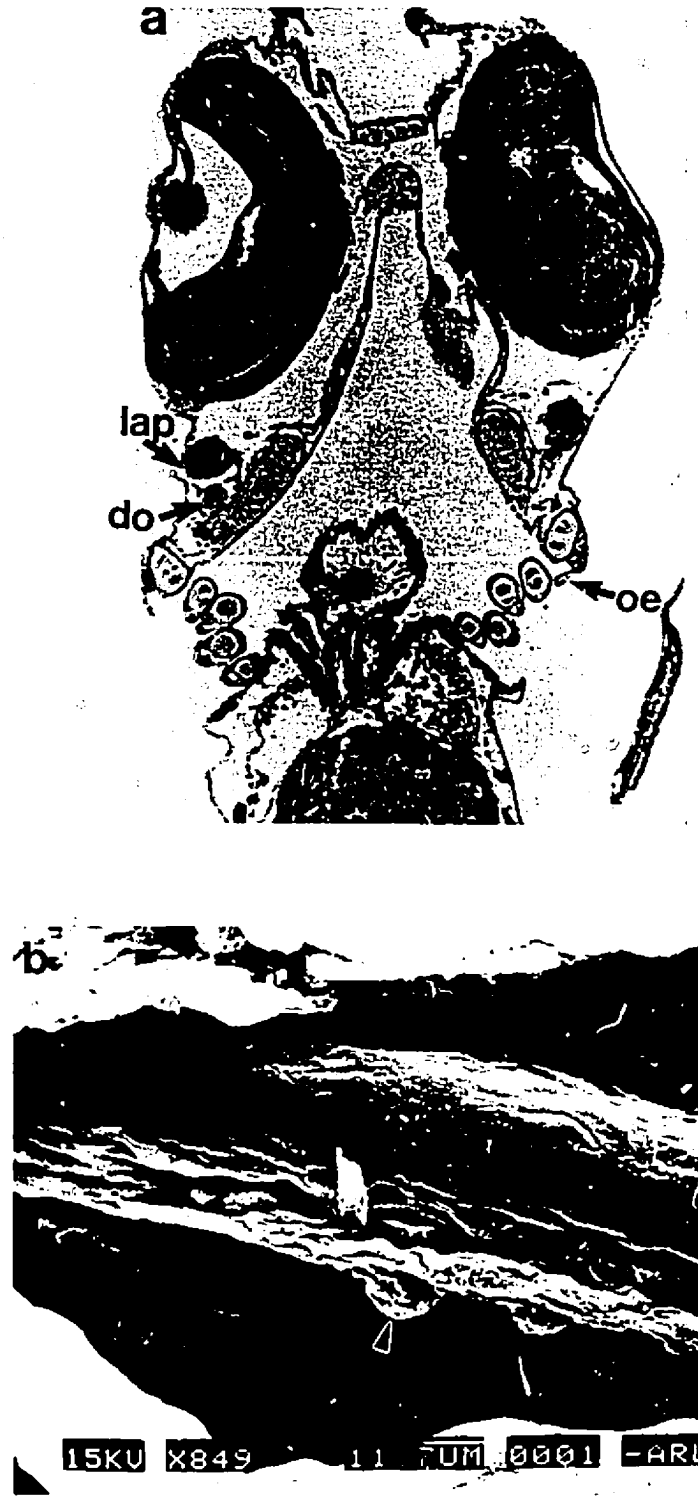


Figure 2.4

together with the opercular apparatus will form the gill cover (Figure 2.4a).

Larval Stage (3–4 weeks) – (stages 7–8; 6.6–7.3 mm)

At this time, the larval cod must feed exogenously since any nutritional benefits from the now internalized yolk–sac (Morrison 1993), are not sufficient for growth and survival. At about 3 weeks post–hatch (stage 7), the cartilaginous structures in the head are thicker and the articulation points larger, e.g. between the dorsal edge of the hyomandibulo–symplectic cartilage and the enlarged otic capsule (Figure 2.1g).

By 4 weeks post–hatch (stage 8), the tip of the trabeculum cranii has thickened and broadened, forming the ethmoid cartilage, which defines and supports the upper jaws and neurocranium. In all specimens growth of the gill filaments has continued on the second, third and fourth gill arches, but no secondary lamellae have formed. Several large pharyngeal teeth are present on the fifth gill arch. Despite the overall growth of the head the opercular apparatus is still composed mostly of connective tissue. However, the operculum bone has begun to develop and it can be seen as osteoid in histological sections. Externally, the opercular epithelium almost encloses the gill cavity.

At the end of the second period of development the same viscerocranial structural linkages exist as in the first period of development (between the lower jaw apparatus, the hyoid apparatus and the suspensory apparatus), however, the skeletal elements composing these mechanical units have all grown in size.

(c) **Third period of development** : (Stages 9–12)

After a period of growth in cranial structures and development of major organ systems, further developmental changes occur in the viscerocranium. The mouth remains sub–terminal/terminal (Figures 1h–1i).

Larval Stage – (5–6 weeks post–hatch): (stage 9; 8.0–9.0 mm)

At 5 weeks post–hatch (stage 9) (Figure 2.1h), increases in the complexity of cranial elements occur simultaneously with those of the intestine, which is now coiled (see Ch. 1, for intestinal staging). In the upper jaw, the premaxilla and maxilla are large and well developed and the upper jaw has become protrusible. The bones do not stain red in cleared and stained specimens but their structure can be clearly identified as osteoid in histological sections. In the lower jaw, the dentary encloses Meckel's Cartilage. Meckel's cartilage process has enlarged (Figure 2.1h) and provides a large surface area for insertion of the adductor mandibulae muscle. Together the dentary, mandibular process and the ventrally extended premaxilla and maxilla define the edges of the mouth. On the dorsal wall of the pharynx, three or four pairs of pseudobranchs extend into the dorsal pharyngeal cavity (Figure 2.5a). Each pseudobranch contains a central cartilaginous rod, and is composed of numerous free lamellae which contain high densities of blood cells. These blood cells move in sinuses between a thin pharyngeal membrane and the central cartilaginous rod. In addition, large numbers of gill filaments are present on all arches and bear the first of the secondary lamellae (Figure 2.5b).

Within the opercular epithelium, a second bone, probably the suboperculum, has

Figure 2.5. (a) Frontal section of a 35 day old cod larva, showing enlarged pseudobranchs (p), and the opercular epithelium, which encloses the gills (oe) Mag = 670x. Note shrinkage of opercular epithelium occurred due to histological preparation, thus the opercular epithelium does not appear to completely enclose gills. (b) Scanning electron micrograph (ventral view) of a secondary lamellae (arrow) forming on the gill filaments.

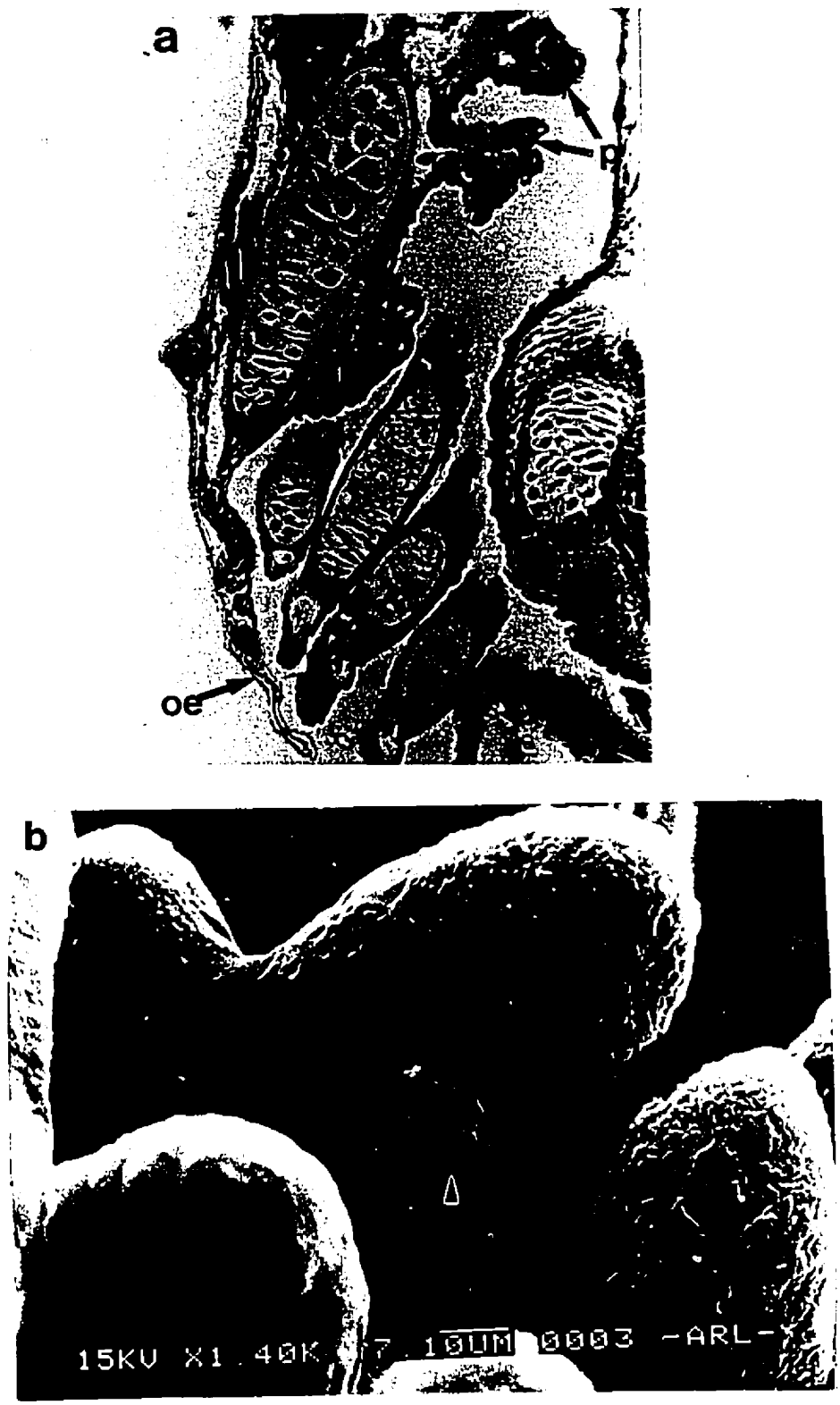


Figure 2.5

formed, in addition to the one (probably the operculum) present at stage 8. The opercular epithelium has developed into a gill cover, enclosing the gills and gill cavity (Figure 2.5a). The posterior edge of the gill cover has thickened to form a valve, which can seal the opercular cavity when adducted.

Larval Stage – (7–8 weeks post–hatch): (stage 10; 9.5 mm)

At this late larval stage (stage 10), changes in both body and cranial morphology occur. Flexion of the notochord occurs and the complexity of cranial structures increases. In the lower jaw, the dentary bone has enlarged and become broader, completely enclosing Meckel's cartilage (Figure 2.1i). The ascending process of the maxilla has developed and articulates with the dentary. Both the lower and upper jaws bear teeth. At the extreme caudal end of Meckel's cartilage, the retroarticular bone has begun to form.

On the gill arches, two rows of filaments have formed (two hemibranchs) and most bear secondary lamellae (Figure 2.6a). Large pharyngeal teeth are present on the fifth branchial arch and 7 branchiostegal rays have developed from the ceratohyal and within the gill cover (Figure 2.6b). In addition, gill rakers have formed on all four gill arches. The gill arches are differentiated into the three elements, ceratobranchial, epibranchial and hypobranchial, which characterise the gill arches in the adult cod. The pseudobranchs have reached their maximum size and the lamellae are beginning to fuse (Figure 2.6c).

All four opercular bones (operculum, subopercular, preopercular and interopercular) are present. They are seen as thin blue strips in cleared and stained specimens, and as

Figure 2.6. (a) Frontal section of a 50 day old cod larva showing one of numerous secondary lamellae forming on the gill filaments (arrow). Mag = 670x. (b) Scanning electron micrograph of a 50 day old cod larva showing 7 branchiostegal rays within the opercular membrane. (c) Frontal section of a 50 day old cod larva showing very large pseudobranchs with fused lamellae (p). Mag = 420x. (d) Frontal section of a 50 day old cod larva showing the presence of one of the opercular bones within the operculum (arrow). Mag = 670x.

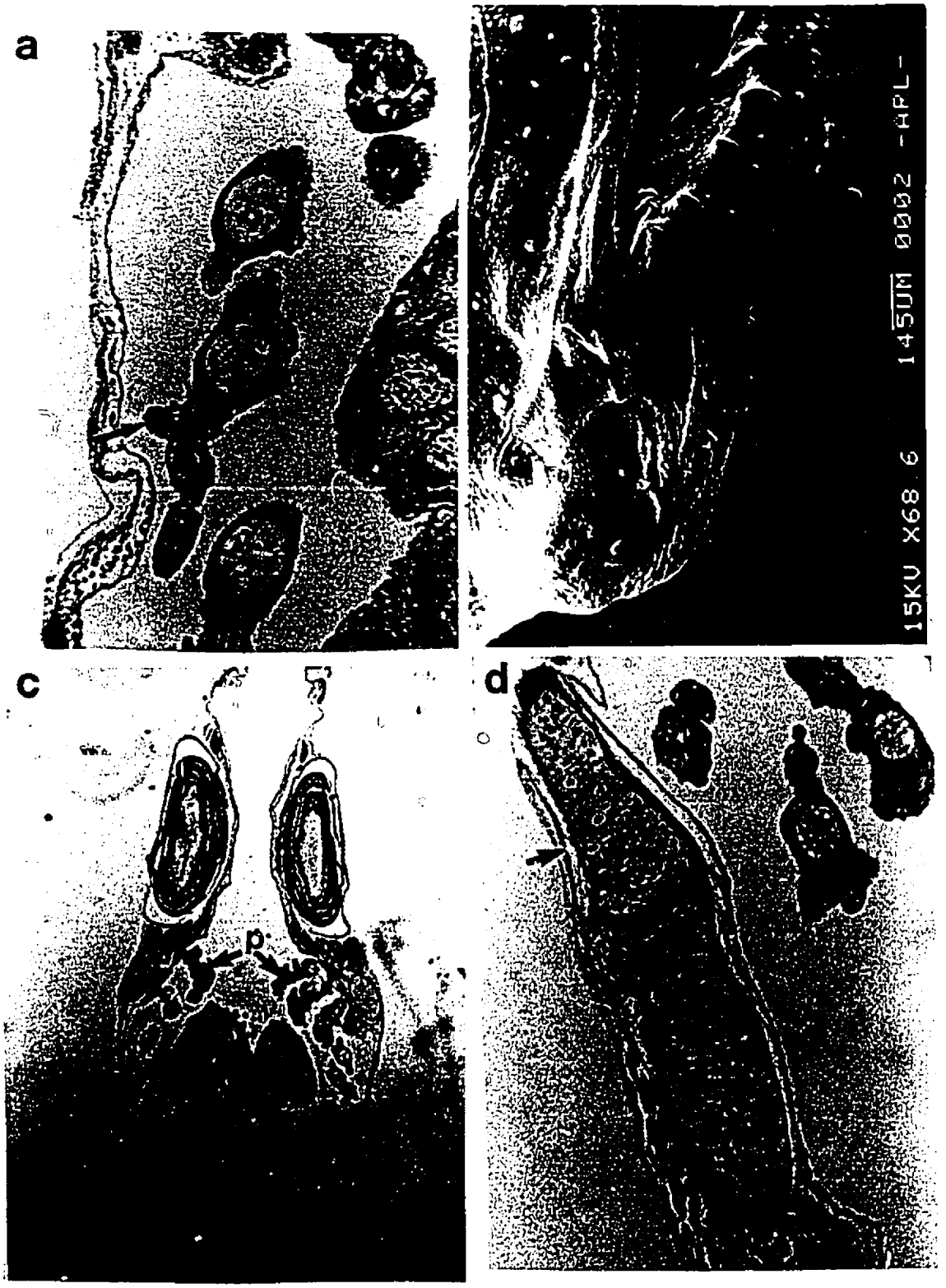


Figure 2.6

osteoid in histological sections (Figure 2.6d). The hyomandibular cartilage has developed a large condyle which articulates with the operculum. At this late larval stage, even though the cartilaginous structures have become more robust, only the cleithrum is completely mineralized.

At the end of this period, cranial structures connect four major mechanical units together the lower jaw, hyoid, suspensory and opercular apparati. Coupling between the mechanical units of the lower jaw apparatus and the upper jaw apparatus, forms an additional mechanical unit.

Transformation stage and pelagic juvenile (9–10 weeks): (stage 11–12; 11.0 – 30.0 mm)

At this stage, the larval cod begins to resemble a young juvenile cod in general body shape. Most cranial structures present in juveniles have formed and are mineralizing (Figure 2.7). After the cleithrum, the next elements to mineralize are the dermal bones, premaxilla and maxilla of the upper jaw apparatus. The dorsal region of the hyomandibular cartilage and the fifth branchial arch also show a progressive formation of perichondral bone. The bones in the opercular apparatus are among the last in the viscerocranium to mineralize.

DISCUSSION

In cod larvae, feeding and respiratory needs are met by changes in the development, growth and integration of viscerocranial skeletal elements. The force exerted by these elements during feeding and respiration will depend upon skeletal size, composition and

Figure 2.7. Alcian blue and alizarin red stained 80 day old cod larva, showing mineralization around the jaws (dark stain) and hyomandibular. Total length = 28mm

Figure 2.7

the associated muscles. However, the rates of development in structure and composition vary throughout the larval life. Therefore, it is important to discuss the changes in structure and composition during each period of development of cod larvae, and to relate these to changing modes of feeding and respiration.

Structures for Feeding

In newly hatched cod (yolk-sac) larvae, structures in the viscerocranium are simple and non-integrated (Figure 2.1a) and the yolk-sac serves as an endogenous food source (see Ch. 1). When the mouth opens at 2 days post-hatch, gill arches trap phytoplankton (Van der Meeren 1991) and possibly small protozoans which pass through the open mouth (during movement). Thus, prey selection depends upon the distance between adjacent gill arches. Since gill arches are present at hatch in all fish larvae studied (Balon 1985, Otten 1982, Dilling 1989, Liem 1991), food trapped by filter-feeding may be important to larval fish survival. Moreover, filter-feeding may be particulary important to yolk-sac cod larvae, since onset of jaw function is delayed until 4-5 days post-hatch. In addition, cod (yolk-sac) larvae inhabit a viscous environment ($Re < 20$; Webb and Weihs 1986), in which active feeding (i.e. suction feeding) is inefficient (Drost 1987, Drost et al. 1988a 1988b, Osse 1990). Therefore, an endogenous food supply and development of structures that provide alternative feeding methods may be critical for survival.

In 4-5 day old cod larvae, articulation of cartilaginous elements and the formation of the interhyal result in the first jaw movements. The interhyal acts as a fulcrum between two mechanical units, the suspensory apparatus and the hyoid apparatus, forming the first

structural linkage (see Ch.3, for functional diagrams). In addition, ventral movement of the hyoid apparatus laterally expands the suspensory apparatus and the buccal cavity via the quadrate. This series of events produces infrequent and uncoordinated 'gulping' movements (Hunt von Herbing et al. 1992), which probably generate very little suction pressure for feeding. Therefore, initial jaw movements in yolk-sac larvae may serve two functions: (1) to provide larval fish with a 'learning period', during which they can increase the coordination of viscerocranial structures with changes in locomotion and growth, while avoiding starvation. Such 'pre-feeding' periods have increase growth rate and survivorship in altricial species (Pedersen et al. 1987, Appelbaum 1989) such as cod, but may not be beneficial for precocial species such as salmonids (Rombough 1988a). (2) Initial jaw movements may facilitate the expansion and growth of the buccal and pharyngeal cavities, which will allow for the ingestion of larger volumes of water during exogenous feeding. Thus, fish larvae have some musculoskeletal control over jaw movement, which facilitates obligate exogenous feeding while relying on endogenous food reserves.

Development of functional systems for exogenous feeding continues in the second period of development, as the key cartilaginous structures (developed in the first period) elongate and thicken. In concert with skeletal growth, integration of structural networks improves. For example: (1) the areas of articulation enlarge between the quadrate and Meckel's cartilage, and between the hyomandibulo-symplecticum and otic capsule; (2) expansion of the floor of the buccal cavity during hyal protraction increases in two ways: ventrally, due to the growth of the basihyal cartilage or 'tongue' and laterally due to the

longer interhyal and growth of the levator arcus palatini muscle, the muscle which laterally expands the suspensory apparatus, and (3) the area for the anterior insertion of the adductor mandibulae muscle increases due to the growth of Meckel's cartilage process; the adductor mandibulae is important in closing the mouth. Increases in skeletal size and width may facilitate an increase of suction pressure generated in the buccal cavity and may thereby increase prey capture success. As the opercular apparatus has not formed by this stage, the buccopharyngeal cavities may be partially sealed by adduction of the gill arches (Lauder 1983), allowing some pressure to be generated in the buccal cavity. However, feeding at this stage is likely to be a combination of suction–feeding and ram–feeding (Ellertsen et. al. 1980). Therefore, as a result of skeletal growth and improved coordination of jaw movements and swimming activity, larvae can feed efficiently by 17 days post–hatch, when the yolk–sac is completely utilized and feeding is entirely exogenous.

Once through the transition period from endogenous to exogenous feeding, the larval fish are totally dependent on external sources of energy. Therefore, rates of prey capture must increase to meet the demands of growth. Larval fish can increase prey capture rates in three ways: (1) increase swimming speed of foraging and prey pursuit, (2) improve aiming accuracy and (3) develop structures that improve prey capture and manipulation. Cod larvae swim faster with increasing size (see Ch. 4), and flexion of the notochord has been suggested to increase swimming thrust (Blaxter 1988). This may result in greater foraging speed and body stability in the water. With the reduction in high amplitude swimming patterns (i.e. anguilliform swimming (Hunter 1981)), head yaw is reduced and

aiming accuracy increases (Drost 1987). In addition, aiming accuracy is likely to increase as the mouth moves from a sub-ventral to a terminal position (a terminal mouth position is better suited to feeding in mid-water; Osse 1990). In concert with these changes, integration of the upper jaw with the lower jaw results in a protrusible upper jaw. Protrusion of the jaws facilitate prey capture in several ways. First, it brings the predator within striking distance with less chance of detection. Second, rotation of the maxilla closes off the angles of the mouth, so the intake of water lateral to the predator's head and not containing the prey, is prevented (Osse 1985). These actions reduce the amount of water ingested with the prey (Osse 1990) and also decrease the "bow wave" effect (Muller and van Leeuwen 1985) and hence increases feeding efficiency. Protrusion may be less important to prey capture in adult cod (Muller and Osse 1984), but its early appearance in other species such as Haplochromis and Cyprinus sp, indicate that it may be important to feeding at the larval stage. However, it is my opinion that while protrusion is important to prey capture, it may not develop until swimming speeds and larval size have increased and hydrodynamic conditions are favourable. This occurs at about 9.0 mm in both cod (this study) and cyprinid larvae (Osse 1990).

By 10 mm (50 days post-hatch), swimming includes an inertial component (Webb and Weihs, 1986) and foraging and prey capture efficiency increases (Hunter 1981, Gamble and Houde 1984). Therefore, further development of viscerocranial structures is important for: (1) exploitation of a greater diversity of prey types and sizes, and (2) reduction of food loss through the gill openings and (3) increase suction pressures necessary for suction feeding. Formation of the dentary and pharyngeal teeth facilitates

the capture of new types and sizes of prey. Teeth are situated on the upper and lower jaws and are designed to grasp and hold prey. Generally, cod larvae ingest their prey whole from the water column (Ellertsen et al. 1980), but they also use teeth to pick prey off the sides of vertical surfaces and from the bottom of the aquarium (personal observations). After capture, prey move from the buccal cavity into the pharyngeal cavity, where they are further processed by the pharyngeal teeth. The pharyngeal teeth crush food and direct it to the gut, thus presumably increasing the rate of food processing and assimilation of nutrients for growth.

Food loss through the gill slits in a 10 mm fish larva is prevented by the formation of the gill rakers, branchiostegal rays and opercular apparatus (operculum, subopercular, interopercular and preopercular). Gill rakers restrict the loss of food particles through the gill openings by creating a barrier between gill arches during gill arch adduction (Lauder 1983). In addition, branchiostegal rays control the extent and rate of flow of water through the opercular valves and therefore aid in controlling food loss and more importantly aid in generating suction pressures during suction feeding. Moreover, during mouth opening, the adduction of the opercular apparatus by the dilatator operculi, as well as adduction of the branchiostegal rays and gill rakers, seal the buccopharyngeal cavities preventing water from entering the posterior opening of the buccal cavity. Adduction of the opercular apparatus also seals the bucco-pharyngeal cavities. Together the formation of these structures probably results in higher buccal pressures, stronger uni-directional flows and more effective suction-feeding than were possible without a completely functional opercular apparatus.

Structures for Respiration

In the first few weeks of larval life, respiration is unaffected by buccal movements, as there are no gill filaments on the gill arches and respiration is cutaneous. Cutaneous respiration is common in early-stage fish larvae (Rombough 1988a), as the large surface-area-to-volume ratios (Osse 1990) and thin dermis (DeSilva and Tytler 1973) favour transcutaneous oxygen diffusion. In cod larvae, cutaneous respiration may be supplemented by haemoglobin (which forms early). However, the importance of haemoglobin to total oxygen uptake is unclear. Salmonid larvae, in which haemoglobin was deactivated, survived for several weeks dependent only on cutaneous respiration for gas exchange and transport (Rombough 1988a, Wells 1993). Consequently, the contribution of haemoglobin to oxygen uptake and transport cannot be assessed until accurate measurements of intravascular oxygen concentrations are possible for small, pelagic fish larvae such as cod.

The structures essential for gill ventilation form at a late stage of development in cod larvae. Musculo-skeletal networks that connect the opercular apparatus with the neurocranium, hyomandibular, hyoid and lower jaw apparatus facilitate the coordinated functioning of the buccal and opercular pumps necessary for branchial respiration (Liem 1985). In cod larvae, the opercular bones (operculum, sub-operculum, pre-operculum and inter-operculum) of the opercular apparatus begin to form at 40-50 days post-hatch. This is much later than in salmonid or cichlid larvae which hatch with partially developed opercular elements (Verraes 1977, Otten 1982). The opercular apparatus is essential to seal the buccopharyngeal cavities, so that generation of high negative pressures and uni-

directional water flow can occur during branchial respiration. Although the adduction of the gills and gill rakers may also provide a barrier to posterior–anterior water flow through the gills (Lauder 1983), it is probably insufficient for generation of large negative pressures presumably needed for ventilation.

In fish larvae, high frequency and strong ventilatory movements are necessary, as viscous effects will rapidly reduce the flow velocities in the buccopharyngeal cavity. One would expect that strong ventilatory movements could occur when size of the elements that constitute the musculoskeletal networks increases with fish growth. As fish size increases, surface area to volume ratios decrease reducing the relative area available to cutaneous respiration (Blaxter 1988). This presumably necessitates transition to branchial respiration (see Ch. 1; Figure 1.9). Thus, growth of the opercular apparatus and associated muscles and the integration of structures facilitate the transition from cutaneous to branchial respiration, presumably in order to meet larval metabolic requirements.

Structures other than the gills, such as the pseudobranch, may also aid in extracting oxygen from water for respiration. The pseudobranch's large volume of blood cells and thin epithelial lining are characteristic of a gas exchange organ. Pseudobranchs begin to develop early in cod larvae and grow to form very large structures (Hunt von Herbing et al. 1992). Their position in the head exposes them to large volumes of oxygenated water each time the mouth opens. In addition, blood sinuses of the pseudobranch are intimately connected to the coronoid gland of the eye (Lagler et al. 1977). The eye is probably one of the most metabolically active organs in fish larvae and is critical for feeding, as larval fish are primarily visual predators (Blaxter 1988). Therefore, one of the functions of the

pseudobranch may be to service the metabolic needs of the eye. In older larvae and in transformation-stage individuals, the lamellae fuse and the pseudobranch becomes surrounded by the membrane of the dorsal pharyngeal cavity (I. Hunt von Herbing, pers. obs.). Its function during juvenile and adult life is unknown, although some studies have suggested that it may be involved in endocrine function (Holliday 1960) or osmoregulation (Mattey et al. 1978).

Skeletal Composition

Mineralization of perichondral and membrane bones in the head occurs late in cod larval development during transformation to the pelagic juvenile stage. This is much later than in many other species studied. In species such as salmonids, cichlids and cyprinidonts many of the structures that are present in the adult have already formed and begun mineralization by the onset of exogenous feeding (Verraes 1977, Langille and Hall 1987, Otten 1992, Dilling 1989). Huntingford (1993) suggests that initial movement of jaws may stimulate ossification of the cartilage near ligament attachment points (e.g. on the lower jaw). However, the major viscerocranial structures in cod are still cartilaginous at 50 days post-hatch (Figure 6, this study). Some variation in rates of mineralization do exist. Morrison (1993) found that mineralization was delayed in laboratory raised cod as compared to field-sampled larvae. Nevertheless, it is clear that thoughout most of the larval life of cod, mechanisms of feeding and respiration are serviced largely by cartilaginous skeletal elements.

Dilling (1989) reported that first feeding in two species of pomacentrids was also

serviced by cranial structures which were still cartilaginous. Cartilage is not as strong as bone, but one study reports that functional use or loading may increase cartilage thickness (Holmdahl and Ingelmark 1948, also see Herring 1993) (e.g. during active prey capture). In contrast, Lanyon and Rubin (1985) suggest that the primary influences on longitudinal growth are genetic and hormonal. Therefore, functional loading may affect cell division, cell nutrition or the physical properties of cartilage itself, rather than being an adaptive modification in response to functional use (Myers and Mow 1983, Currey 1984, Lanyon and Rubin 1985, Biewener and Bertram 1993). Consequently, larvae may be predisposed to faster growth rates through the added advantage of having longer, thicker cranial structures. This would result in earlier articulation and larger generation of suction pressures, which would lead to increased prey capture efficiency, higher growth rates and an earlier transition from endogenous to exogenous feeding (and from larva to juvenile).

Bone requires energetically expensive mechanisms to function, e.g. a vascular system, and a complicated mineral metabolism and nutrient exchange system (Lanyon and Rubin 1985, Herring 1993). Therefore the rate of ossification may depend on the type and amount of nutrients that larval fishes are able to obtain. The reason for differences in the rate of differentiation and extent of ossification of skeletal elements in the larval fish viscerocranium are not entirely clear, but may be related to (1) historical (genetic) differences, (2) the nature of larval fish habitat (e.g. temperature in tropical fish can accelerate growth and metabolism, which could be related to the rate of ossification), or (3) inter– or intra – specific differences in the level of feeding and respiration required for survival.

In cod larvae, the opercular apparatus is one of the last series of skeletal structures to completely mineralize. Formation of the opercular apparatus significantly alters the number and types of structural organization involved in feeding and respiration. Therefore, it appears that for cod and perhaps most fish larvae, initial viscerocranial structures are specialized for feeding. Once fish larvae have survived the critical transition from endogenous to exogenous feeding, the subsequent formation of the opercular apparatus facilitates the transition from cutaneous to branchial respiration.

Chapter 3

THE ONTOGENY OF FEEDING AND RESPIRATION IN ATLANTIC COD LARVAE: (II) FUNCTION.

INTRODUCTION

To date, all investigations of the functional morphology of larvae have been conducted on four fish groups: salmonids, cichlids, pomacentrids, and embiotocids (Verraes 1977, Otten 1982, Dilling 1989, Liem 1991, respectively). In contrast, the functional morphology of feeding and respiration in marine species, such as Atlantic cod Gadus morhua, is unstudied. Gadids such as Atlantic cod are structurally 'simple' at hatch; i.e. only a few skeletal elements are present (Ch. 2) compared to cichlids and pomacentrids (Otten 1982, Dilling 1989). Cod develop from a yolk-sac larva to the pelagic juvenile stage over 60-90 days at 5 – 9°C (Lough et al. 1989, Lough and Potter 1993), whereas cichlids and pomacentrids develop into adults within 2 weeks at higher temperatures (e.g. 27°C). Skeletal structures that develop at the egg stage of cichlids and pomacentrids do not develop until the early larval stage in cod. However, cod larvae are still able to feed exogenously soon after hatch, despite their less integrated viscerocranial structures (Ch. 2). Larvae of this type are even more remarkable since most of their skeletal structures remain cartilaginous and incompletely formed throughout their long exogenous feeding period.

Mechanisms of feeding and branchial ventilation in juvenile and adult stages of several

species of teleost fish have been thoroughly described (Alexander 1967, Osse 1969, Ballintijn 1972, Liem 1978, Van Leewen and Muller 1984, Lauder 1985, Sibbing 1986). In these older stages, the structures are large, and feeding and respiratory kinetics can be determined through invasive techniques such as electromyography and pressures-sensitive recordings in buccal and opercular cavities. Although fish larvae are much too small for such invasive procedures, high speed video recordings and reconstructive morphology have been used to determine the ontogenetic changes in feeding and respiratory mechanisms (Verraes 1977, Liem 1980, Otten 1982 1983, Dilling 1989).

The relative rates of growth and development of skeletal elements in the skull (Ch. 2). affect the functional design of the teleost skull for feeding and respiration throughout ontogeny. Otten (1983) constructed a mathematical model to describe the functional morphology of biting and suction of cichlids. He determined the importance of anatomical points "hot spots" the position of which is critical in determining the biting force (Otten 1983). Application of this model showed that the teleost skull undergoes major transformation in form and function during the early life history (Otten 1983). Moreover, in pomacentrid larvae, growth-dependent changes in the proportions of the head transform the buccal cavity from a cylindrical cavity to a truncated conical cavity (Dilling 1989), affecting the ratio of head depth to head length. As a result, a simultaneous shift in feeding strategy occurs, from ram-feeding to suction feeding, as fish grow from the larval to the juvenile stage (Brainerd 1985).

In addition to structural changes, changes in feeding and respiratory mechanisms occur through growth (Otten 1982, Liem 1991). For example, Otten (1982) demonstrated

that a critical ontogenetic stage occurs in cichlid larvae during which there is a rapid change in mouth-opening mechanisms. At this threshold stage, the geniohyoideus muscle transforms from a depressor to an adductor of the mandible, and the levator operculi coupling becomes operational to depress the mandible and open the mouth (Otten 1982). If the initiation of this coupling is delayed, asphyxiation and subsequent death of the larva occurs (Liem 1991). This coupling results from growth and integration of structures in the head which trigger changes in feeding mechanisms. Thus, previous studies have shown that growth and integration of structures and subsequent shifts in functional mechanisms may be critical to survival.

The objectives of this study are: (1) To determine the mechanisms of feeding and respiration in larval cod throughout ontogeny, and (2) to compare the functional morphology of feeding and respiration in cod larvae to other species, and determine if any common patterns emerge that may influence larval survival.

METHODS AND MATERIALS

Animals

Fertilized Atlantic cod eggs were obtained from four sources: captive populations at St. Andrews Biological Station (St. Andrews, New Brunswick), Dalhousie University (Halifax, Nova Scotia), Memorial University (St. John's, Newfoundland) and wild Sable Bank populations. Eggs and larvae were raised at 5-7°C in 80 l glass aquaria. High concentrations of the green alga Isochrysis sp. were added to each aquaria and 'green' cultures were maintained throughout the study period. Rotifers and wild caught

zooplankton, sieved to appropriate sizes, were always maintained at densities of >20 prey/ml to ensure good feeding conditions for all larvae (see Ch. 2 for details of culture procedures). Larvae were sampled every day for the first 10 days after hatching and then less frequently as numbers declined. Samples of transformation (i.e. metamorphosed) stage individuals were supplemented from mesocosm studies (Hunt von Herbing unpublished data).

Skeletal preparation, histology and scanning electron microscopy.

Samples for whole mount skeletal staining, scanning electron microscopy and histology were fixed in modified Karnovsky's fixative (after Sire 1987). For whole mount skeletal staining specimens were treated with Alcian Blue and Alizarin red S for cartilage and bone. Cartilage stains blue, bone red and ligaments pick up a blue stain. Identity of all elements were confirmed using histology (see Ch. 2 for details of cartilage and bone identification). Representative stages of skeletal morphology were drawn using a camera lucida – equipped Wild dissecting microscope. Specimens for light microscopy were processed for embedding by standard techniques. For scanning electron microscopy, fixed samples were dehydrated in a graded alcohol series and prepared with Peldri II (Peldri Inc.) as a sublimation dehydrant (in place of critical point drying). After coating with silver, samples were viewed by a Nanolab 2000 Scanning Electron Microscope (SEM) (Bausch and Lomb).

Kinematic analysis

Jaw movements of 1 week, 2 week and 7 week old larvae, were recorded using two methods: (1) a Sony video camera with a macrolens and (2) a Sony video camera mounted on a Zeiss Tessovar macromicroscope. In the first method, 4 or 5 larvae of the same stage, were placed in a perspex container (10 cm x 10 cm x 5 cm) and filmed continuously for 3 hours to record a series of ventilatory sequences. Water temperature within the container was regulated by performing the entire experiment in a 5°C cold room. In the second method, larvae were placed individually in a Petri dish which was then placed on ice to maintain recorded temperatures of 5–6°C. Approximately 30 larvae were used to obtain a series of jaw movements. Drawings of one cycle of jaw movement (one cycle = from a closed mouth position to peak gape to closed mouth) for each stage were made from projected video recordings.

Skeletal measurements

Measurements of cranial skeletal structures were made from whole mounts stained as described above. Specimens were filmed with a monochrome video camera mounted on a dissecting microscope. Images were captured using Image software (developed by Dr. W. Rasband at the National Institutes of Health, U.S.A.) in Pixeltools (Perceptions Corp., Knoxville, Tennessee). Five skeletal measurements were made: head length was measured from the front of the eye to the first vertebra of the vertebral column; head depth was measured as the total dorso – ventral difference of the head at the midpoint of the eye (Figure 3.1). Variations in the ratio of head depth:head length, were used to

Figure 3.1. Schematic drawing showing location of skeletal measurements. a = head depth, c = jaw length, d = head length, q = quadratal angle, vc = vertebral column.

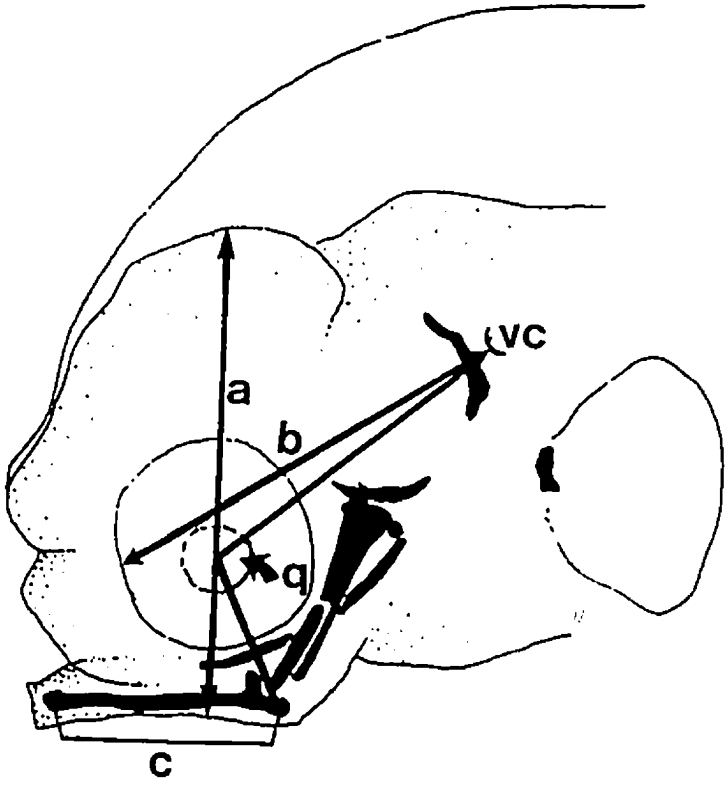


Figure 3.1

estimate changes in the shape of the head. A large ratio indicates a deep head, suitable to generating suction pressure, while a relatively smaller ratio represents a narrower head, suitable to ram feeding (after Brainerd 1985). The quadratal angle was measured as the angle between the line connecting the eye–lens with the jaw joint, and the line connecting the center of eye–lens with the rostral tip of the notocord in lateral projection (Figure 3.1). Small changes in the quadratal angle represent changes in head proportions due to growth, while large shifts in the angle indicate shifts in kinematic mechanisms for mouth opening (after Otten 1982).

Changes in both the head depth:head length ratio and the quadratal angle with standard length or age, may also result from growth of other elements in the head. Therefore, jaw length (from the extreme caudal end to the tip of the Meckel's cartilage) and eye length (eye diameter), were also measured.

RESULTS

Viscerocranial structures in cod larvae throughout development have been described in detail in Ch. 2. The stages referred to within each kinematic period are the general stages Ch. 2 (e.g. the yolk–sac, larval and transformation stage). The structural and functional interconnection between two or more mechanical units (e.g. suspensory and branchial apparati, see Ch. 2) is referred to as a 'coupling' and performs a specific function (after Liem 1980). The following descriptions of morphology, including ligamentous connections and musculoskeletal couplings, are specific to kinematic mechanisms and musculoskeletal couplings in cod larvae.

Morphology and Kinematics

(a) The Hyoid Period

This period extends from the time of initial jaw movement (5 to 6 days post-hatch; general larval stages 3-4; see Ch. 1) to 33 days post-hatch (larval stage 10; see Ch. 1). The cartilaginous elements of the hyoid period are shown in Figure 3.2a-c. The hyoid period is characterised by jaw movements which result from hyoid coupling (i.e. the musculoskeletal coupling between the hypaxial muscles, sternohyoideus, hyoid apparatus and mandible). This coupling governs mandibular depression via the mandibulohyoid ligament. The mandibulohyoid ligament connects two cartilaginous elements: the ceratohyal and Meckel's cartilage. It extends from the lateral side of the caudal portion of the ceratohyal cartilage to the caudal end of Meckel's cartilage (Figure 3.2a). Although not illustrated in the figure, well defined geniohyoideus muscles attach from the ceratohyal to the anterior end of Meckel's cartilage. The geniohyoideus originates laterally on the ceratohyal above the junction between the hypohyal and ceratohyal. In addition, a well developed sternohyoideus muscle originates on the cleithrum and inserts on the 3rd hyobranchial. No opercular bones or opercular membrane are present at this stage.

The first jaw movements occur between 5 to 6 days post-hatch. One jaw movement cycle (one cycle = closed mouth to peak gape to closed mouth) of a 5 day old cod larva is shown in Figure 3.3 (a-d). Contraction of the sternohyoideus muscle causes a posterior retraction of the hyoid apparatus. This movement produces depression of the lower jaw via the mandibulohyoid ligament (Figure 3.3 a-b). As the sternohyoideus relaxes and the

Figure 3.2. Development of cartilaginous elements in cod larva head during the hyoid period which extends from 6 days post– hatch (a) to 33 days post–hatch (c). hy = hyoid, hs = hyomandibulo–symplecticum, m = Meckel's cartilage,. The position of the mandibulohyoid ligament is indicated by the arrow.

HYOID PERIOD

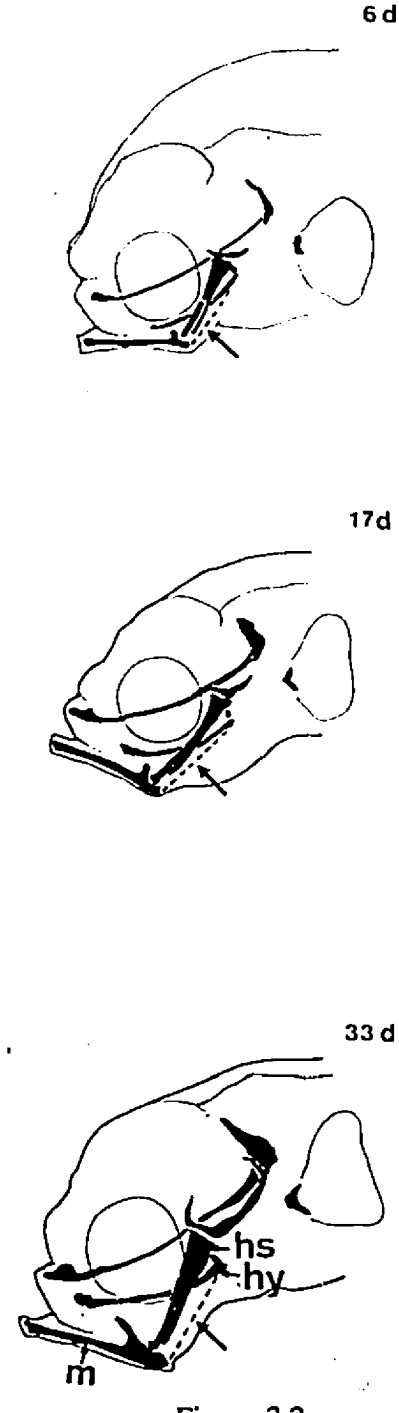


Figure 3.2

Figure 3.3. A series of video images and illustrations of a 5–6 day old cod larva showing the movement of the jaw during one mouth opening cycle. hy = hyoid, hs = hyomandibulo–symplecticum, m = Meckel's cartilage, q = quadrate.

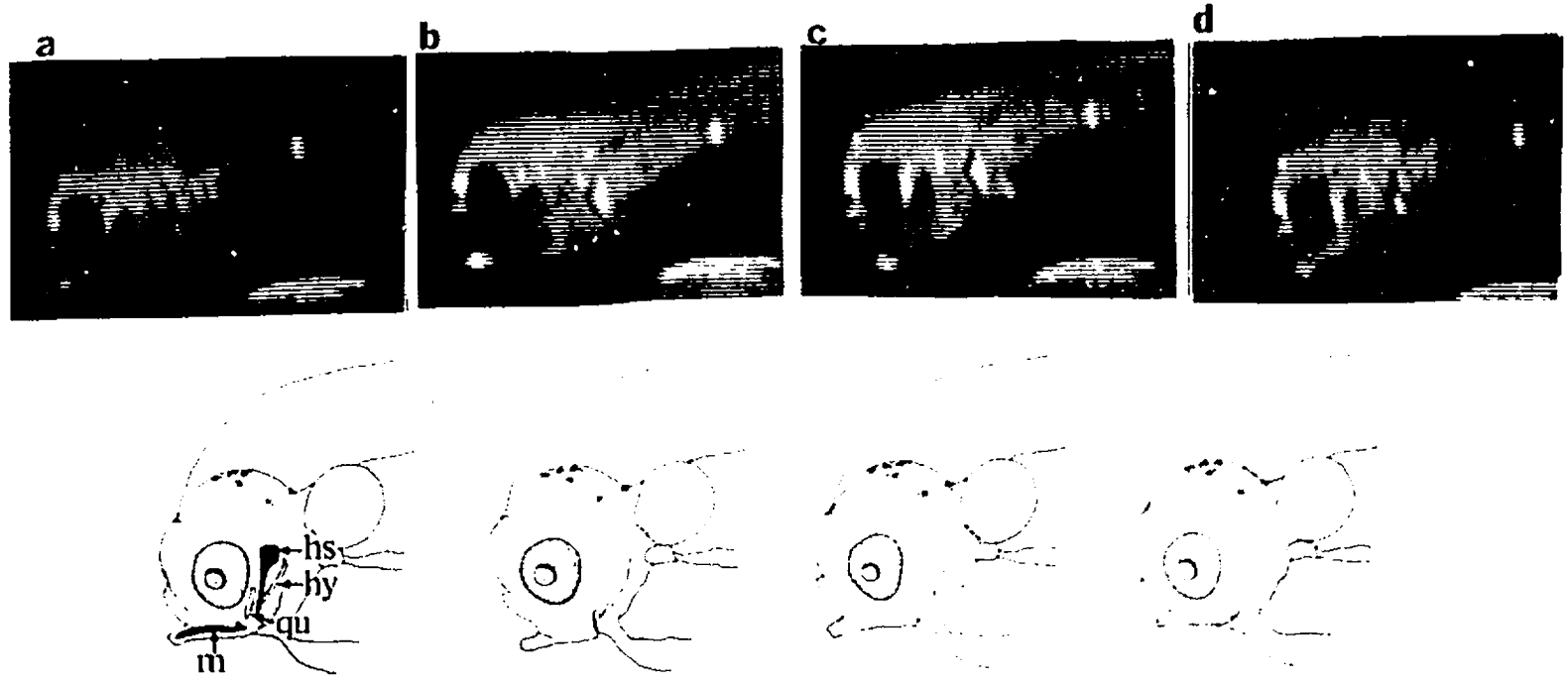


Figure 3.3

geniohyoideus contracts, the hyoid is protracted back into the mouth cavity and the jaw and mouth close (Figure 3.3 c–d). Figure (3.4a) shows that the movement of the lower jaw and hyoid are synchronous ; the beginning of depression of the lower jaw is immediately accompanied by depression of the hyoid. Although the adductor mandibulae muscle is small at this early stage, it may also assist in closing the mouth. In addition, a second coupling occurs between the hyoid and suspensory apparatus via the interhyal. As a result, during the posterio–ventral movement of the hyoid apparatus, a small degree of lateral expansion occurs. At this stage, the levator arcus palatini muscle is small (Ch. 2, Figure 3.2a) and is unlikely to supplement the lateral abduction of the suspensory apparatus. In general, jaw movements are infrequent and uncoordinated and movement is largely dorsal–ventral.

In the second week after hatching (stage 6; see Ch. 1), skeletal structures and muscles have increased in size and are integrated (Figure 3.2b). As in 6 day old larvae, jaw movement and mouth opening are controlled by the transmission of force, exerted by the sternohyoideus muscle on the hyoid arch, to the mandible via the mandibulohyoid ligament. Figure 3.5 (c–h) shows one full cycle of jaw movement for a 10 day old cod larva. As the sternohyoideus contracts it retracts the hyoid posterio–ventrally and the mouth begins to open. At peak gape, the mandibulohyoid ligament is maximally extended between the hyoid and the mandible (Figure 3.5e). In addition, the relaxed geniohyoideus is observed between the Meckel's cartilage and the hyoid (Figure 3.5e). Upon closing, the hyoid retracts into the buccal cavity at the same time as the mouth closes (Figure 3.5f–h). Therefore, movement of the hyoid and jaw are synchronous in

Figure 3.4. The distance (mm) moved by the lower jaw and the hyoid during one cycle of jaw movement in stage 3 (5–6 day old larvae), stage 6 (10 day larvae), and stage 10 (50 day old larvae). Note that the in stages 3 and 6 the jaw and hyoid move synchronously while in stage 10 they move asynchronously.

= distance moved by the lower jaw, = distance moved by the hyoid.

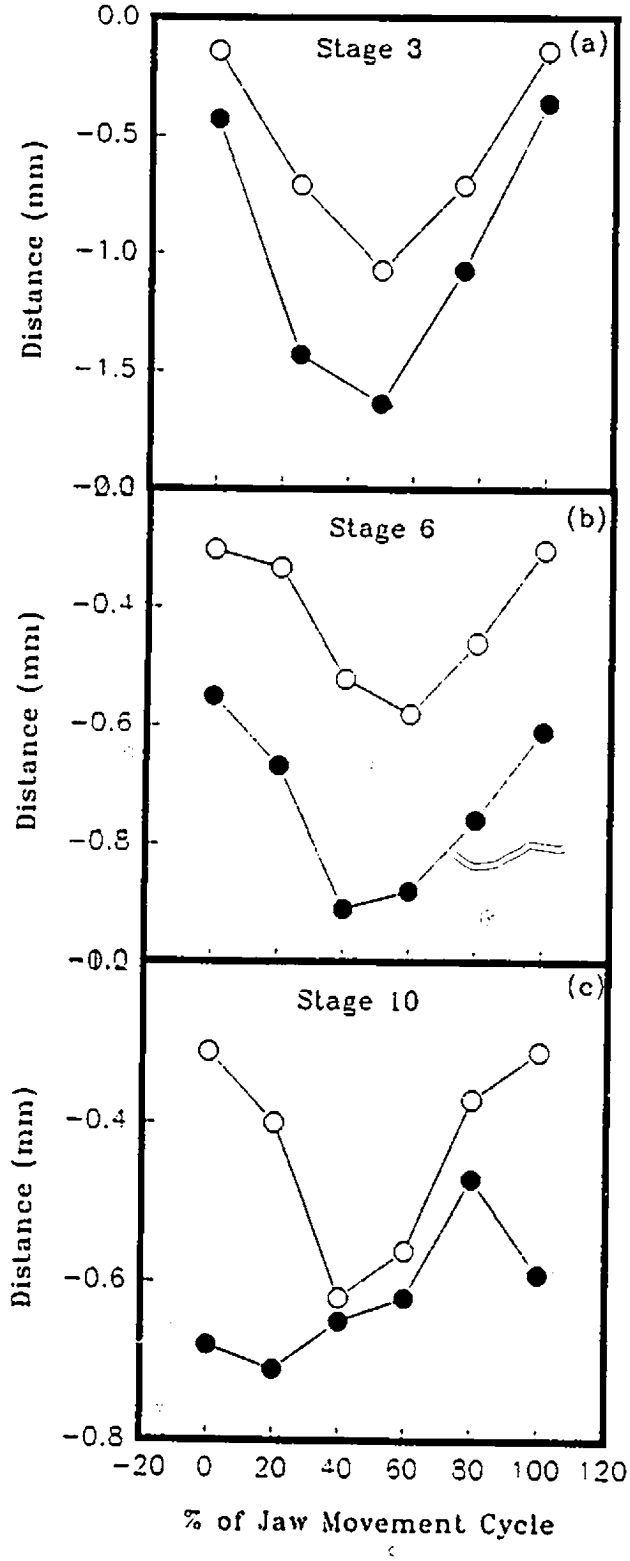

Stage 3
(a)
Distance (mm)
0.0
-0.5
-1.0
-1.5
-0.0
Stage 6
(b)
Distance (mm)
-0.4
-0.6
-0.8
-0.0
Stage 10
(c)
Distance (mm)
-0.4
-0.6
-0.8
-20 0 20 40 60 80 100 120
% of Jaw Movement Cycle

Figure 3.4

Figure 3.5. A series of video images of a 10 day cod larva showing one full cycle of jaw movement. md = mandible, hy = hyoid, relaxed geniohyoideus = g. The fully extended mandibulohyoid ligament is indicated by the arrowhead.

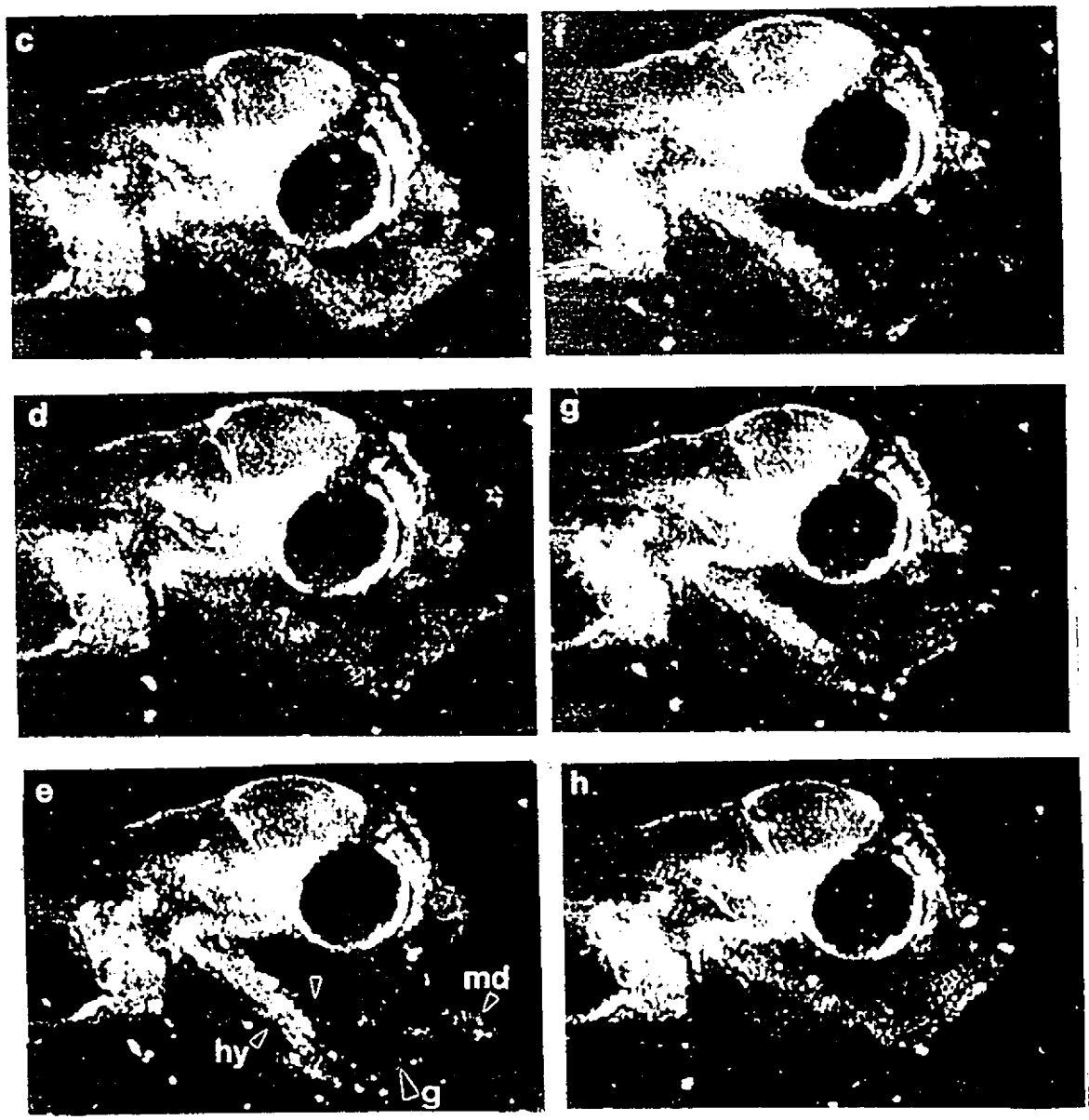


Figure 3.5

the first two weeks after hatching (Figure 3.4 a,b). This synchrony is maintained throughout the hyoid period. Lateral expansion through the abduction of the suspensoria is still limited at this stage. However, as the cartilaginous elements and muscles (e.g. the levator arcus palatini) grow in size, the amount of lateral expansion during jaw depression and mouth opening increases.

By 33 days post–hatch (Figure 3.2c), small opercular bones are present in the growing opercular epithelium (see Ch. 2). However, the mechanism for jaw movement and mouth opening remains unchanged. The major ligament to open the mouth is still the mandibulohyoid ligament, as no ligament coupling the opercular series to the mandible has formed.

Note that in all stages of the hyoid period the hyoid apparatus remains dorsal to the jaw joint when the mouth is closed. As a result contraction of the geniohyoideus muscle cannot depress the mandible.

(b) The Opercular Period

The jaw movements characterising this period occur during late larval stages and the transformation stage. The major mouth opening mechanism shifts from the hyoid coupling to the levator operculi coupling (i.e. a musculoskeletal coupling between the levator operculi muscle, operculi series and mandible). This coupling mediates mandibular depression via the opercular apparatus and the interoperculomandibular ligament. Major morphological changes important to jaw movement and mouth opening are: the formation of the four opercular bones (operculum, sub–operculum, pre–operculum

and interoperculum), and the hyomandibular condyle that articulates with the operculum and the opercular series (Figure 3.6b). In the lower jaw, the dentary bone has enveloped Meckel's cartilage and small retroarticular bones have formed posterior to the Meckel's cartilage.

At 50 days post-hatch, the opercular bones are small and lie in the opercular epithelium lateral to the suspensory arch (Figure 3.6a). The opercular epithelium has grown (its distal edge has thickened and formed the opercular valve) to completely cover the gills. In concert with the branchiostegal rays, the operculum can seal the buccal and opercular (pharyngeal) cavities and prevent posterior to anterior water flow. The mandibulohyoid ligament is still present, but the anterior portion of the interoperculum has become closely associated with it (Figure 3.6a).

At 62 days post-hatch, all opercular bones have further increased in size (Figure 3.6b). The interoperculum has grown rostrally, reducing the distance between it and the mandibular arch. As a result, the interoperculum is closely associated with the mandibulohyoid ligament and the rostral tip of the interoperculum 'inserts' into the ligament (Figure 3.6b). The rostral portion of the mandibulohyoid ligament thickens and forms the interoperculomandibular ligament (or retroarticular ligament) (Figure 3.6b).

During the opercular period in cod larvae, the mouth is opened and the mandible depressed by two musculoskeletal linkage systems: (1) levator-operculi coupling and (2) the mandibulohyoid coupling. In the first coupling, the levator operculi muscle, which attaches from the neurocranium to the operculum, contracts and rotates the left operculum counter-clockwise. The operculum, pivoted by its articulation with the hyomandibular

Figure 3.6. Development of cartilaginous (solid) and bony (stippled) elements in a cod larval head during the opercular period. In the 50 day cod larva, the interoperculum is in close association with the mandibulohyoid ligament (indicated by the arrow). In the 62 day old cod larva, the interoperculum inserts into the mandibulohyoid ligament, the rostral end of which has become the retroarticular ligament (indicated by the arrow). D = dentary, HS = hyomandibulo-symplecticum, HY = hyoid, I = interoperculum, O = operculum, S =subopercuIum, P = preoperculum. Arrowhead indicates position of hyomandibular condyle.

OPERCULAR PERIOD

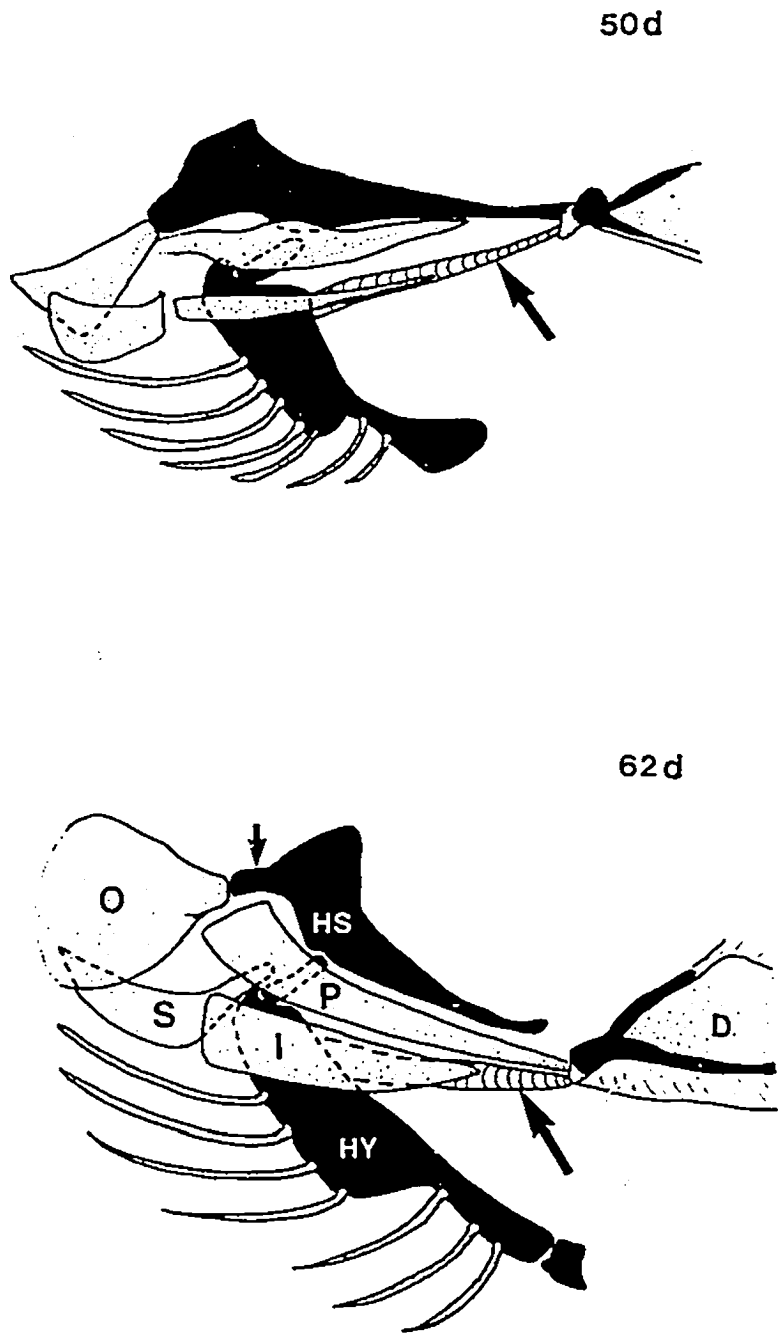


Figure 3.6

condyle, rotates the subopercular caudally. Through a system of ligaments, which connect the opercular series, force is transmitted from the interopercular via the interoperculomandibular ligament, to the mandible which is depressed. In the second coupling, mouth gape and depression of the hyoid is increased by the hyoid coupling, the elements of which are still present and functional.

A full cycle of jaw movement for a 50 day – old cod larva is shown in Figure 3.7 (a–f). In the first frame, the mouth is closed and the hyoid fully retracted. As the hyoid begins to descend, the mouth begins to open. As the hyoid begins to move dorsally, the mouth continues to open, reaching maximal gape when the hyoid is retracted into the buccal cavity. The asynchronous movement of the mouth opening and hyoid movement (i.e. the phase in which the lower jaw is still depressing while the hyoid is elevating), is indicative of a second mouth–opening mechanism (i.e. the levator–operculi coupling). This asynchronous movement differentiates the levator–operculi coupling from the hyoid coupling, in which the hyoid and mandible depress and elevate synchronously (Figure 3.4c vs Figures 3.4 a,b).

In addition to the extensive dorsal–ventral expansion, lateral expansion of the buccal cavity also occurs. This results from the lateral movement of suspensory arch, which is mediated by the large levator arcus palatini muscle and ventral movement of the hyoid apparatus.

Skeletal measurements in cod larvae

Skeletal measurements were transformed into ratios of head depth:head length, jaw

Figure 3.7. A series of video images and illustrations of a 50 day old cod larva showing the movement of the mouth and buccal cavity during one ventilatory movement. crb = ceratobranchial, hs = hyomandibulo-symplecticum, hy = hyoid, Mc = Meckel's cartilage, qu = quadrate.

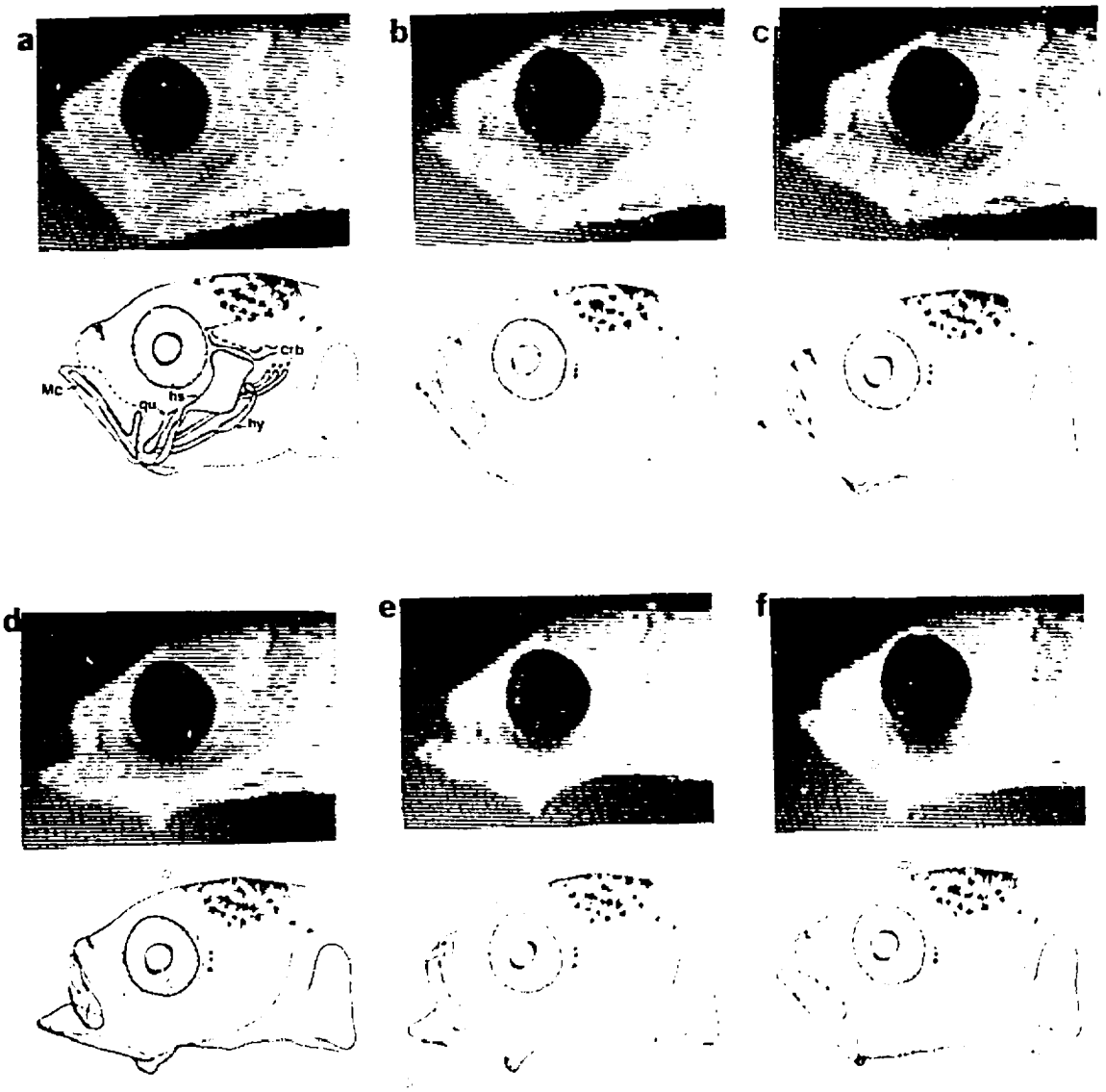


Figure 3.7

length : head length, eye diameter:head length. To determine how skeletal growth varies over the course of development, these ratios were plotted against standard length and days post-hatch.

A second order regression provided the best fit for the growth data, measured as an increase in standard length over days post-hatch ($r^2 = 0.98$, $p < 0.05$; Figure 3.8). The shape of the curve indicates two long developmental time periods separated by a shorter transition interval. The first period extends from hatching to approximately 30 days post-hatch or from 4 to 8 mm standard length. The second period extends from 35 days to 90 days post-hatch or from 10 mm to 30 mm standard length. The interval between periods on the growth curve (i.e. the transition interval) occurs at 30-35 days post-hatch or 9-10 mm standard length. Note that the first period includes only larval fish, whereas the second period is composed of larvae and pelagic juveniles (>80 days old and 29 mm standard length).

Figures 3.9 (a,b) show the changes in the ratios of head depth:head length vs standard length and days post-hatch. In the first period of growth, the ratio of head depth:head length was high and variable ranging from 0.95 to 1.4. In the second period of growth, i.e. after 35-40 days post-hatch, the ratio of head depth:head length decreased rapidly to 0.59 by 90 days post-hatch or 30 mm standard length. In contrast, the quadratal angle increased rapidly from hatching to 35 days or from 4 to 10 mm standard length (Figures 3.8 c,d). At this time the jaw joint reached its most rostal position relative to the eye. Thereafter, the quadratal angle remains high until the end of the larval period (about 60 days post-hatch), at which time it begins to decrease. Note that the largest and oldest

Figure 3.8. The relationship between growth vs. standard length (mm) in cod larvae and pelagic juveniles. H = hyoid period, O = opercular period.

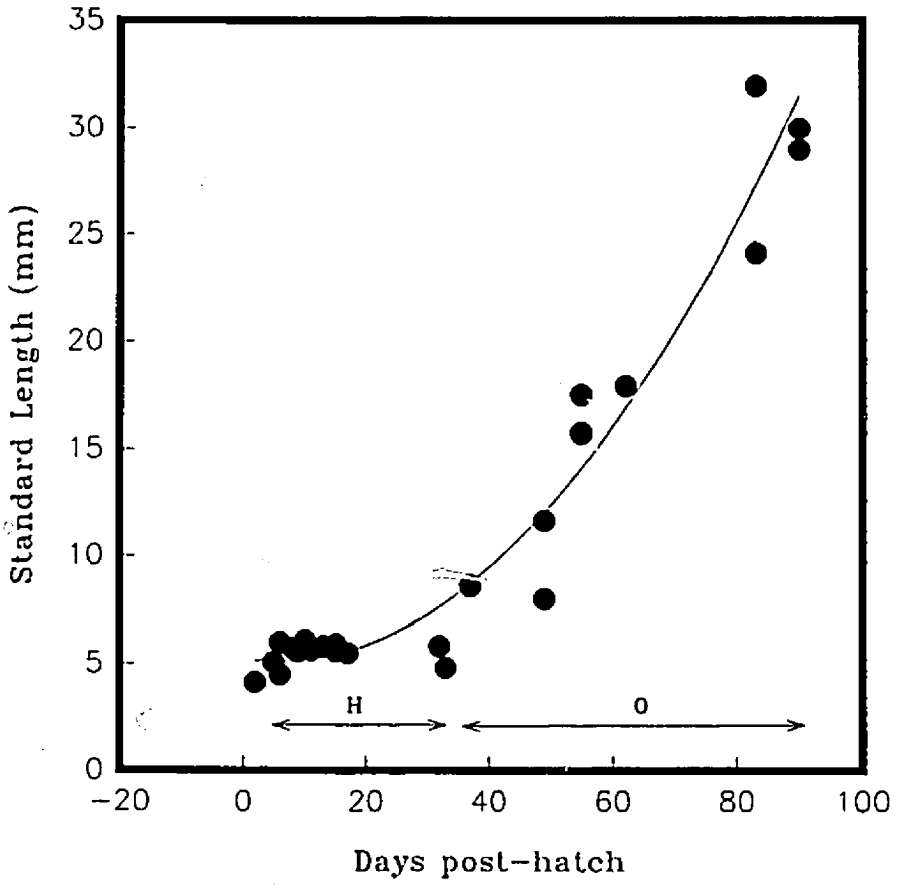

Standard Length (mm)
35
30
25
20
15
10
5
0
−20
0
20
40
60
80
100
Days post−hatch
H
O

Figure 3.8

Figure 3.9. The relationship between the ratio of head depth:head length vs standard length and days post-hatch (a–b), and between the quadratal angle vs standard length and days post-hatch (c–d), in cod larvae and pelagic juveniles. Symbols represent one individual.

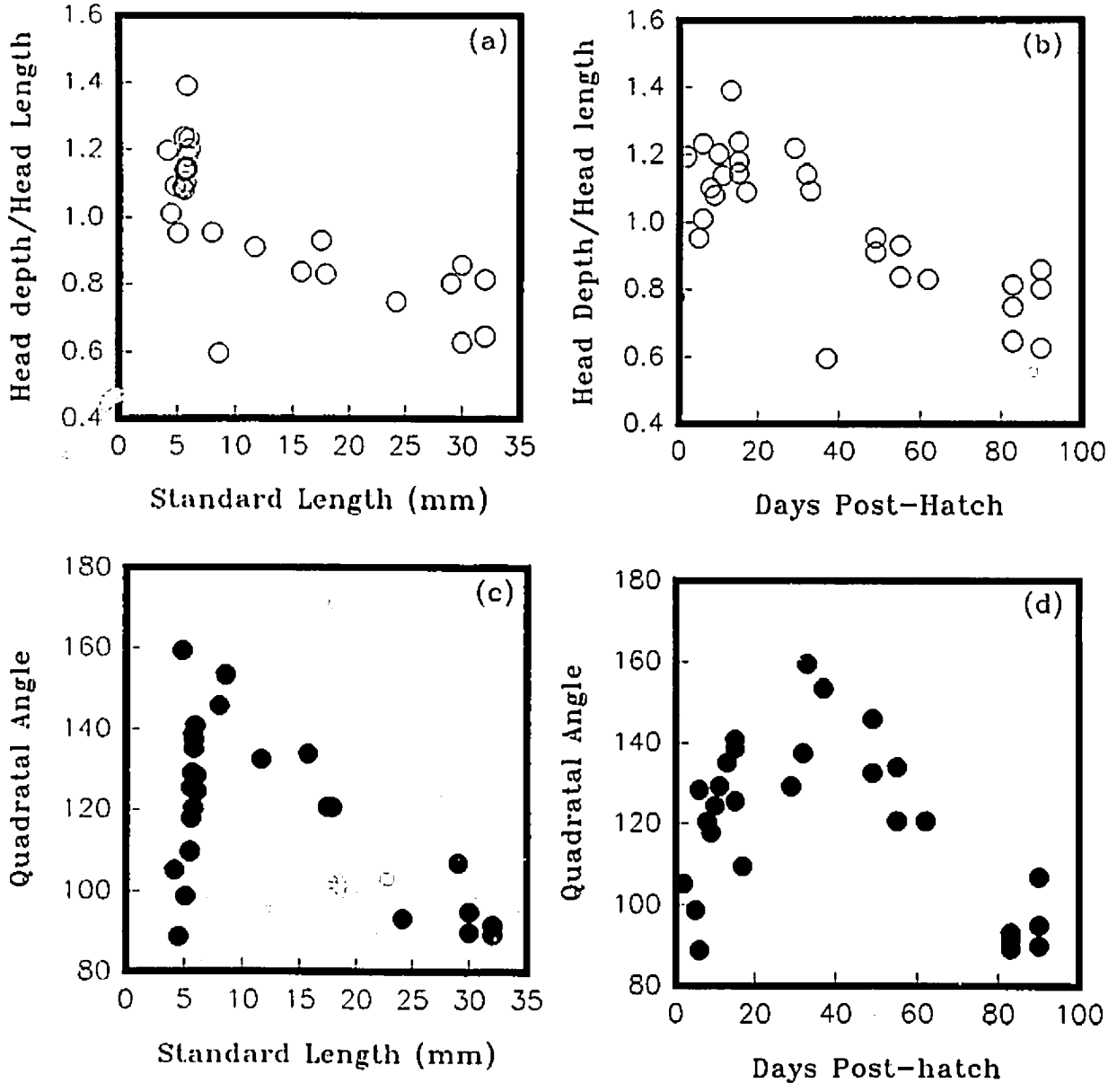

(a)
Head depth/Head Length
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0 5 10 15 20 25 30 35
Standard Length (mm)
(b)
Head Depth/Head length
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0 20 40 60 80 100
Days Post-Hatch
(c)
Quadratal Angle
180
160
140
120
100
80
0 5 10 15 20 25 30 35
Standard Length (mm)
(d)
Quadratal Angle
180
160
140
120
100
80
0 20 40 60 80 100
Days Post-hatch

Figure 3.9

individuals were pelagic juveniles (>80 days post-hatch and 29 mm). These individuals were characterized by small quadratal angles and low head depth:head length ratios. The ratios of jaw length:head length and eye diameter:head length showed no size dependent or age dependent relationships, but remained relatively constant through both the larval and pelagic juvenile periods (Figures 3.10 a,b).

DISCUSSION

Feeding and buccal respiration are intimately linked in the early life history stage of Atlantic cod. Mouth opening mechanisms change throughout ontogeny in concert with the development of new cranial structures and musculoskeletal linkages. In cod larvae, the hyoid coupling serves as the major musculoskeletal linkage for opening the mouth during most of the larval life. With growth and differentiation of new structures, a second musculoskeletal linkage, the levator-operculi coupling, supplements the first linkage. This second linkage becomes the major mechanism responsible for opening the mouth in late stage larvae, juveniles and adults.

This study is one of only a few which investigates the functional morphology of feeding and respiration in fish larvae. To determine whether the general patterns of jaw movements in cod are similar to jaw movements of larval stages in other species, it is necessary to compare mechanisms of mouth opening across species. One might expect that because of the relatively homogenous environment of the water column, interspecific differences in fish larval mechanisms for feeding and respiration may not occur. However, interspecific differences may result from historical (heritable) factors that are

Figure 3.10. Ratio jaw length:head length vs days post-hatch (a) and eye diameter:head length vs days post-hatch (b), in cod larvae and pelagic juveniles. Symbols represent one individual.

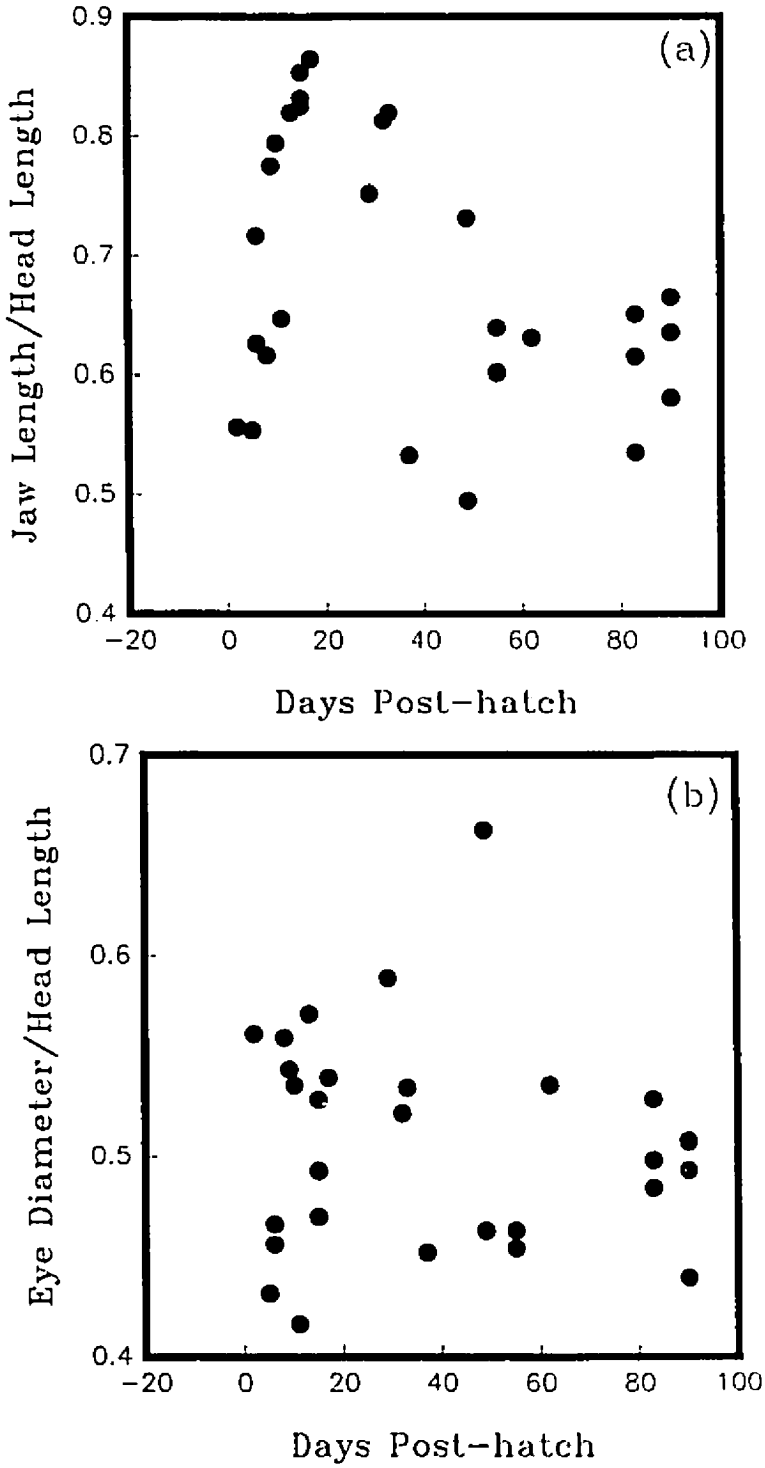

(a)
Jaw Length/Head Length
0.9
0.8
0.7
0.6
0.5
0.4
−20
0
20
40
60
80
100
Days Post−hatch
(b)
Eye Diameter/Head Length
0.7
0.6
0.5
0.4
−20
0
20
40
60
80
100
Days Post−hatch

Figure 3.10

species-dependent.

Mouth opening mechanisms of early larval life.

The 'hyoid period' describes the primary mechanism that is responsible for mouth opening in the early part of larval life of four species of fish : a gadid (Gadus morhua), salmonid (Onchorhynchus mykiss), cichlid (Haplochromis elegans) and an embiotocid (Micrometrus minimus) (Figure 1, Verraes 1977, Otten 1982, and Liem 1991 respectively). In addition, opercular structures are either small or nonexistent as in cod and are non-integrated in the hyoid stage of all of the species above. Although retraction of the hyoid apparatus causes expansion of the buccal cavity, the specific musculoskeletal linkages, that are responsible for mouth opening differ among species.

In cod (Figures 3.4) and rainbow trout larvae (Verraes 1977), the hyoid is retracted and the mouth is opened by the contraction of the sternohyoideus and the depression of the mandible through the mandibulohyoid ligament. This results in the synchronous movement of the jaw and hyoid (Figure 3.4 a & b). In cichlid and embiotocid species, the mandibulohyoid ligament is absent from all stages of the life history (Otten 1982, Liem 1991). Thus, larvae begin life with the jaw joint positioned such that the working line (i.e. the line over which the force is transmitted) of the geniohyoideus is ventral to the jaw joint (see Figure 7, Otten 1982). Through contraction of the sternohyoideus and the geniohyoideus, the hyoid is retracted synchronously with the lower jaw. Such a mouth-opening mechanism is not possible in cod, as the working line of the geniohyoideus lies dorsal to the jaw joint in all stages of the larval life, and the mandible

cannot initially be depressed by contraction of the geniohyoideus. Thus, similar synchronous movements of the hyoid and lower jaw occur in all species studied, but the mechanisms that produce the movements differ among species.

Mouth opening mechanisms of the late larval and juvenile life history stage.

As ontogeny progresses, new structures develop and become integrated or 'coupled' by differentiation of muscles and ligaments. In older larvae and in juveniles, the musculoskeletal coupling responsible for mouth opening is the levator–operculi coupling. However, in contrast to the 'hyoid period', the coupling that produces jaw movement during later stages of larval life is similar in all species studied to date. The key differences among species occur in the timing of the formation of the necessary skeletal elements, muscles and ligaments that constitute the coupling.

The opercular apparatus and the interoperculomandibulo ligament form late in both the larval life of cod (45–50 days post–hatch; stage 10, Ch. 1) and rainbow trout (juvenile stage; stage 97, Verraes 1977). However, contractions of the levator–operculi muscle may be transmitted to the lower jaw through connective tissue of the opercular epithelium, which is well formed by the middle of the larval period in both cod (see Ch. 2) and trout (Verraes 1977). Verraes (1977) has suggested that it is these early contractions that facilitate the formation of the opercular series in the same order in all fish larvae studied, the first bone (the operculum) being nearest the muscle.

In cichlids and embiotocids the operculi coupling develops much earlier than in cod and salmonids. The hyoid period constitutes only 6% of the larval period in cichlids and

only one day elapses between the hyoid and opercular periods (Otten 1982). Due to skeletal growth, changes in the proportions of the head drive the transition from the hyoid to opercular coupling as the opercular apparatus is complete soon after hatching. Rapid skeletal growth causes the jaw joint to be displaced rostrally, shifting the working line of the geniohyoideus dorsal to the jaw joint. Consequently, the function of the geniohyoideus is switched from mandibular depression to mandibular adduction (Otten 1982). At the same time, growth is coordinated with the development of the levator operculi coupling which substitutes for the geniohyoideus to open the mouth. Thus, in all species studied the levator–operculi mechanism is conserved and the asynchronous movement patterns of the jaw and hyoid observed in both cichlids and cod larvae are produced by the same musculoskeletal linkage.

The transition between musculoskeletal couplings in all larval species studied, coincides with sharp changes in growth–dependent components such as head proportion and the quadratal angle. The shift from hyoid to opercular coupling occurs simultaneously with a change in head proportions (e.g. head depth:head length or quadratal angle). In cod this transition occurs just after the head depth begins to decrease, and after the quadratal angle reaches a peak (Figures 3.8 a–d). These changes also coincide with an increase in growth rate which occurs at 9–10 mm, or 35–40 days post–hatch (Figure 3.7). In rainbow trout, the transition to the opercular period also occurs after similar, though smaller increases in quadratal angle (Otten 1982).

In cichlids, embiotocids and pomacentrids, the transition between hyoid stage and opercular stage also follows sharp changes in the head proportions and quadratal angle

(Otten 1982, Liem 1991). In all cases, head proportions (head depth:head length) increase with growth. In addition, an increase in head depth is accompanied by a change of buccopharyngeal cavity shape, which shifts from a cylindrical to a truncated cone design (Dilling 1989, Liem 1991). A cylindrical buccopharyngeal cavity with a shallow suspensory apparatus and wide gape is conducive to ram feeding, whereas a truncated cone – shaped cavity with a deeper suspensory apparatus and small gape is conducive to suction feeding (Brainerd 1985). Therefore, shifts in mouth opening mechanisms, head proportions and quadratal angles co-occur and facilitate shifts in feeding strategy. Corresponding shifts in feeding strategies may also occur in cod. However, because of the decreasing head depth with growth, feeding strategies would be expected to move in the opposite direction, i.e. from suction feeding to ram feeding. Juvenile and adult cod display a combined ram/suction feeding behaviour (Muller and Osse 1984). Therefore, changes in growth rate, head proportions, and quadratal angles occur in concert, albeit in different directions, with changes in feeding strategy in all larval fish studied. Such preprogrammed and coordinated functional shifts in larval ontogeny may be essential to meet changes in larval fish survival needs.

Why did the second musculoskeletal linkage develop?

Emerging structures often appear in specific sequences thereby establishing the functions critical to fish larval survival. For example in cichlids, the shift of the quadratal angle through growth renders the geniohyoideus ineffective as a mandibular depressor. Without the development of the second musculoskeletal linkage, larvae would be unable

to feed or respire (opercular movements also provide for gill irrigation) and would either starve or asphyxiate (Liem 1991). In contrast, the hyoid coupling serves feeding needs for over 70% of the larval life in cod and 100% of the larval life in trout (Verraes 1977). Respiratory needs for most of the larval life are largely met by cutaneous respiration (see Ch. 2, Rombough 1988 and Osse 1991). In addition, when opercular coupling develops, the hyoid coupling continues to function, providing most of the buccal cavity expansion. Therefore, in cod and rainbow trout, the opercular mechanism may develop because : (1) high velocity unidirectional flow generated by buccal/opercular pumps is needed to facilitate the transition from cutaneous to branchial respiration, (2) increases in larval size may make hydrodynamic conditions more favourable for opercular coupling; a shift of mechanisms occurs at 9–10 mm, when larger size and swimming speed lead to higher Reynolds numbers (Webb and Weihs 1986) and (3) development of the opercular apparatus provides an efficient seal so that large negative pressures can be potentially generated in the buccal cavity. Such increases in suction pressure lead to an increase in feeding efficiency, as well as facilitating buccal – opercular ventilation. Therefore, although the onset of the second musculoskeletal linkage is delayed in cod and rainbow trout compared to cichlids and pomacentrids, its appearance is finely tuned to the requirements for survival and may constitute a critical stage for all fish larvae.

Phylogeny and the development of function

Differences exist in mouth – opening mechanisms between species that cannot be explained as an adaptational response to the environment, but perhaps can only be

explained in the context of different phylogenetic origins. Cichlids, pomacentrids and embiotocids are phylogenetically closely related (Liem 1991). Their larval stages undergo similar changes in mouth opening mechanisms and feeding strategies that are important for survival through ontogeny. Alternative to the primary mouth opening mechanism (i.e. the contraction of the geniohyoideus), development of a mandibulohyoid ligament in cod and salmonid larvae, does not occur in the cichlid, pomacentrid and embiotocid species discussed here (Otten 1982). Though a connective tissue which resembles the mandibulohyoid ligament, was observed between the rostral part of the dentary and the hyoid in the cichlid Astatotilapia elegans (Aerts et al. 1987), no mention of its function as part of an alternative mouth opening mechanism for cichlids has been suggested for adults or larvae. Therefore, despite the structural and functional similarities of larval mouth opening mechanisms, species specific differences exist in key structural points or 'hot spots' (see Otten 1983). These reflect adult differences in feeding modes which are already expressed at the larval stage.

Otten's (1983) 'hot spots' model has not been utilized in my comparison of functional design between cod and salmonid larvae, since most of the structures important to the model are not present until late in the larval life in cod. While cod and salmonids are phylogenetically not closely related (Lauder and Liem 1983), both develop a mandibulohyoid ligament by virtue of a longer lower jaw which reduces the distance between the hyoid and mandible (Verraes 1977). Both species show progression from the simple hyoid coupling to the more complex opercular coupling as structural and functional complexity in the head increases with the functional demands of growth,

whereas cichlids, pomacentrids and embiotocids have taken an entirely different pathway to realize the same opercular coupling.

Summary. Similarities in mouth opening mechanisms exist among diverse teleost species in the larval period. These mechanisms produce the same preprogrammed changes necessary to survive in similar environments. However, variation in larval strategies to environmental challenges appear to reflect the phylogenetic differences characteristic of adults. Such differences may provide sufficient interspecific variation between larval fishes, to create species-specific feeding niches within an environment perceived to be homogeneous. Such variation could then be acted upon by selection pressures, thought to be intense in the larval stage (Balon 1985), and in turn influence adult functional design.

Chapter 4

ACTIVITY METABOLISM IN LARVAL ATLANTIC COD: AN INTER - POPULATION COMPARISON.

INTRODUCTION

Activity is thought to be one of the most important factors affecting energy turnover in fishes, as activity metabolism can become the major component of total metabolism at any stage of the life history. The relationship between activity and oxygen consumption has been extensively studied in adults and juveniles (e.g. Fry 1947, Saunders 1963, Brett 1964 1970, Beamish 1978, Fry 1971, Brett and Groves 1979). Only recently have studies considered the importance of metabolic costs of activity in early stages undergoing rapid growth and development (Hunter 1972, Hunter and Kimbrell 1980, Wieser et al. 1985, Wieser and Forstner 1986, Dabrowski 1986, Kauffman 1990). However, detailed study of the energetics of swimming in fish larvae has been plagued with methodological problems related to the small size of the animals. In larger, older stages (juveniles and adults), the metabolic cost of swimming has been determined by forcing the fish to swim against currents in a flow-tunnel respirometer while measuring the relationship between swimming speed and oxygen uptake (Fry 1947, Fry 1971). Early-stage larval fish do not readily swim against currents (Rombough 1988a). For larvae and small juveniles the relationship between oxygen consumption and swimming speed has been investigated using two methods. In the first, larval fish were made to

swim at progressively faster speeds in a circular respirometer making use of their optomotor response to a moving background (Dabrowski 1986). In these conditions fish were induced to swim against small currents in a modified flow-tunnel respirometer, tailored to meet the limited endurance of fish larvae (Kauffman 1990). In both of these studies, freshwater species were used, and the larvae tested ranged from 2-500 mg (Kauffman 1990) and 20-45 mg (Dabrowski 1986). Pelagic larvae of marine fishes are typically much smaller than freshwater larvae (e.g. for Gadus morhua, a yolk-sac larva weighs less than 1mg wet weight (Laurence 1978)). In addition, some marine fish larvae (e.g. cod larvae) do not readily display a optomotor response until a few days after hatch (Budgey 1992) and cannot swim constantly against even small currents until they grow larger. Therefore, in order to determine the metabolic cost of swimming in small larvae, techniques and procedures building on past methodologies need to be developed.

The necessity for specialized techniques to determine the energetics of locomotion in fish larvae emphasizes the differences between larvae and older stages of fish. Unlike juvenile and adult fish, larvae undergo dramatic changes in morphology, physiology, and growth (Cohen 1984, Blaxter 1988, Rombough 1988a). As fish larvae grow, they experience changes in their hydrodynamic environment, moving from a viscous environment to one dominated by inertial forces (Webb and Weihs, 1986) and larval swimming patterns change from continuous tail-beat swimming to 'beat and glide' swimming (Hunter 1972, 1981, Weihs 1980, Batty 1984). In addition, decreases in surface area-to-volume ratios and growth of gills shift the method of oxygen uptake from cutaneous to branchial respiration. Despite these extensive developmental changes, only

a few studies have investigated the importance of activity metabolism to changing larval requirements for locomotion (Wieser et al. 1988, Wieser et al. 1990). Therefore, further studies are necessary to: (1) determine the proportion of total metabolism devoted to activity and (2) to estimate the cost of transport at different developmental stages of small fish larvae. Such studies are important, as there is little if any information about the relative importance of activity to the total energy budget of these small fish larvae.

Swimming speed, growth rate and metabolic rate of fish larvae vary with temperature (Batty and Blaxter 1992, Laurence 1978, Rombough 1988a). However, it is unclear how temperature affects the quantity of energy available for activities such as foraging and predator avoidance. Previous work revealed that the effects of temperature on energy devoted to swimming varied with species; in some cases energy available for activity increased with increasing temperature (e.g. salmonid larvae; Gruber and Wieser 1983), and in others, activity metabolism was independent of temperature change (e.g. in cyprinids; Wieser and Forstner 1986). The effect of temperature change on the proportion of energy available for activity is important, as it may represent a fitness index in which variation could indicate differential environmental adaptation between populations of the same species. For example cod populations along the east coast of Canada may differ in their activity metabolism as Newfoundland cod larvae are spawned in much colder water (0 – 5°C) (Lear and Green 1984) than Scotian Shelf larvae (8–10°C) (Griffin and Lochmann 1993). These populations may differ in their thermal optima. The presence of antifreeze proteins in juvenile and adult Newfoundland cod and their absence in Scotian Shelf cod (Fletcher 1993), suggests that Scotian cod could be 'warm-adapted'

while cod from Newfoundland are 'cold-adapted'.

To date, only a few studies have examined patterns of activity in larval Atlantic cod, Gadus morhua (Ellertsen et al. 1980, Solberg and Tilseth 1984, Skiftesvik and Huse 1987, Yin and Blaxter 1987, Skiftesvik 1992). Generally, activity increased in exogenously feeding larvae only after yolk-sac absorption and reached high levels in starving larvae (Skiftesvik 1992). However, metabolic costs of activity were not measured. The objectives of my study are to: (1) determine activity and metabolic patterns during cod larval development, (2) determine the proportion of total metabolism devoted to activity, (3) determine how temperature affects the cost of activity, and (4) determine whether intraspecific differences in activity metabolism occur between two geographically and genetically separate stocks of Atlantic cod.

METHODS AND MATERIALS

Animals

Fertilized Atlantic cod eggs were obtained from two populations considered to be genetically distinct (Pogson et al 1993): Newfoundland central (NF) (adults are held at Memorial University, St.John's, Newfoundland) and Scotian Shelf stock (NS) (adults are held at Dalhousie University, Halifax, Nova Scotia). Eggs and larvae of both populations were raised separately at two controlled temperatures of 5 ± 0.5 and 10 ± 0.5°C in static 80 l glass aquarium. High concentrations of the green alga Isochrysis sp. were added to each aquaria and 'green' cultures were maintained throughout the experimental period.

Rotifers and wild caught zooplankton, sieved to appropriate sizes, were always maintained at densities of >20 prey/ml to ensure good feeding conditions for all larvae. Subsamples of larvae (n = 32–40) were taken from each population every day and for the first ten days after hatching. As the total numbers of larvae declined, daily subsamples were reduced to n = 16–20 larvae. Samples were taken simultaneously with those of Chapter 1 and are comparable with respect to developmental stage (see Chapter 2 for more detailed explanation of culture methods).

Respirometry

Figure 4.1 illustrates the electrode array used to measure metabolic rates. Measurements of oxygen consumption were made using four pulsed O_2 – electrodes (Endeco Inc.) housed in 250 μl Plexiglas chambers. Each electrode was linked to one of four cylindrical glass chambers, 30 mm long x 0.8 mm internal diameter (Cyclobios Inc.). Oxygen saturated seawater (31 ‰) was circulated via stainless steel tubes through the experimental chambers and across the electrodes by a peristaltic pump. Seawater was circulated for 1 – 2 hrs from a reservoir until the electrodes stabilized to within ± 0.1 ppm between consecutive readings. Two to four cod larvae (depending on larval stage, decreasing numbers with growth) were inserted into each of the four experimental chambers and the system closed. After allowing a 20 minute acclimation period for the larvae to recover from any stress related to transport into the chambers (Hunt von Herbing, unpublished data), oxygen concentrations were recorded every 4 minutes for one hour. Four minutes allowed the electrode to stabilize between readings and produced the

Figure 4.1. Diagram of electrode array and respirometers.

E= pulsed electrode, C=experimental chamber, PP=peristaltic pump,

Rv=resevoir

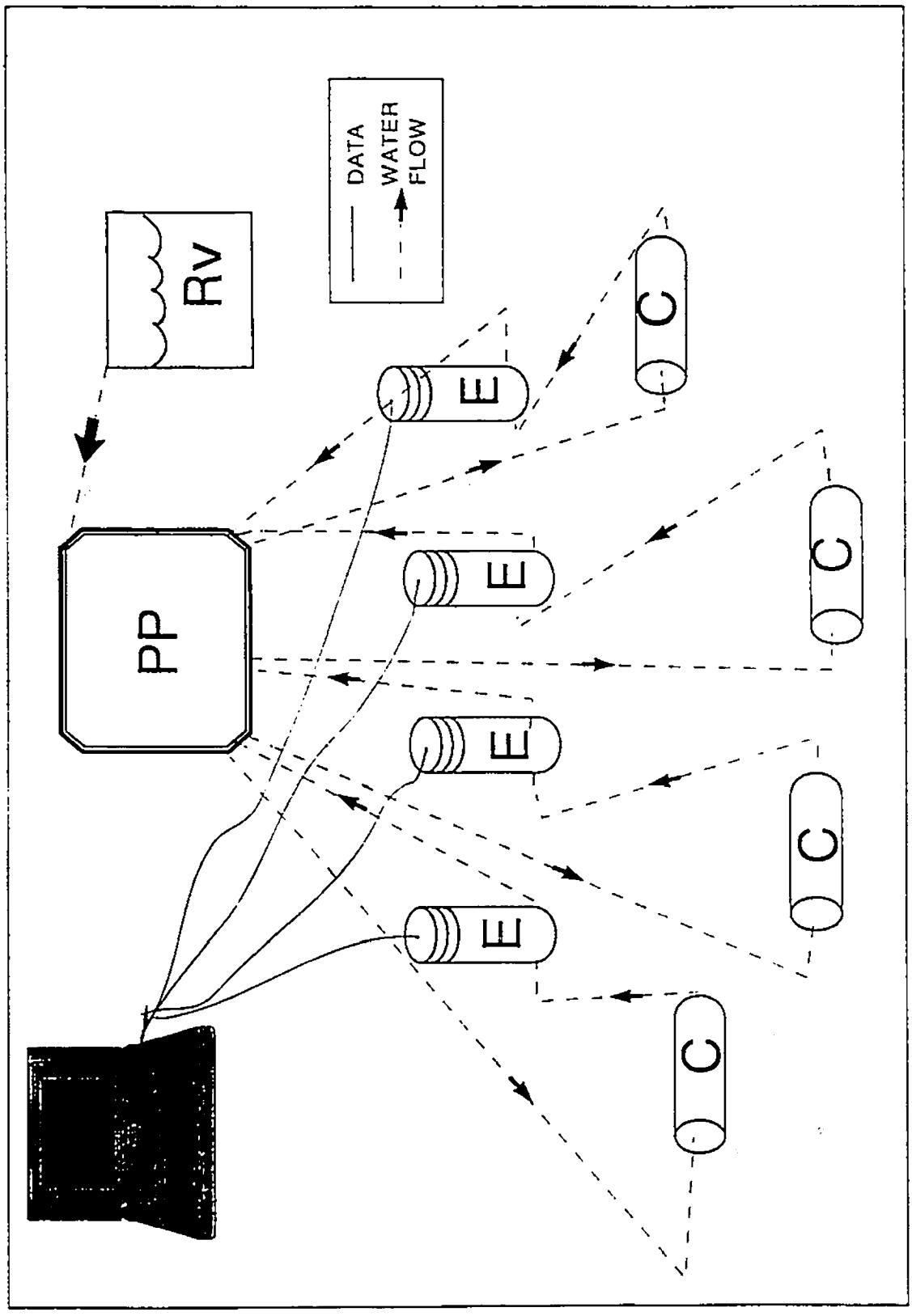


Figure 4.1

most reliable readings at the flow rate used (0.25 ml/min). Higher flow rates would have produced more stable electrode readings, but would have been harmful to the larvae in the chambers. Oxygen concentrations were recorded on a Tandy laptop computer. Oxygen consumption (mg O_2/hr) was calculated from regressions of oxygen concentration over time for each electrode. All experiments were conducted at 10°C in a constant temperature cold room.

Blanks were run for each chamber and electrode after each experimental run and the values obtained were subtracted from those values during the experiment. In addition, nitrogen equilibrated seawater was pumped through the system every week to check that no environmental oxygen was leaking into the system. For calculation of specific oxygen uptake, larvae were removed from the chambers, viewed for developmental stage after each experimental run, rinsed in distilled water and then dried for 24–48 hr in an oven (60–80°C), to a constant weight. They were then weighed to the nearest 0.01 mg on a Cahn Gram electrobalance (precision ± 0.1μg). Yolk-sacs were dissected from early larvae, and the body proper and yolk-sac were weighted separately.

Experiments were conducted under the following thermal conditions for both populations: (1) larvae raised at 5°C were tested at 10°C (R5T10) and (2) larvae raised at 10°C were tested at 10°C (R10T10).

Activity

The movements of each larva in each experimental chamber were recorded by a Sony HI-8 video camera. Each recording was made concurrent with a respirometric run.

Activity recordings were made for each larva for a total of 20 minutes, three times throughout each experiment. HI-8 tape was transferred to VHS format and the activity of each larva was analyzed using the Peak Performance Image Analysis Program (Peak Performance Inc.). Swimming speed was determined through frame-by-frame analysis of the distance each larva moved over time. The center of the larval eye was selected as an easily-determined and reliable point of reference. Only positive movements (i.e. movements of the larva against the water current) were used in the calculations of swimming speed. The Peak Performance system enabled activity to be resolved into 3 components: (1) % time active (2) mean swimming speed (mm/s) and (3) maximal swimming speed (mm/s) (the highest swimming speed recorded within a 60 second period) and was seen during distinct pulses of activity. Swimming speed was measured as the change in position (i.e. distance moved) of a larva during a 1 second interval. Mean swimming speed was calculated was calculated as the average swimming speed over 60 seconds. Figure 4.2 shows a representative example of one activity spectrum for one larva. Locomotion in young larval cod (at low Reynolds numbers) is characterized by intermittent pulses of continuous tail-beat swimming. Each pulse lasted only a few seconds.

All numeric data were subjected to normal probability plots and Bartlett's Test for homogeneity of variance (SYSTAT, Wilkinson 1990). Student-t tests were used to determine significant differences of mean swimming speeds, maximal swimming speeds and mass specific metabolic rates between stages and among populations. T-tests between regression slopes (Zar 1984) were used to test for significance of metabolic costs

Figure 4.2. Example of one activity spectrum for one cod larva showing average and maximal swimming speeds.

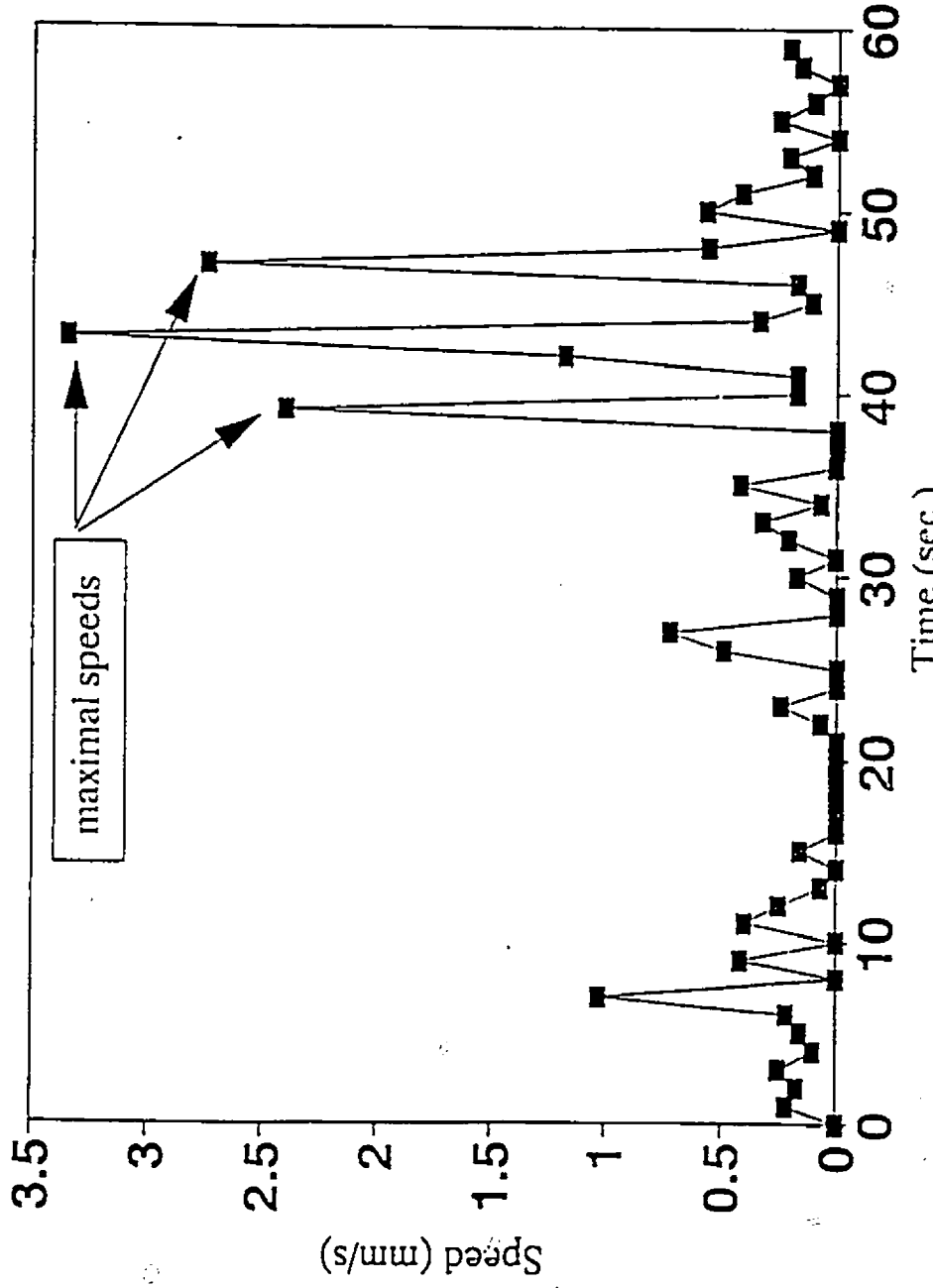

maximal speeds
Speed (mm/s)
Time (sec.)
0
0.5
1
1.5
2
2.5
3
3.5
0
10
20
30
40
50
60

Figure 4.2

of swimming between populations.

RESULTS

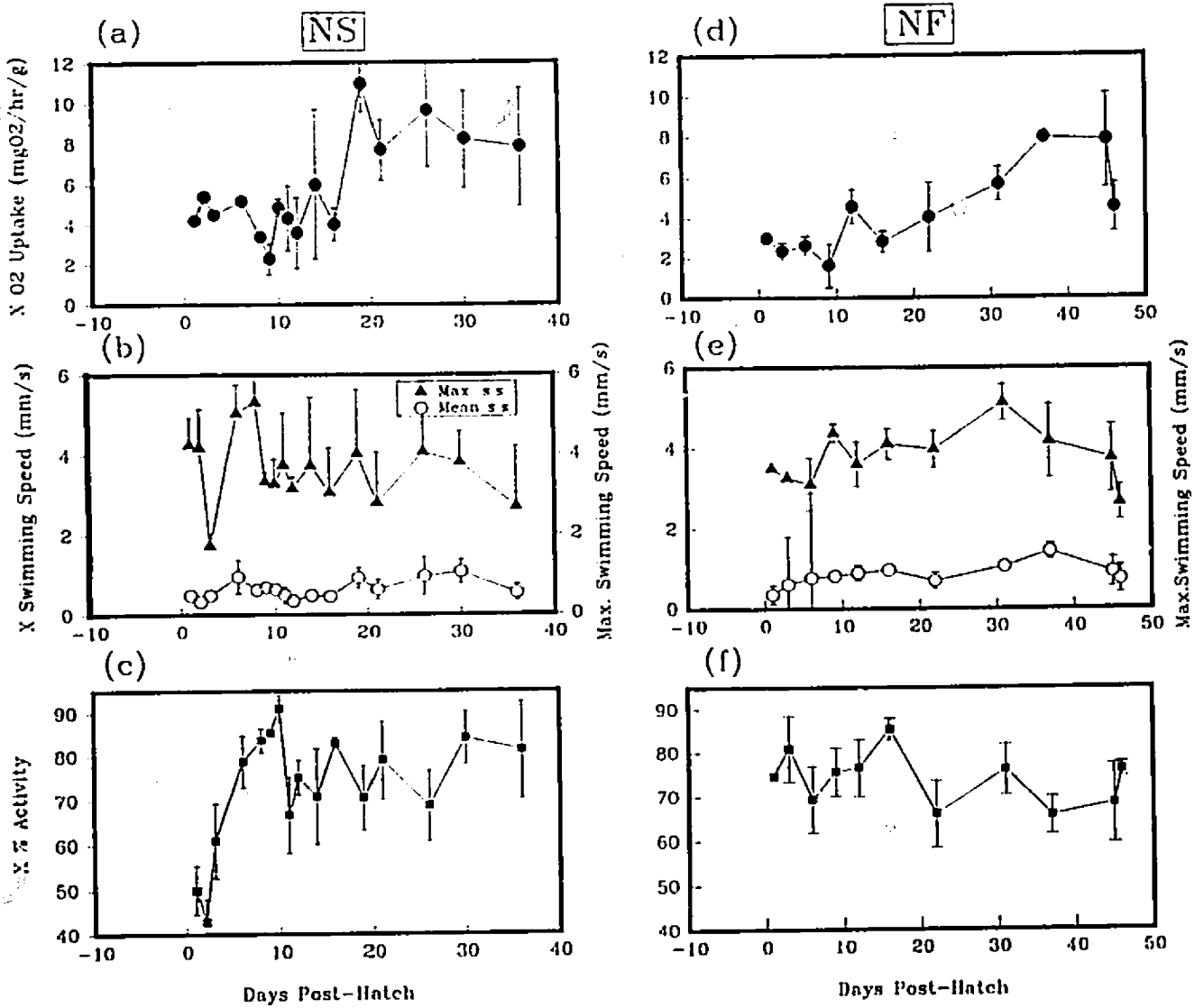
Patterns of activity and metabolism throughout ontogeny.

Within–population comparisons

The patterns of activity and metabolism are similar for both Newfoundland (NF) and Scotian Shelf (NS) populations, raised at 5°C and tested at 10°C (Figure 4.3a–f).

For the Scotian Shelf population, percent of time spent active was low (40–50%) in the first 3 days of life (Figure 4.3a) but increased with the onset of first–feeding, reaching over 90% by 10 days post–hatch. Thereafter, percent activity declined, stabilizing at 70–75% activity for the remainder of the exogenous feeding period (Figure 4.3a). Mean swimming speed of the NS larvae was significantly lower for the first few days after hatching (0.49 ± 0.043 mm/s) than after yolk–sac resorption (0.87 ± 0.12 mm/s) (Figure 4.3b; t–test, $t = 3.2$, $p < 0.005$). Swimming of cod larvae in the first few days of the yolk–sac stage was characterized by short periods of continuous tail–beat swimming. In contrast, swimming during later larval periods (at larger body sizes and larger Reynolds numbers) was characterized by a beat–and–glide swimming mode. However, the maximal swimming speeds attained in yolk–sac stage were not significantly different from those in later larval stages (t–test, $t = 4.05$, $p > 0.05$; Figure 4.3b). Mass specific metabolic rates reflected the stage differences in swimming speed and percent activity, and were significantly higher in the later larval stages than the yolk–sac stage (t–test; t

Figure 4.3. Patterns of mean oxygen uptake (mgO2/hr/gdwt), mean swimming speed (mm/s), and mean percent activity in cod larvae from two populations Scotian Shelf (NS) (a–c) and Newfoundland (NF) (d–f) vs. days post–hatch. Corresponding changes in activity, feeding and development are also shown. Error bars represent ± 1 s.e.



Activity : Burst → Beat & Glide
Feeding:: Endo→Trans.Exog.
Stage : yslarva → larva

Activity : Burst → Beat & Glide
Feeding : Endo→Trans Exog.
Stage : yslarva → larva

Figure 4.3

= 3.94, $p < 0.005$; Figure 4.3c).

In the NF population, percent activity was maintained at 60–80% throughout the experiment (Figure 4.3d). Mean swimming speed was low immediately after hatching, but increased following the onset of first feeding. However, mean swimming speed was not significantly different between the later larval stage and the yolk–sac stage (t–test; $t = 1.44$, $p > 0.05$; Figure 4.3e). Moreover, no differences in maximal swimming speeds were found between the yolk–sac and larval stages (t–test; $t = -8.64$, $p > 0.05$; Figure 4.3e). In contrast, mean metabolic rates were significantly higher in the larval stage than in the yolk–sac stage (t–test; $t = 3.38$, $p < 0.005$; Figure 4.3f).

Between–population comparisons

Mean swimming speed of the NF population was significantly higher than that of the NS population in the yolk–sac stage (t–test; $t = 3.26$, $p < 0.005$), but was not significantly different in the later larval stage (t–test; $t = 8.46$, $p > 0.05$). In addition, no significant differences occurred in maximal swimming speed between the two populations at the yolk–sac or later larval stages (t–test; $t = 5.61$ and $t = 1.37$, $p > 0.05$ for both cases; yolk–sac and later larval stages respectively). In contrast, mean metabolic rates were significantly higher in the NS than the NF population in both yolk–sac and later larval stages (t–test; $t = 3.78$ and $t = 2.51$, $p < 0.001$ for both cases; yolk–sac and later larval stage respectively).

Given that the NF population had higher growth rates than the NS population (see Ch. 1), the above comparisons were also carried out using length – specific swimming speeds

(i.e. swimming speed/body length). However, repeating the above analyses using specific swimming speed yielded identical patterns of variation. For the NS population, mean specific swimming speed was still significantly higher in the later larval stage than in the yolk-sac stage (t-test; $t = 2.8$, $p<0.01$). For the NF population, no differences existed in mean specific swimming speed between the two stages (t-test; $t = 8.13$, $p>0.05$). Between-population comparisons showed a significantly higher mean specific swimming speed at the yolk-sac stage for the NF population (t-test; $t = 2.68$, $p < 0.001$), but no difference between the later larval stages (t-test; $t = 3.66$, $p>0.05$). Maximal speed in body lengths was not significantly different between populations (0.5 bl/s for both NF and NS; t-test; $t = 1.72$, $p>0.05$).

Activity metabolism

To approximate the metabolic cost of swimming over the first 40 days of cod larval life (approximately 75% of the total larval life), specific metabolic rate was regressed against swimming speed (the mean activity of all larvae within one experimental chamber). A first order regression proved to be the best fit for the data from each population. Figures 4.4a – b showed that specific metabolic rates regressed significantly against swimming speed for both the NS and NF populations, and showed that 25-35% of the variance in specific oxygen uptake was due to swimming speed (for NS, $r^2 = 0.36$, $p < 0.05$; for NF, $r^2 = 0.26$, $p < 0.05$). The slope or metabolic cost of swimming for the NS population was almost twice that of the NF population (t-test between slopes; $t=3.5$, $p<0.05$; Figure 4.4 a,b).

Figure 4.4. Mass specific oxygen uptake ($mgO_2/hr/gdwt$) vs swimming speed (mm/s) are shown for cod larvae form the Scotian Shelf (NS) (a) and Newfoundland (NF) (b). Symbols represent individual larva.

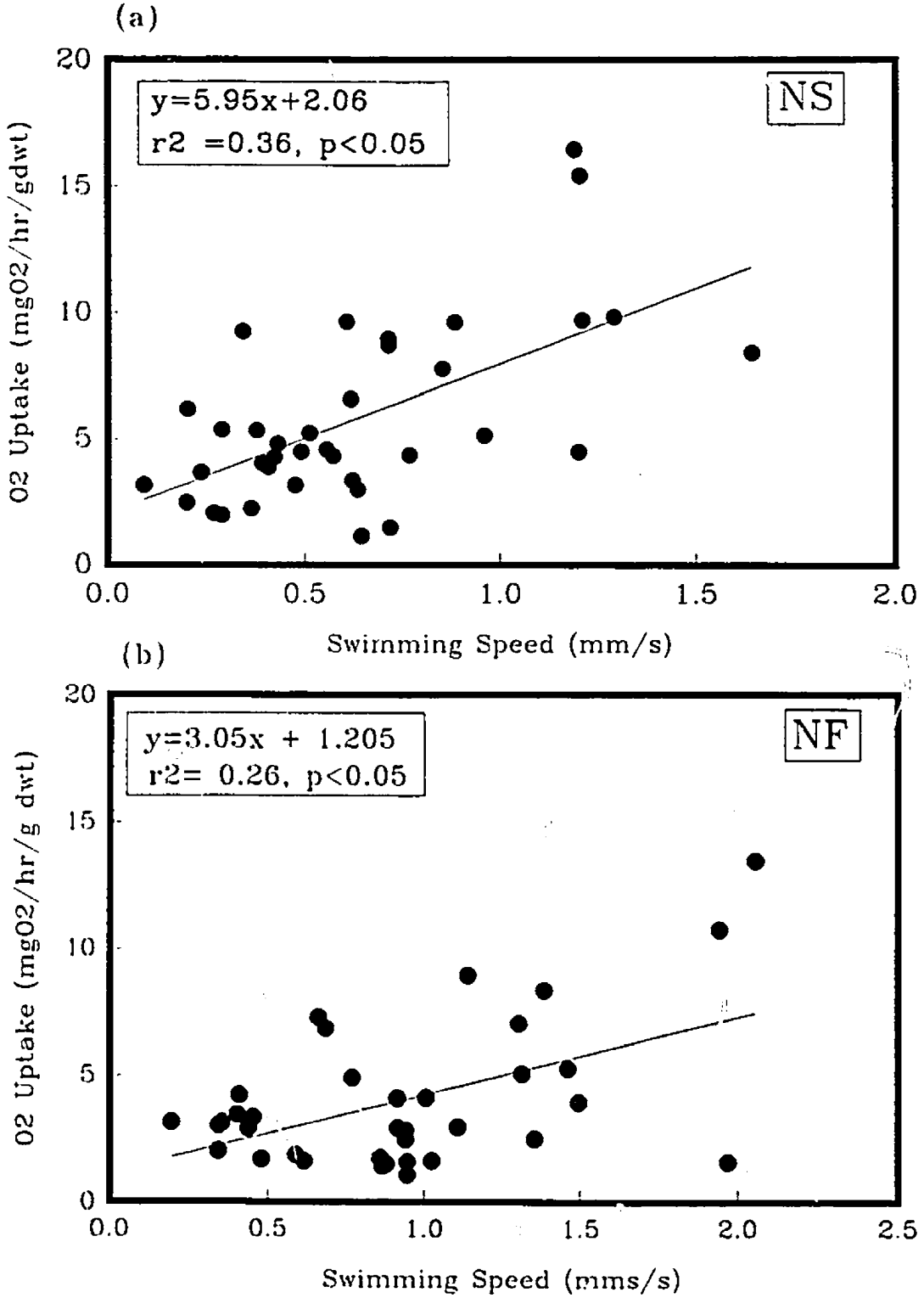

(a)
y=5.95x+2.06
r2 =0.36, p<0.05
NS
02 Uptake (mg02/hr/gdwt)
Swimming Speed (mm/s)
(b)
y=3.05x + 1.205
r2= 0.26, p<0.05
NF
02 Uptake (mg02/hr/g dwt)
Swimming Speed (mms/s)

Figure 4.4

Although these data do not represent individual fish larvae swimming against proportionately faster current speeds (which would be necessary to determine true aerobic scope), it does represent an index of the cost of activity. Similarly, the y-intercept represents an index of the standard or basal metabolic rate. To determine the oxycaloric equivalent of the cost of swimming, specific metabolic rate was converted to J/mg/hr by multiplying by the factor 13.96 (the average energy released in Joules from: protein (13J), fat (14J) and carbohydrates (15J), (Elliot and Davison 1975)) (Figure 4.5). Therefore, the difference between the energetic cost at maximal activity (derived from mean maximal speeds obtained for both populations, i.e. 3.4 mm/s for NS and 3.9 mm/s for NF and the relationship between mass specific oxygen uptake and swimming speed for each population) and energetic cost at zero activity represents the total (aerobic + anaerobic) scope for activity (Figure 4.5). The energetic cost of activity between the populations are different (Figure 4.5): (1) NS larvae have a higher maximal oxygen consumption (VO_2 max) than NF larvae, (2) NS larvae have a higher total scope for activity than NF larvae, (3) NS larvae are apparently less efficient swimmers than NF larvae since they have to expend more energy at all levels of activity.

Activity metabolism at different ontogenetic stages

To determine if the cost of locomotion is similar throughout ontogeny, the early life history period was divided into three basic periods based on feeding and swimming behaviour and morphology (see ch. 1-3).

Figure 4.5. The energetics of activity for two populations of cod Scotian Shelf (NS) and Newfoundland(NF), showing both the relationship of mass specific oxygen uptake (mgO2/hr/gdwt)and energetic cost vs swimming speed. NS =Scotian Shelf larvae, NF = Newfoundland larvae. VO_2 max = maximal mass specific oxygen uptake, Max. s.s = maximal swimming speed.

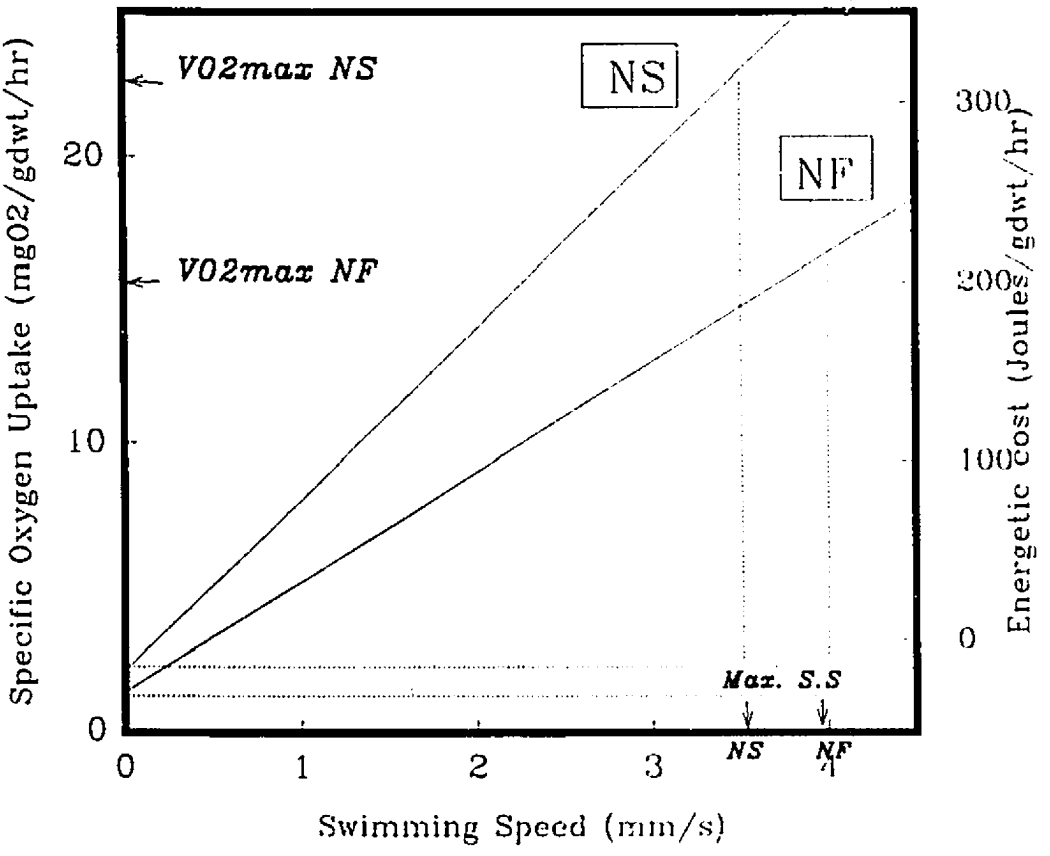

VO2max NS
VO2max NF
NS
NF
Max. S.S
NS
NF
Specific Oxygen Uptake (mgO2/gdwt/hr)
Energetic cost (Joules/gdwt/hr)
Swimming Speed (mm/s)
0
10
20
0
100
200
300
0
1
2
3

Figure 4.5

Period I (yolk-sac period).

Morphology: the presence of a large yolk-sac (50% utilized (see Ch. 1, Table 1.2), uninflated swim-bladder, rapid change in cranial skeletal structures, no functional jaw (1-6 days post-hatch (see Ch. 2, Figure 2.1)).

Behaviour: swimming patterns characterized by periods of intermittent burst swimming, and little or no activity related to feeding.

Period II (Mixed-Feeding period).

Morphology: yolk-sac (>50% to 100% utilized), partial inflation of swim-bladder (swim-bladder is entirely inflated at the end of the second period), a functional jaw.

Behaviour: swimming patterns still intermittent but changing to 'beat-and-glide' patterns, activity more related to feeding, but exogenous feeding still supplements endogenous feeding.

Period III (exogenous feeding period).

Morphology: no yolk-sac left, dorsal fin regressing (juvenile fins forming) changing body shape.

Behaviour: swimming patterns sub-carangiform and 'beat-and-glide', solely exogenous feeding, therefore, large proportion of activity related to feeding.

Figures 4.6a-f show the cost of activity for all three periods in both populations. In the first 'yolk-sac' period, mass - specific oxygen uptake was unrelated to swimming speed for both the NF and NS populations (6a and 6d). Thus, in the first period no portion of total specific oxygen consumption could be explained by changes in activity. In the second mixed-feeding period, there was a possible tendency ($r^2 = 0.5$, $p = 0.08$)

Figure 4.6. Relationships of mass specific oxygen uptake (mgO2/hr/gdwt) vs. swimming speed for three discrete periods of cod larval life in two populations Scotian Shelf (NS) (a–c) and Newfoundland (NF) (d–f).

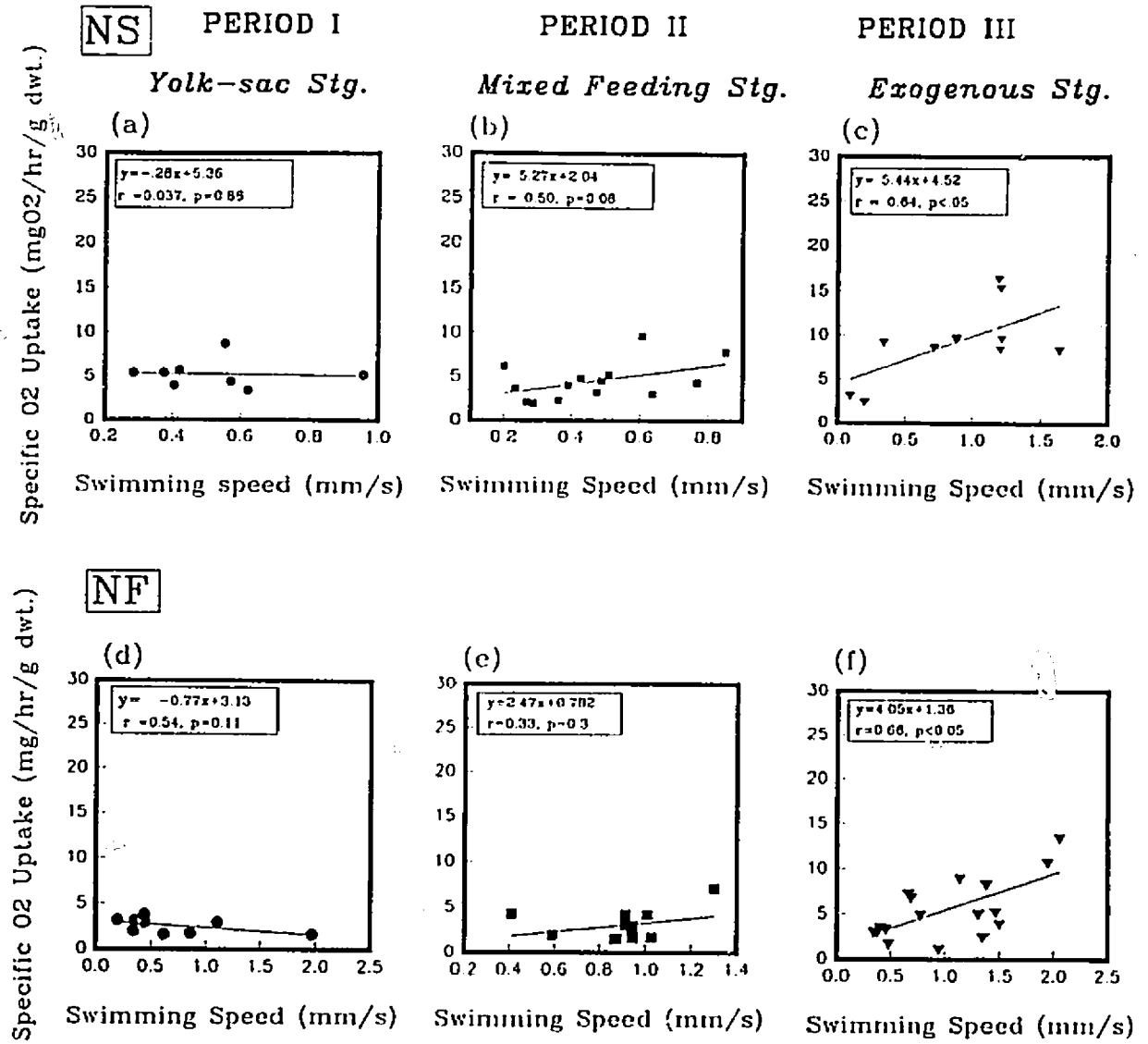

NS
PERIOD I
PERIOD II
PERIOD III
Yolk–sac Stg.
Mixed Feeding Stg.
Exogenous Stg.
(a)
(b)
(c)
y=-.28x+5.36
r =0.037, p=0.86
y= 5.27x+2.04
r = 0.50, p=0.08
y= 5.44x+4.52
r = 0.64, p<.05
Specific O2 Uptake (mgO2/hr/g dwt.)
Swimming speed (mm/s)
Swimming Speed (mm/s)
Swimming Speed (mm/s)
NF
(d)
(e)
(f)
y= -0.77x+3.13
r =0.54, p=0.11
y=2.47x+0.782
r=0.33, p=0.3
y=4.05x+1.36
r=0.66, p<0.05
Specific O2 Uptake (mg/hr/g dwt.)
Swimming Speed (mm/s)
Swimming Speed (mm/s)
Swimming Speed (mm/s)

Figure 4.6

for changes in mass – specific oxygen consumption to be explained by changes in swimming speed in the NS population (Figure 4.6b). However, for the NF population, no significant relationship existed between specific oxygen uptake and swimming speed (Figure 4.6e). In the third 'exogenous feeding' period, mass – specific oxygen consumption increased with swimming speed in both populations (for NS, $r^2 = 0.41$, $p < 0.05$; for NF $r^2 = 0.44$, $p < 0.05$; Figures 4.6c and 4.6f). Therefore, 41% (NS) to 44% (NF) of the variance in mass – specific oxygen consumption can be explained by changes in swimming speed during the exogenous feeding period.

Overall, for both genetic stocks, mass – specific oxygen consumption and activity are generally unrelated in the early larval stages, while a greater proportion of total oxygen consumption can be explained by variation in swimming speed in the later stages of larval life.

Temperature effects on the cost of activity in period III of the larval life.

Oxygen consumption and swimming speed were measured for larvae raised at 10°C. However, due to the high variance at this temperature, oxygen consumption did not regress significantly against swimming speed in either the NS population ($r^2 = 0.031$, $p > 0.05$) or in the NF population ($r^2 = 0.126$, $p > 0.05$). Therefore, in order to compare cost of activity between larvae raised at 5°C and larvae raised at 10°C, mean cost of activity (mgO_2/gdwt/m) [specific oxygen uptake (mgO_2/gdwt/h) / swimming speed (mm/s)] was determined for each population and for each temperature treatment. Characteristics that defined period III larvae (see previous section) were also used to

determine period III larvae raised at 10°C. Morphological and behaviourial characteristics were the same between temperature treatments, although they were displayed earlier in larvae raised at 10°C than at 5°C (see Ch. 1 for rates of development).

Between-population comparison

The mean cost of activity for larvae raised at 10°C and tested at 10°C, in larval period III, was not significantly different between populations (t-test, $t = 1.11$, $p>0.05$; Figure 4.7a). In contrast, the mean cost of activity for larvae raised at 5°C and tested at 10°C was significantly higher in the NS population than in the NF population (t-test, $t = 2.4$, $p<0.005$; Figure 4.7 a,b).

Within-population comparison

For the NF population, the mean cost of activity for larvae raised at 10°C and tested at 10°C was significantly higher than the mean cost of activity for larvae raised at 5°C and tested at 10°C (t-test, $t = 2.43$, $p<0.005$; Figure 4.7 a,b). However, for the NS population, no significant differences exist between temperature treatments (t-test, $t = -7.9$, $p> 0.05$; Figure 4.7 a,b). Therefore, while the cost of activity of larvae from the NS population seemed unaffected by an increase of 5°C in temperature, an equivalent rise in temperature increased the cost of activity in larvae from the NF population.

Figure 4.7. A comparison of the cost of activity during the third period of cod larval life, between two populations, Scotia Shelf (NS) and Newfoundland (NF), for each temperature treatment. R10T10 = raised at 10°C and tested at 10°C. R5T10 = raised at 5°C and tested at 10°C. Error bars respresent ± 1 s.e.

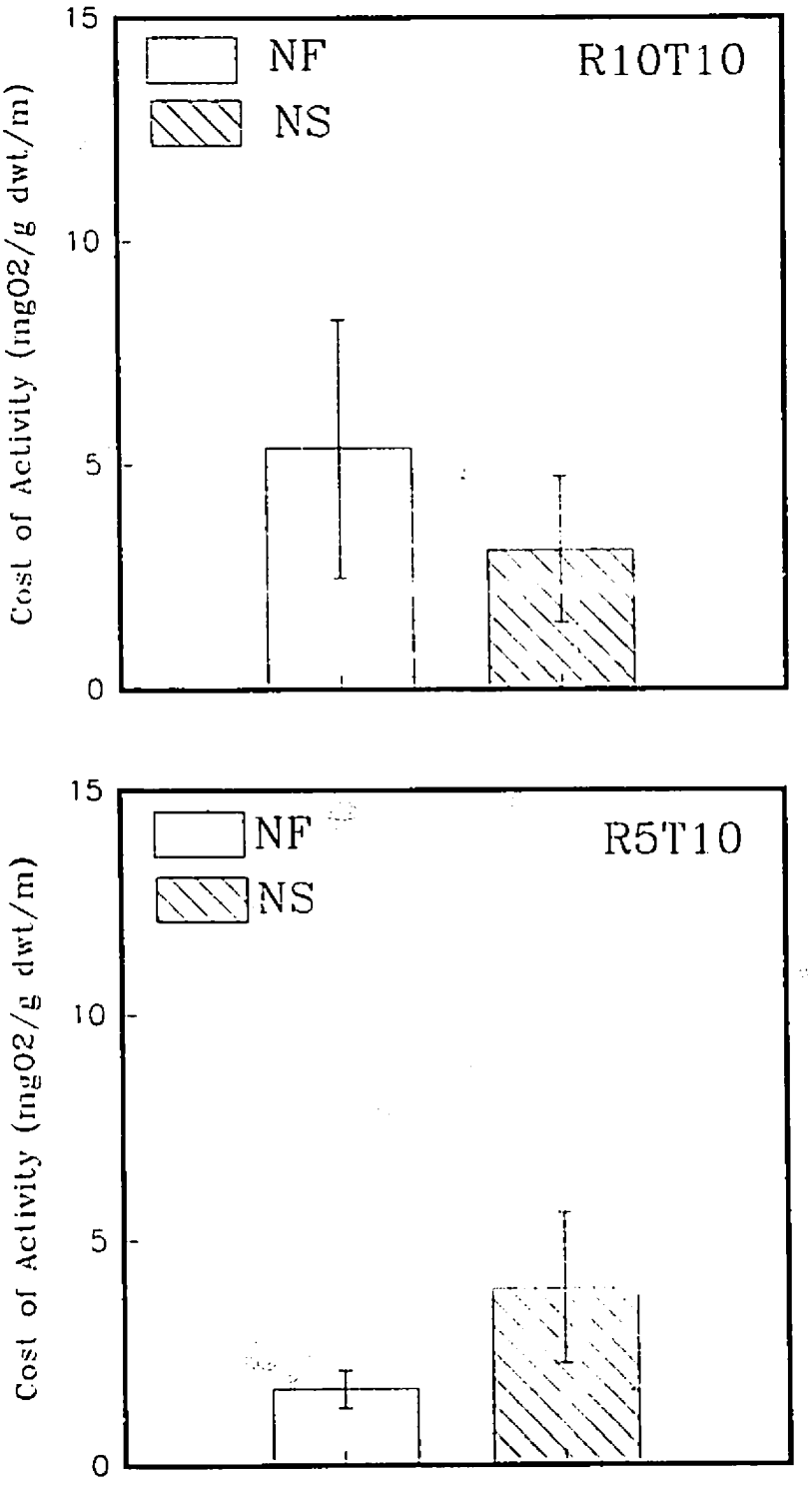

15
10
5
0
NF
NS
R10T10
Cost of Activity (mgO2/g dwt/m)
15
10
5
0
NF
NS
R5T10
Cost of Activity (mgO2/g dwt/m)

Figure 4.7

DISCUSSION

The methods used to determine the proportion of total metabolism devoted to activity worked well for small cod larvae in this study. The high sensitivity and quick response of the pulsed electrodes and small respirometers enabled oxygen consumption to be recorded for a smaller number of larvae (1–4 larvae) than was needed in previous studies (e.g. 50–100 individuals (Dabrowski 1986), 250 individuals (Kauffman 1990)). In the present study, this reduced (and in most cases eliminated) increases in metabolic rate due to interactions with other animals in the respirometer – a factor which perhaps affected metabolic rates in the above studies. In addition, the time course for the experiments in the present study was shorter (1 hr) compared to the times more commonly used (e.g. 7–10 hr ; (Dabrowski 1986) and 4 hr (Kauffman 1990)). This reduced the period over which nitrogenous wastes could build up in the experimental chamber, allowed numerous replicates to be run (avoiding large changes in developmental stage), and also avoided the effects of hypoxia by maintaining the oxygen concentrations above 80% saturation. Although short time-course experiments seem to be well suited for small fish larvae, the procedures used in this study did not enable either simultaneous feeding and activity recordings to be made or high current speeds to be used. Therefore, future modifications of this procedure to accommodate in situ feeding and higher current speeds (specifically for larger marine larvae), would enable the construction of a more robust energy budget.

Ontogenetic differences in mass – specific metabolic rate and swimming speed.

During the ontogeny of Atlantic cod, energy demands for swimming increase as the larvae grow. The mode of feeding switches from endogenous to exogenous food resources, and respiration shifts from cutaneous to branchial. Given these profound changes, cod larval life was divided into three periods based on changes in morphology and feeding behaviour.

Endogenous Feeding Stage – Period I

In the endogenous feeding 'yolk-sac' stage, larvae are small (4-5 mm) and shaped essentially like a sphere with a tail (Webb and Weihs 1986). They have no gills and a limited vascular system (see Ch. 2). Yolk-sac larvae move at slow speeds and in low Reynolds number regimes ($Re < 20$), where viscous forces dominate (Webb and Weihs 1986). In order to overcome viscous drag, they 'burst' swim (Hunter 1981) and may rely on anaerobic power to do so. This is supported indirectly by the evidence that the specific metabolic rate does not increase with increasing activity (Figures 4.6a & 4.6c; Period I). More direct evidence of anaerobiosis, such as changes in the composition of trunk muscle fibres and changes in the proportion of aerobic/anaerobic enzymes, appears to vary with species. For example, in yolk-sac larvae of rainbow trout, white muscle fibres and high levels of glycolytic enzymes (Forstner et al. 1983), support the hypothesis that swimming movements are powered by anaerobiosis. In contrast, coregonid and cyprinid larvae possess a red muscle layer and high levels of oxidative enzymes, which suggest that activity is primarily driven by aerobic metabolism (Forstner et al. 1983,

Wieser 1991).

The amount and type of activity exhibited by the fish larvae can be a reflection of the source of energy utilized. For example, just-hatched rainbow trout move very little (Wieser et al. 1985), while coregonids swim actively searching for food almost immediately after hatching (Forstner et al. 1983). This sustained swimming creates the need for aerobic energy production in coregonids, while the small burst – like movements of rainbow trout are indicative of those likely fuelled by anaerobiosis at least in adults. In cod yolk-sac larvae, the predominance of white muscle fibres in the trunk muscle (Morrison 1993, Hunt von Herbing, unpublished data) and erratic, infrequent swimming movements are further indirect evidence that locomotion may be partially powered by anaerobiosis. However, analyses of oxidative and glycolytic enzymes, in addition to calorespirometry (see Gnaiger 1983), may be required to fully discern the extent to which activity is driven by anaerobic processes in cod larvae.

The amount of energy gained by aerobic sources may be limited in the yolk-sac stage, as oxygen uptake is solely dependent on the diffusion rate and surface area of the larva (Blaxter 1988, Rombough 1988a). Thus, oxygen is supplied directly to the muscle under the skin through diffusion, but is not convected away as the vascular system is still in its early developmental stages (see Morrison 1993 and Ch. 2). With a fixed surface area, the rate of oxygen uptake can only be increased by increasing the gradient between the ambient oxygen levels and internal or muscular levels of oxygen. Larvae may also burst-swim to dissipate the surrounding boundary layers (Weihs 1980) and increase the transepithelial PO_2 gradient and therefore cutaneous respiration. In time, larvae must

further decrease the oxygen levels in the muscle by developing a functional vascular system which carries away the oxygen taken up by the skin to other organs or tissues. However, the development of the cutaneous vasculature is poorly known in the larval stages and cutaneous respiration in adult fish makes only a minor contribution to over all oxygen consumption (Feder and Burggren 1985).

Burst-swimming movements at the yolk-sac stage are often considered in the context of predator avoidance (Webb 1981, Yin and Blaxter 1987). Such movements may also provide the larvae for future feeding (i.e. 'learning to feed; Ch. 2), when exogenous food resources become exhausted. In either event, burst swimming results in large amplitude lateral (anguilliform) movements which produce large head yaw and reduce prey capture efficiency (Drost 1987). Should the larvae need to feed exogenously at this stage, foraging efficiency (energy cost vs energy gain) would be low especially if burst locomotion is powered anaerobically (i.e. incurring an oxygen debt). If the latter is true, there is probably a trade-off in an energetic sense, between the acquisition of exogenous food and its anaerobic catabolism. Other types of swimming modes that facilitate efficient prey capture (e.g. 'beat-and-glide' swimming) are not possible at this stage, as the viscous drag is too high (Vlymen 1974) and the anaerobic conversion of glucose to ATP too low (Hochachka and Somero 1984) to allow a continuous-type swimming. Therefore, while growth and development are probably fuelled almost exclusively by aerobic combustion of yolk-sac reserves, we cannot discount the possibility that anaerobic metabolism may play some role in powering burst - swimming movements.

Mixed Feeding Stage – Period II

During the mixed-feeding stage, the jaw is fully functional, and external feeding supplements energy derived from the yolk-sac. The large dorsal fin present in the previous stage is unchanged, but due to growth, different swimming patterns have changed. The period between continuous swimming bouts is reduced, while swimming frequency has increased (Figure 4.3a – b, this study) and the larvae now swims in an intermediate Re environment ($20 < Re < 200$) (Webb and Weihs 1986). Thus, inertial forces begins to play an important role enabling the larvae to adopt a 'beat-and-glide' mode of swimming (or cruising). Cruising speed becomes important as it determines the frequency that larvae encounter prey and also accounts for increases in the metabolic expenditure (Hunter 1981; this study; Figure 4.6b).

In addition, extra energy is probably also spent on maintenance of position in the water column. In the endogenous feeding stage, the yolk-sac, being composed largely of proteins and lipids (Stroganov 1962) provides buoyancy for the larvae in place of an oil globule present in other species (e.g. labrids). As the yolk is utilized to fuel development and growth, and cartilaginous structures become more dense, the larvae begin to sink (I. Hunt von Herbing unpublished data). In response, the swimbladder inflates to reduce sinking rate. However, at the onset of inflation (9 days post-hatch at 5°C, see Ch. 1), the swimbladder does not appear to be fully functional (Hunt von Herbing, unpublished data), and the larvae are negatively buoyant. As a result, energy demand for locomotion increases for two reasons: (1) to facilitate foraging and exogenous prey capture and (2) to reduce sinking rate and maintain larval position in the water

column. The latter is important as it may enable the larvae to stay in a patch of high prey density, thereby maximizing prey encounter rate with minimal movement.

As a result of growth, swimming speeds and Reynolds numbers increase in cod larvae, but surface area–to–volume ratio decreases (Blaxter 1988). Thus, the area over which gas exchange can occur effectively decreases, reducing the oxygen available for aerobic activities. Although gill filaments begin to appear during this period (see Ch. 1, Figure 4.8), they are probably non–functional as blood supply is not fully developed. However, arteries and pigmented blood cells begin to appear from 6 days post–hatch in cod larvae (Ch. 2, Figure 2.3a). Therefore, it is possible that oxygen uptake is not solely diffusion–limited and some convection of oxygen to other parts of the body may be possible. Branchial respiration is essentially prevented at this stage, because the suction pressures within the buccopharyngeal cavities are evidently too small to provide the high velocity flow needed to break down large boundary layers within the cavities (Osse 1989). Therefore, despite the tendency for increased vascularization and blood pigmentation, and because of the lack of branchial respiration, the supply of oxygen for aerobic activities is still limited predominantly by diffusion. Thus, because increases in swimming activity still occur without large corresponding increases in oxygen consumption (Figure 4.6 b,c) there is reason to suppose that anaerobic respiration continues to supplement locomotion at this stage as we suspect it might in the earlier yolk–sac larvae.

Exogenous feeding stage – Period III

After passage through the mixed–feeding stage, yolk–sac reserves are exhausted, and

feeding is solely exogenous. As a result, larvae are very active (percent activity increases) and 40% of the total energy budget is devoted to foraging and locomotion (Figure 4.6c – f). In comparison, demersal juvenile cod of the Scotian Shelf allocate approximately 22% of their total energy budget to foraging and locomotion (M. Tupper, Dalhousie University, unpublished data). In addition, morphological changes may improve swimming performance, for example: the dorsal fin regresses and is replaced by paired fins (this aids maneuverability), and flexion of the notochord occurs (this may provide increased thrust) (Ch.1, Table 1.2). Thus changing body shape, along with larger size, results in a shift in swimming patterns from anguilliform to subcarangiform (Webb and Weihs 1986). This occurs in concert with the transition to the inertial regime (Re > 200), in which pressure drag replaces that viscous drag as a major component of drag (Webb and Weihs 1986). Moreover, the increases in percent activity are more likely to be composed of higher swimming speeds (Skiftesvik 1992, this study (Figures 4.3a & 4.3f). Thus, changes in swimming patterns and swimming speed increase the accuracy and efficiency of prey location and capture (Drost 1987, Drost and Muller 1988). High foraging efficiency is essential at this stage, as energy supply for growth and eventually transformation to the juvenile stage, depends on larval capability to obtain exogenous food.

Increases in size are accompanied by growth and proliferation of the gills and secondary lamellae (see Ch. 1, Figure 1.8), which may alleviate the further reduction of surface area–to–volume ratio. In cod larvae, gills and the buccal–opercular pump become functional at about 9–10 mm standard length (see Ch. 3). Gills are also perfused with

blood at this stage; the epithelium increases extensively in surface area and is also made thinner thereby facilitating gas diffusion (Morgan 1974), by reducing the distance over which oxygen must travel from the surrounding water to the gill artery. In addition, the number of blood cells increases (sufficiently for pigmentation to be easily observed) and this presumably also increases the amount of oxygen that can be conveyed around the body. All of the above changes may enable the larvae to rely to a greater extent upon aerobic respiration to provide energy for activities such as foraging and locomotion. In general, oxygen uptake is considered to be the limiting factor for energy production. Other factors such as the transfer of CO_2 or NH_3 could also be potentially limiting, however CO_2 is much more diffusible than O_2 in body fluids and NH_3 is readily diluted in the ocean (Dejours 1975).

In light of the differences between all three stages in cod larvae, it is of interest to directly compare swimming performance and its importance to larval survival. It appears that in period I, cutaneous respiration over a fixed surface area and possible reliance on anaerobiosis to power 'burst' swimming for predator avoidance (Yin and Blaxter 1987) are sufficient to meet energy demands as feeding is entirely endogenous. However, the second 'mixed feeding period' period is more critical for cod larvae as exogenous feeding supplements endogenous supplies, but total energy output may still partially rely on anaerobiosis. Since a large amount of substrate (glycogen/glucose) would be needed to power foraging activity through anaerobiosis larvae could, if food were limiting, expend more energy than they were able to acquire. In this situation the yolk-sac acts as a substrate reserve to prevent starvation. As a result however, these larvae may enter

Period III energy depleted, and this may affect their potential for survival. While activity in Period III is probably powered to a large extent by aerobic processes (although anaerobiosis may still be used for occasional swimming bursts to avoid predators), it is also the period most likely to be affected by changes in environmental variables; i.e. any factor that affects the partial pressure of oxygen in the water, such as temperature, will also affect the energetics of swimming.

Temperature effects on activity metabolism between and within the Scotian Shelf and Newfoundland populations.

Measurements of the changes in metabolism which result from environmental perturbations is a useful indicator of intrinsic differences between fish stocks (Nelson et al. 1993). When cod larvae of the two different populations acclimated to 5°C in this study were acutely exposed to 10°C, Newfoundland larvae were found to have a lower intrinsic cost of activity that Scotian Shelf larvae, over the entire larval life (Figures 4.4 & 4.6). Such differences between populations could be explained by differences in morphology (e.g. Newfoundland larvae may be more hydrodynamically efficient), or the differences may reflect the way in which total energy is partitioned between aerobic and anaerobic metabolism.

In the wild, adaptive differences between stocks may affect survival. For example, if larvae acclimatized in situ to 5°C were engulfed by a convection current of 10°C; an event that could represent a warm core ring from the Gulf Stream being advected onto the Scotian Shelf (Scott and Scott 1988), or a typical yearly anomaly in sea surface

temperatures in Newfoundland (Pepin 1991), then I would predict a greater likelihood for Newfoundland larvae to survive the event. Since Newfoundland larvae have lower intrinsic metabolic rates for the same activity (Figure 4.5), they require reduced levels (or quality) of food to fuel the activity. This would result in reduced rates of foraging and energy expenditure and reduced predator encounter rates (Gerritsen and Strickler 1977), but higher growth and survival rates.

On the Scotian Shelf, most spawning occurs between October and April, with a peak of intensity in December. Sea surface temperatures vary from 8–10°C in the late fall to 3–5°C in the spring (Drinkwater and Trites 1991). Therefore, most NS eggs and larvae experience high temperatures (8–10°C) during development, though a smaller proportion are also spawned in the colder waters of spring. Recent studies have shown that cod larvae are retained over Sable Bank, and indeed prefer regions of higher temperatures (9°C) to that of the surrounding colder water (Griffin and Lochmann 1993). This may be partially related to the high densities of prey items found contiguous with high densities of fish larvae (Griffin and Lochmann 1993). In contrast, Newfoundland stocks spawn from March to July (temperatures range from 1–5°C) and eggs and larvae drift in the cold Labrador current to shallow nursery grounds 600–1000 km to the south (Lear and Green 1984). As a result, Newfoundland larvae experience, on average, much colder temperatures throughout their larval life than do NS larvae.

During the exogenous feeding stage, the average metabolic cost of activity for NF larvae raised at 10°C and tested at 10°C was significantly higher and more variable than that of larvae raised at lower temperatures (Figure 4.7). Thus, when raised at

temperatures above those they would normally experience in the wild, the cost of activity increases for the normally 'cold – adapted' Newfoundland larvae. Higher temperatures would mean that greater amounts of energy from food would be needed to fuel the elevated metabolic rates. In addition, the costs of foraging could be so high that large energy debts might accumulate, and inhibit growth. For example a study of energy allocation in juvenile (150 g) cod by Priede (1985) indicated that at 10°C, the maximum power expenditure on foraging (standard metabolism + swimming + apparent SDA) was 181 mW, while the maximum power supplied by aerobic metabolism (VO_{2max}) was 141 mW. At 15°C, the situation worsened as the maximum expenditure on foraging rose to 304 mW, while VO_{2max} rose to only 223 mW.

In contrast to Newfoundland larvae, larvae from the Scotian shelf population showed no significant difference in the cost of activity between the two temperature treatments (Figure 4.7). Therefore, these 'warm –adapted' larvae are probably less likely to suffer mortality if temperatures are high and stable throughout the larval life. Scotian Shelf larvae may however, be particularly sensitive to acute temperature increases. This is shown by the higher metabolic costs compared to NF larvae when larvae were transferred from 5°C to 10°C (Figure 4.7b). In contrast, the Newfoundland cod larvae show less of a stress related increase in metabolic cost during the exogenous feeding stage (Figure 4.7b).

Clearly, early life history stages of cod must be able to withstand a multitude of different types of changes in environmental parameters, and tolerance to stress may be genetically determined. Recently, Pogson et al. (1993) used cDNA clones to establish for

the first time that contiguous populations of cod along the Northeast coast of Newfoundland and along the coast of Nova Scotia are genetically discrete. Genetic differences may also partially explain the regional differences observed in the timing and location of spawning (Fletcher 1993). Together these findings suggest that interpopulation differences have arisen by environmental selection and that genetically distinct groups of cod are adapted to their regional conditions. In this study, these differences were reflected in the different swimming energetics in the Newfoundland and Scotian Shelf larvae. Since selection is thought to be most intense at the larval stage (Balon 1985), factors which differentially affect energy budgets of larval fish may have significant consequences for the future of the populations.

Chapter 5

EFFECTS OF TEMPERATURE ON GROWTH AND METABOLISM IN DEVELOPING ATLANTIC COD: AN INTER-POPULATION COMPARISON.

INTRODUCTION

Oxygen consumption of fish eggs and larvae can be partitioned into two major compartments: growth (change in size and weight) and metabolism (to support activities such as locomotion and feeding). These compartments are not independent of each other, as energy is being exchanged continually from one compartment to another (Brett and Groves 1979). The balance between the energy-demanding processes and the energy supplied from the combustion of food and from respiration is critical to the overall physiological fitness of an individual. Theoretical models predict that optimum patterns of resource allocation are those that lead to greater reproductive output, thereby transmitting more genes to future generations (Arnold 1988). The energetic balances of fish larvae may be disrupted by limited capability to capture food (see Ch. 2), coupled with variations in prey availability. These factors likely contribute to the high mortality that is typical of fish larvae (Hewitt et al. 1985, McGurk 1986, Houde 1989).

Eggs and larvae of temperate marine fishes also experience marked temperature changes – either rapid changes due to short-term anomalies (Pepin 1991), or more gradual changes due to seasonal cycles. Changes in temperature can affect early-stage energetic balances by varying both embryonic and larval rates of growth and metabolism

(Houde 1989, Blaxter 1992). However, growth and metabolic rate do not necessarily vary at the same rate with changes in temperature (Kamler 1992).

The relationship of oxygen consumption and mass is often expressed by using the allometric power function $VO_2 = aM^b$, where VO_2 is oxygen consumption, M is animal body mass and a and b are fitted parameters, and b is often referred to as the "metabolic mass exponent". Alternatively, this relationship can also be expressed as mass-specific oxygen consumption ($VO_2/M = aM^{(b-1)}$). Generally, metabolic mass exponents are high for fish eggs and larvae (i.e. > 0.8; Raciborski 1987, Guigerre et al. 1990) compared to juveniles and adults (<= 0.8; Kamler 1976), although values seem to be highly variable among species (e.g. b = 0.65 for larval plaice, b = 0.82 for herring larvae (DeSilva and Tytler 1973)) and among development stages (e.g. > 1.0 in yolk-sac stages vs < 1.0 in exogenously feeding stages (Raciborski 1987, Rombough 1988a, Kamler 1992)). Only a few studies have examined the effects of temperature on metabolic mass exponents in larval fish (Laurence 1978, Quantz and Tandler 1982, Almatar 1984), and comparisons between stages and between species are difficult because metabolic rates are generally not normalized to activity and developmental stage.

It is important to study the effects of temperature on the amount of energy allocated to growth in species such as Atlantic cod, Gadus morhua. The adults of this species spawn in many regions of the north Atlantic, and their early life history stages consequently encounter large differences in temperature (e.g. 0°- 3°C off the coast of Newfoundland (Lear and Green 1984) to 12°C in late fall on Sable Bank (Drinkwater and Trites 1991). To my knowledge, only 4 studies have documented the metabolism and

growth of eggs and larvae of Atlantic cod (Davenport and Lonning 1980, Laurence 1978, Solberg and Tilseth 1984, 1988). In three of these studies, growth and metabolic rates were measured only to the end of the yolk-sac stage (Davenport and Lonning 1980, Solberg and Tilseth 1984, 1988). Only one study has investigated growth and metabolic rates from hatching to metamorphosis in Atlantic cod raised at different temperatures (Laurence 1978). Laurence (1978) found that while both growth and metabolic rates increased with temperature, metabolic mass exponents appeared to decrease. However, he did not investigate the metabolic mass exponents during the embryonic period; nor did he determine how the total available energy was allocated to growth and metabolism at different stages in the larval life.

In my study, the growth and metabolic rates of 2 stocks (central Newfoundland and Scotian Shelf), considered to be genetically distinct (Pogson 1993), were studied under 3 different temperature treatments. Eggs and larvae of the Newfoundland population are spawned from April to June and are generally exposed to much colder temperatures (0–3°C, (Lear and Green 1984)) than those of the Scotian Shelf. The latter are spawned from November to March (with a peak in December) and are most commonly exposed to temperatures of 5–9°C (Griffin and Lochmann 1993). My laboratory studies of the effects of temperature change on the partitioning of energy between growth and metabolism was designed to simulate conditions that eggs and larvae of different stocks experience in the ocean. In this way we may better understand how changes in ambient conditions affect energy allocation and survivorship of the early life history stages in two discrete stocks. Such information may be important for future management of the fishery.

METHODS AND MATERIALS

Animals

Atlantic cod eggs and larvae were obtained from two stocks: central Newfoundland stock (NF) (adults (originally of the 2J, 3KL stock) are held at Memorial University, St.John's, Newfoundland) and Scotian Shelf stock (NS) (adults (originally of the 4VsW stock) are held at Dalhousie University, Halifax, Nova Scotia). Eggs and larvae were raised and maintained at two controlled temperatures of 5°C ± 0.5 and 10°C ± 0.5 in 80 l closed-system rectangular glass aquaria. Tanks were shielded by black plastic to avoid disturbance of the larvae and to aid the larvae in locating prey. Seawater in each aquaria was treated with antibiotics (0.2g/l) (Penicillin-G and Streptomycin sulphate, SIGMA) to reduce bacterial levels. Gentle aeration was provided by air stones which kept the eggs suspended and reduced the build up of fungus. In addition, high concentrations of the green alga Isochrysis sp. were also added and maintained throughout the experimental period. Rotifers and wild caught zooplankton, sieved to appropriate sizes, were fed to the larvae throughout the experimental period. Prey density was kept high (>20 prey/ml) to ensure good feeding conditions for all larvae.

Respirometry and Growth

The electrode array described in Ch.4 (Figure 4.1) was used in the present study to determine total oxygen uptake (mgO_2/hr). Oxygen uptake was measured for 6-8 cod eggs and 2-4 cod larvae (4 of early stage larvae and 1-2 late stage larvae) of both populations (i.e. NF (Newfoundland) and NS (Scotian Shelf)). To ensure that the experimental larvae

did not have food in their guts, approximately 20–30 larvae were isolated in beakers from the aquaria and held over night at controlled temperatures. For each experiment, larvae were placed in the respirometer and acclimated to the chamber for 20 min to reduce stress – related effects on oxygen consumption. Following the acclimation period, the respirometer systems were closed and the decrease in oxygen concentration was recorded every 4 minutes for 1 hour. The eggs and larvae from each population were subjected to 3 temperature treatments: R5T5 (eggs and larvae were raised at 5°C and tested at 5°C), R5T10 (eggs and larvae were raised at 5°C and tested at 10°C) and R10T10 (eggs and larvae were raised at 10°C and tested at 10°C). All experiments were conducted in a cold room, in which the temperature was regulated to either 5°C ± 0.5 or 10°C ± 0.5.

Blanks were run for each experimental chamber and electrode after each experiment: these values (which ranged from 10–20% of total oxygen uptake) were subtracted from the values obtained during the experiment. For calculation of specific oxygen uptake, eggs and larvae were removed from the chambers, viewed for developmental stage after each experimental run, rinsed in distilled water and then dried for 24–48 hrs in an oven (60 – 80°C), to a constant weight. They were then weighed to the nearest 0.1 mg on a Cahn Gram electrobalance. For the eggs and for the yolk–sac larvae, yolk–sac was dissected from the embryonic tissue and weighed separately.

Dry weights obtained from the above procedures were also used to determine growth rates for both the NF and NS populations raised at 5°C and 10°C. Specific daily growth rates (SG) were calculated from the equation:

$$SG = 100 \ln WT - \ln wt / T \ ,$$

where WT = dry weight at the end of the time interval; wt = dry weight at the beginning of the time interval; T = time interval in days (after Laurence 1975).

Samples of larvae were taken simultaneously with those of Chapter 1 and the developmental stages herein correspond to the descriptions outlined in Ch.1 (see Table 1.2 in Ch. 1). In addition, activity estimates determined in Ch. 4 were also recorded in concert with oxygen consumption in the R5T10 and R10T10 temperature treatments in the present study.

Energy budgets, were calculated using caloric values of 5.2 cal/mg ash free dry weight for yolk-sac larvae and 4.8 cal/mg ash-free dry weight for exogenous feeding larvae (1 cal = 4.184 J). The caloric values were obtained from Solberg and Tilseth (1984), who utilized a microbomb microcalorimeter. Energy budgets in this study were only calculated in terms of energy utilization for growth and activity related metabolism (i.e. net energy budgets). The budgets do not include costs of assimilation, urinary or fecal losses (i.e. these would be included in calculations of gross energy budgets). Total oxygen consumption was determined from raw data and converted to energy equivalents using the averaged oxycaloric value of 13.96 J/mgO_2 (Elliot and Davison 1975). Total daily rates of energy consumption (Joules/day) by yolk-sac, mixed feeding stage and exogenous feeding larvae were estimated by summing the energy equivalents of the absolute growth rate (G) and the metabolic rate (R) of each sample period. The proportion of energy expended on growth (or net growth efficiency) was calculated from (G/(G+R)) and the proportion of energy expended on metabolism was calculated from (R/(R+G)).

All numeric data were subjected to normal probability plots and Bartlett's Test for homogeneity of variance (SYSTAT, Wilkinson 1990). Data were log – transformed where necessary to meet the demands of normality and homoscedasticity. Linear regressions were used to determine growth rates (μg dry weight day_{-1}) for each population and for each ontogenetic period (i.e. embryonic and larval) at 5°C and 10°C. Differences between growth rates and metabolic exponents among periods, between populations, and among temperature treatments were determined by t–tests between linear regression slopes (Zar 1984).

RESULTS

Growth

Temporal patterns of embryonic and larval dry weights and standard lengths are shown for both populations and at both temperatures in Figure 5.1 (a–b) and Figure 5.2 (a–b). In all cases, the dry weight of embryonic tissue increased at a greater rate than that of the embryo plus yolk–sac. The weight difference between these two measurements equalled the dry weight of the yolk, which fuelled the growth of the embryo and hence decreased over time.

During the embryonic period, the daily specific growth rates were higher at 10°C than at 5°C for both NS and NF populations (Table 5.1). For eggs raised at 5°C, peak hatching occurred at 14 and 18 days post–fertilization (NF and NS populations respectively), while at 10°C, eggs hatched in less time, i.e. 9–10 days for both populations (Figures 5.1 and 5.2 (a–b)). In addition, the total time over which hatching occurred was greater at 5°C (approximately 5 days) than at 10°C (approximately 2 days). In the initial

Figure 5.1 Mean dry weight (μg) and standard length (mm) vs days post–fertilization at two temperatures, 5°C and 10°C for the Scotian Shelf (NS) population of Atlantic cod. H = hatching, YS = yolk–sac, YSE = yolk–sac extinction. Vertical error bars represent ± 1 standard error

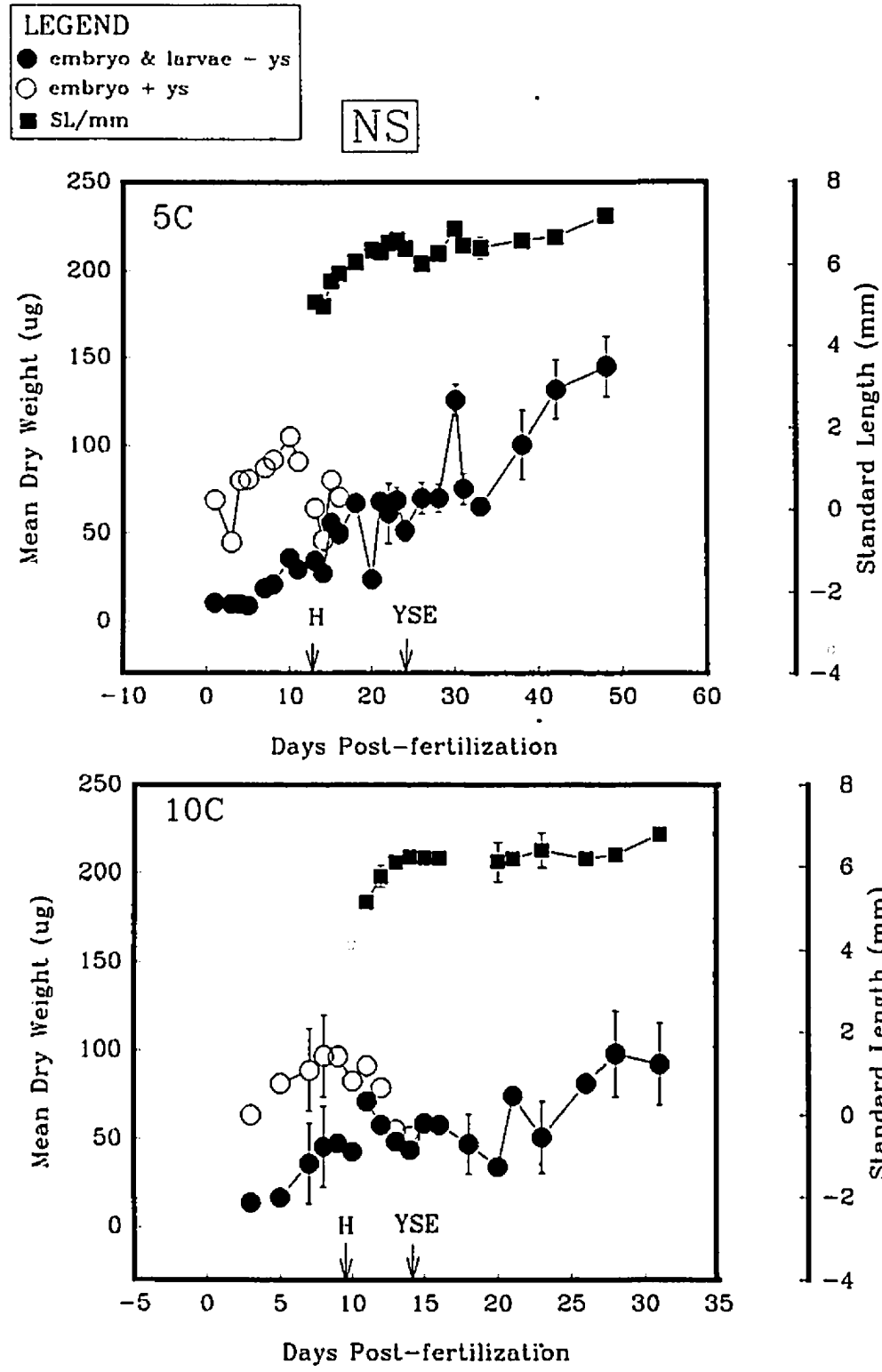

LEGEND
embryo & larvae - ys
embryo + ys
SL/min
NS
5C
Mean Dry Weight (ug)
Standard Length (mm)
H
YSE
Days Post-fertilization
10C
Mean Dry Weight (ug)
Standard Length (mm)
H
YSE
Days Post-fertilization

Figure 5.1

Figure 5.2 Mean dry weight (μg) and standard length (mm) vs days post-fertilization at two temperatures, 5°C and 10°C for the Newfoundland (NF) population of Atlantic cod. H = hatching, YS = yolk-sac, YSE = yolk-sac extinction. Vertical error bars represent ± 1 standard error

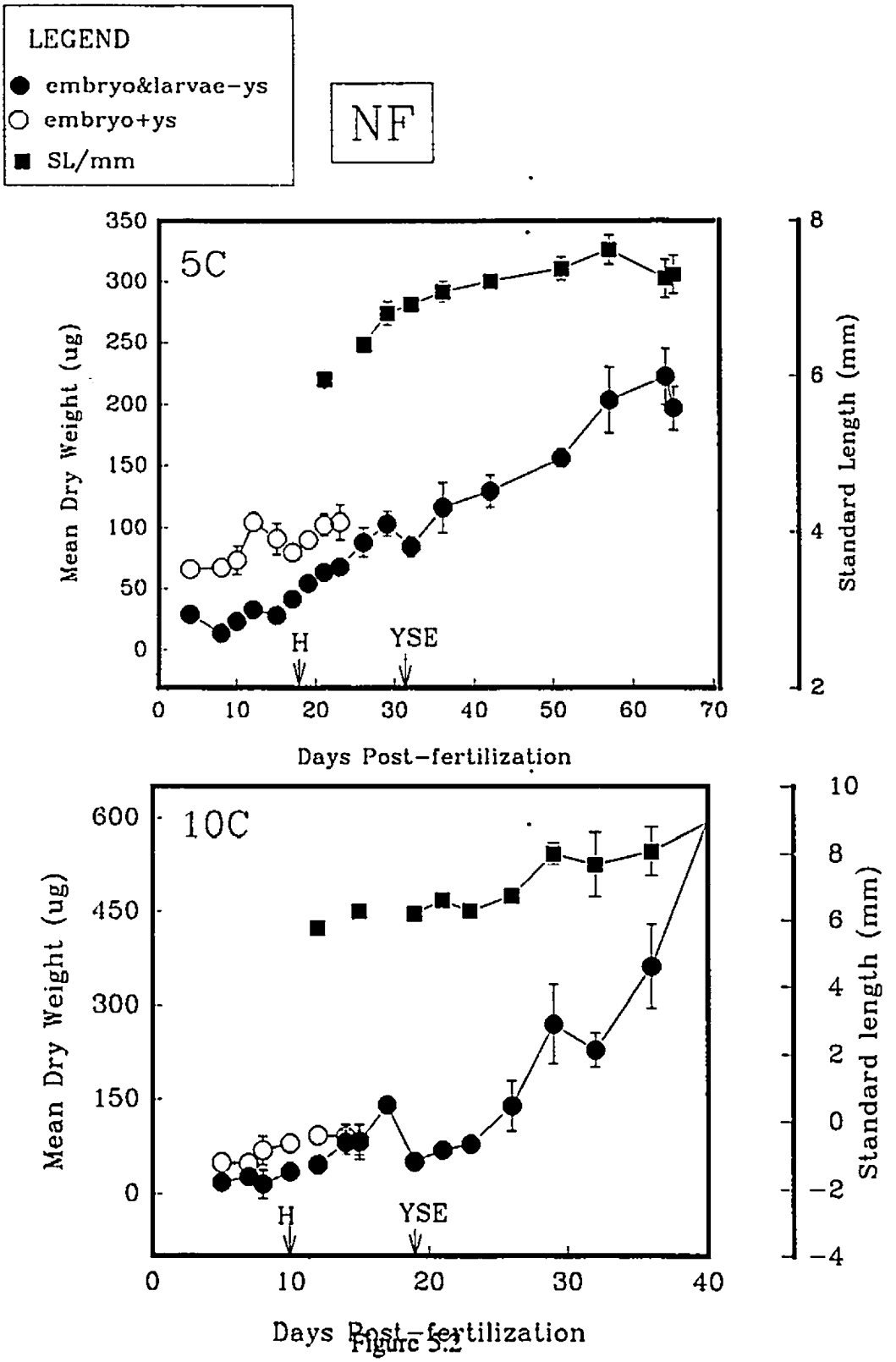

LEGEND
embryo&larvae-ys
embryo+ys
SL/mm
NF
5C
Mean Dry Weight (ug)
Standard Length (mm)
H
YSE
Days Post-fertilization
10C
Mean Dry Weight (ug)
Standard length (mm)
H
YSE
Days Post-fertilization

Figure 5.2

Table 5.1 : Specific daily growth rate (%/day) of three developmental stages in Atlantic cod, Gadus morhua in two populations: NS (Scotian Shelf) and NF (Newfoundland), at two temperatures.

Stage	NS 5°C (%/day)	NS 10°C (%/day)	NF 5°C (%/day)	NF 10°C (%/day)
Embryonic	8.6	13.5	9.9	16.8
Yolk-sac	9.1	8.4	8.9	18.6
Exogenous	4.4	7.6	5.2	9.3

period following hatching (the yolk–sac period), specific daily growth rates decreased from those of the embryonic period (with the exception of NF at 10°C) (Table 5.1). The lowest specific daily growth rates for both populations and temperatures occurred during the exogenous feeding period, but as with the embryonic and yolk–sac periods, growth rates were higher at 10°C than at 5°C (Table 5.1). Despite the lower specific daily growth rates, neither population exhibited symptoms of starvation. Both populations at all temperature treatments continued to increase in dry weight and standard length after yolk–sac extinction (YSE), albeit at a slower rate than during the embryonic and yolk–sac periods (Figure 5.1 (a–b) and Figure 5.2 (a–b)). Yolk–sac extinction occurred at approximately the same time at a given temperature in both populations, but differed between temperatures (12–15 days post–hatch at 5°C and 7–8 days post–hatch at 10°C) (Figures 5.1 and 5.2 (a–b)).

Comparisons of growth rates between populations and temperatures were made by regressing log dry weight against days post–fertilization for the embryonic and larval periods of each population; at 5°C (Fig. 5.3a) and at 10°C (Fig. 5.3b). Log dry weight regressed significantly against days post–fertilization, for 5°C (embryonic, $r^2 = 0.87$ and $r^2 = 0.62$, $p<0.05$; larval, $r^2 = 0.72$, $p < 0.0001$ and $r^2 = 0.50$, $p < 0.0001$; for NF and NS respectively) and for 10°C (embryonic, $r^2 = 0.94$, $p < 0.05$ and $r^2 = 0.932$, $p < 0.05$; larval, $r^2 = 0.85$, $p < 0.001$ and $r^2 = 0.44$, $p < 0.001$; for NF and NS respectively). Embryonic growth rates were significantly higher in NF population than in the NS population at 5°C (t–test; $t = 2.07$, $p < 0.05$) and at 10°C (t–test; $t = 24$, $p < 0.001$). Similarly, larval growth rates were also significantly higher in the NF population than in

Figure 5.3 Regressions of log dry weight (μg) vs days post-fertilization at two temperatures, 5°C and 10°C for the Newfoundland (NF) and Scotian Shelf (NS) population of Atlantic cod.

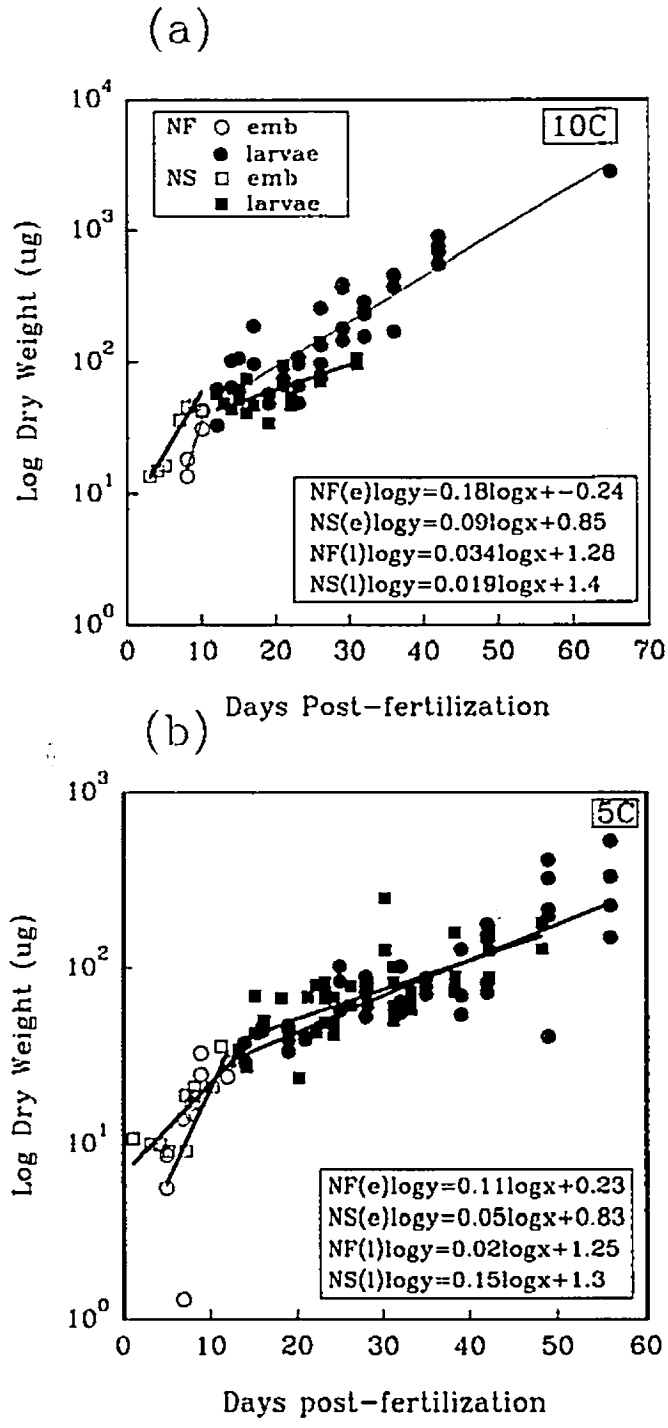

(a)
NF ○ emb
● larvae
NS □ emb
■ larvae
10C
NF(e)logy=0.18logx+−0.24
NS(e)logy=0.09logx+0.85
NF(l)logy=0.034logx+1.28
NS(l)logy=0.019logx+1.4
Log Dry Weight (ug)
Days Post−fertilization
(b)
5C
NF(e)logy=0.11logx+0.23
NS(e)logy=0.05logx+0.83
NF(l)logy=0.02logx+1.25
NS(l)logy=0.15logx+1.3
Log Dry Weight (ug)
Days post−fertilization

Figure 5.3

the NS population at 5°C (t-test; $t = 3.99$, $p < 0.05$) and at 10°C (t-test; $t = 5.14$, $p < 0.05$). This suggests that trends in growth rate exhibited in the embryonic stage are maintained throughout the larval stage. In addition, within each population, growth rates were significantly higher at 10°C than at 5°C (t-test; $t = 1.8$, $p < 0.05$, for NS) and (t-test; $t = 1.89$, $p < 0.05$, for NF).

Metabolism

(a) Changes in metabolism with development

Temporal patterns of mass-specific oxygen uptake for both populations and for all three temperature treatments are shown in Figure 5.4 (a-c) and Figure 5.5 (a-c). In all cases, mass-specific oxygen uptake during the embryonic period gradually increased from fertilization to hatching. Upon hatching, a small increase in mass-specific oxygen uptake occurred in both populations and in all temperature treatments, with the exception of NF (R5T5). The metabolic cost of hatching appeared to be independent of temperature, but was higher in the NS population than in the NF population (Table 5.2).

To determine whether patterns in mass-specific oxygen uptake are linked to changes in morphology or behaviour of the developing larvae, mass-specific oxygen uptake was plotted against days post-fertilization, in conjunction with developmental stages (described in Ch. 1). In all cases, mass-specific oxygen uptake continued to increase after hatching until first feeding (i.e. from stages 1 to 3) (Figures 5.4 and 5.5 (a-c)). Metabolic rates decreased from stage 3 to 5, in concert with yolk-sac utilization (Figures 5.4 and 5.5 (a-c)). Between stages 5 (> 50-75% of the yolk-sac utilized) and 7 (no yolk

Figure 5.4 Mass specific oxygen uptake ($mgO_2/hr/gdwt$) vs days post–fertilization for three different temperature treatments, R5T5 (raised at 5°C and tested at 5°C), R5T10 (raised at 5°C and tested at 10°C) and R10T10 (raised at 10°C and tested at 10°C), for the Scotian Shelf (NS) population of Atlantic cod. Numbers represent developmental stages defined in Chapter 1. ys = yolk–sac Vertical error bars represent ± 1 standard error

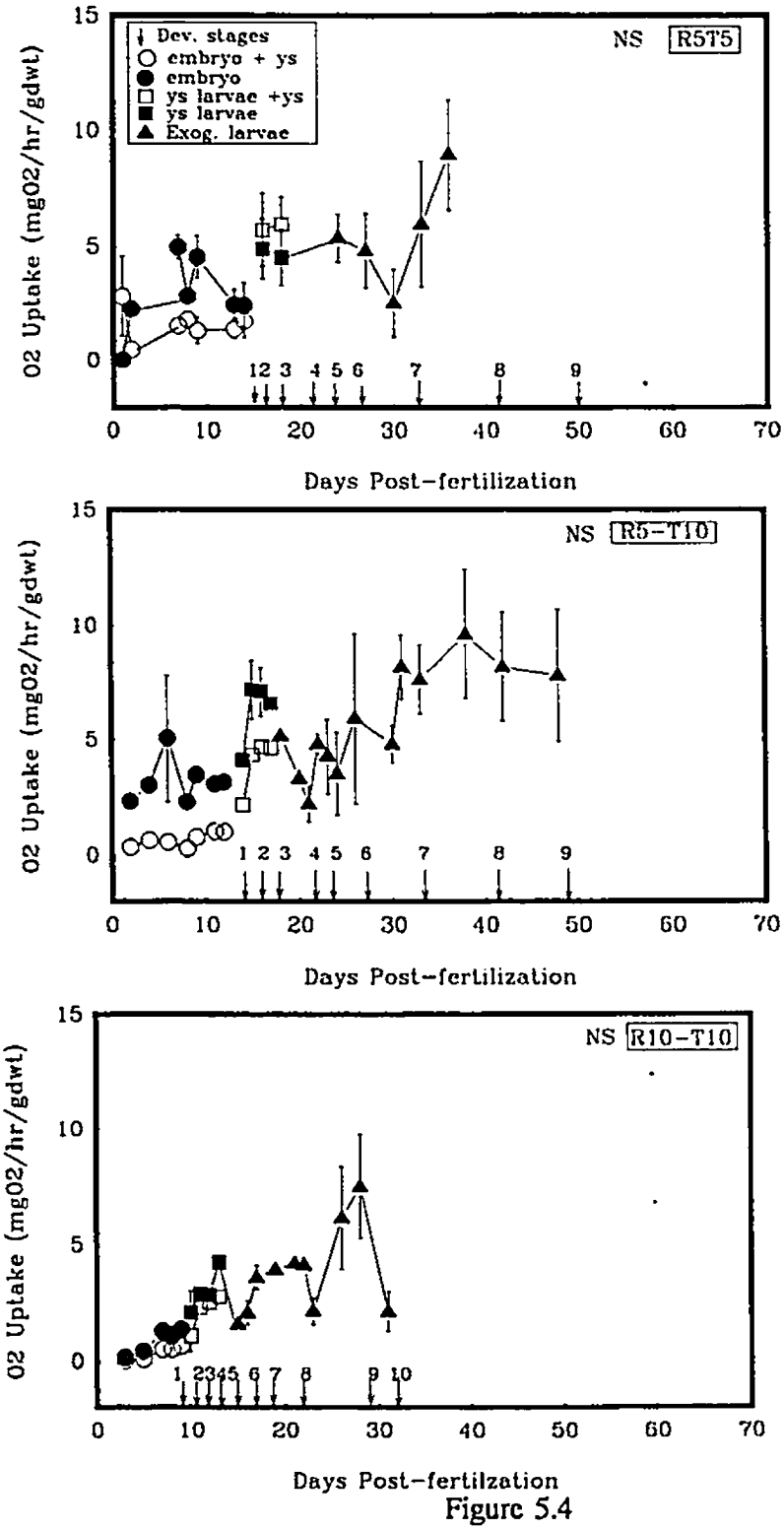

Dev. stages
embryo + ys
embryo
ys larvae +ys
ys larvae
Exog. larvae
NS R5T5
NS R5-T10
NS R10-T10
02 Uptake (mg02/hr/gdwt)
Days Post-fertilization

Figure 5.4

Figure 5.5 Mass specific oxygen uptake ($mgO_2/hr/gdwt$) vs days post–fertilization for three different temperature treatments, R5T5 (raised at 5°C and tested at 5°C), R5T10 (raised at 5°C and tested at 10°C) and R10T10 (raised at 10°C and tested at 10°C), for the Newfoundland (NF) population of Atlantic cod. Numbers represent developmental stages defined in Chapter 1. ys = yolk–sac Vertical error bars represent ± 1 standard error

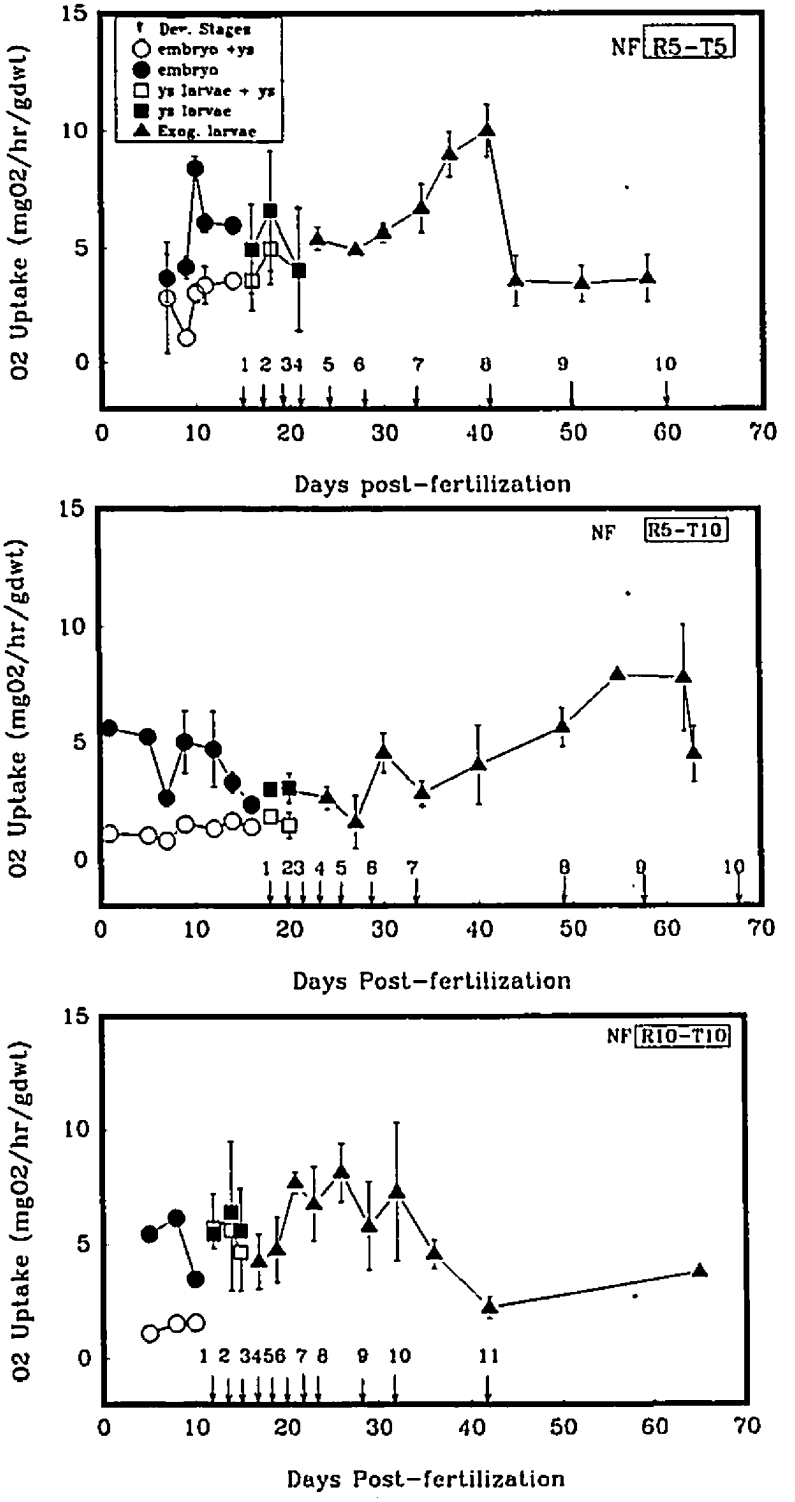

Dev. Stages
embryo +ys
embryo
ys larvae + ys
ys larvae
Exog. larvae
NF R5-T5
O2 Uptake (mgO2/hr/gdwt)
Days post-fertilization
NF R5-T10
O2 Uptake (mgO2/hr/gdwt)
Days Post-fertilization
NF R10-T10
O2 Uptake (mgO2/hr/gdwt)
Days Post-fertilization

Figure 5.5

Table 5.2 : The cost of hatching (mgO_2/hr/gdwt) in Atlantic cod, Gadus morhua, at three different temperature R5T5 (raised and tested at 5°C), R5T10 (raised at 5°C and tested at 10°C), and R10T10 (raised and tested at 10°C).

Temperature Treatment	Cost of hatching (mgO_2/hr/gdwt)
NS R5T5	3.27
NF R5T10	3.38
NF R10T10	2.89
NF R5T5	−1.89
NF R5T10	0.69
NF R10T10	2.1

remaining) mass-specific oxygen uptake again increased, as larvae increasingly relied on obtaining exogenous food supplies for energy.

Patterns of mass-specific oxygen uptake were highly variable and depended on temperature in the exogenous feeding period. In general, mass-specific oxygen uptake increased throughout the exogenous feeding period until developmental stages 8 or 9 (in stage 8 gill filaments are numerous and by stage 9 secondary lamellae had formed and buccal pumping had begun (see Ch. 3)). Thereafter, (i.e. after stage 10) as the larvae approached transformation, levels of mass-specific oxygen uptake decreased (Figure 5.4c and Figures 5.5 a-c) and approached that seen in the initial stages of larval development.

The highest rates of mass-specific oxygen uptake occurred during the exogenous feeding period (Figures 5.4 and 5.5 (a-c)). The extent to which rates of mass-specific oxygen uptake increased during this period, compared to those during the embryonic period, differed between populations. Mean embryonic rates of mass-specific oxygen uptake were significantly higher in the NF population than in the NS population (ANOVA, $F = 2.92$, $p < 0.05$) (Table 5.3). However, the mean maximal larval rates of mass specific oxygen uptake were not significantly different between populations or temperature treatments (ANOVA, $F = 0.01$, $p > 0.05$) (Table 5.3). In addition, the highest rates of oxygen uptake were recorded between stages 7 and 9 for both populations and temperatures (Table 5.3). The largest increases in mass-specific oxygen uptake occurred in the NS population (Table 5.3). However, increases in metabolic rates appeared to be independent of temperature for both populations.

Table 5.3 : Mean embryonic oxygen uptake (mgO_2/hr/gdwt), highest mass specific oxygen uptake (O_2/hr/gdwt), factor of increase and developmental stage at which the highest metabolic rates occurred.

Temp. Treatment	$\bar{X}$ Embryonic Rate (mgO_2/hr/gdwt)	Max. Larval Oxygen Uptake (mgO_2/hr/gdwt)	Factor of Increase	Developmental Stage
NFR5T5	5.7± 0.82	10.05±1.1	1.77	8
NFR5T10	4.13±0.50	7.92±0.3	1.91	8 – 9
NFR10T10	5.02±0.80	8.11±1.3	1.61	8 – 9
NSR5T5	3.27±0.5	8.9±1.5	2.74	7 – 8
NSR5T10	3.23±0.3	9.6±1.5	2.97	7 – 8
NSR10T10	1.23±0. 23	7.56±0.2	6.14	8 – 9

(b) Changes in metabolism with growth

To determine the effects of temperature on the relationship of metabolism with growth, the allometric power relationship ($VO_2 = aM^b$) was used. The log of absolute oxygen consumption ($\mu gO_2/hr$) was regressed against log dry weight for both populations and for all temperature treatments (for the NS population: R5T5 at the embryonic stage (e); $r^2 = 0.28$, $p > 0.05$, and for the larval stage (l); $r^2 = 0.20$, $p > 0.05$; for R5T10 (e) $r^2 = 0.81$, $p < 0.05$; for R5T10(l), $r^2 = 0.5$, $p < 0.05$; for R10T10(e), $r^2 = 0.90$, $p < 0.5$; for R10T10, $r^2 = 0.51$, $p < 0.05$; for the NF population: for R5T5(e), $r^2 = 0.94$, $p < 0.05$; for R5T5(l), $r^2 = 0.40$, $p < 0.05$; for R5T10(e) $r^2 = 0.5$, $p = 0.08$; for R5T10(l) $r^2 = 0.60$, $p < 0.05$; for R10T10(e) $r^2 = 0.86$, $p = 0.10$; for R10T10(l) $r^2 = 0.73$, $p < 0.05$; Figure 5.6 a–f). Embryonic and larval periods were treated separately.

In the embryonic period, acute temperature change significantly reduced the metabolic mass exponent (b), compared to those determined from the non-stressed treatments (i.e 0.89 and 0.54 for NSR5T10 and NFR5T10 respectively, versus 2.00 and 1.37 for NSR10T10 and NFR5T5 respectively (t–test; 3.53, $p < 0.05$, for NS) and (t–test; $t = 2.59$, $p < 0.05$, for NF). Note that for the embryonic period in NFR10T10 and NSR5T5, the relationships between oxygen uptake and dry weight were not significant and could not be used in the above comparisons.

For comparisons of mass exponents between the embryonic and larval periods, embryonic mass exponents were significantly higher than those of the larval period in 2 cases (i.e. NFR5T5 and NSR10T10) out of 4 (t–test; $t = 1.86$, $p < 0.05$, for NF) and (t–test; $t = 9.39$, $p < 0.001$, for NS). Note that for the embryonic period in NFR10T10 and

NSR5T5, the relationships between oxygen uptake and dry weight were not significant and could not be used in the above comparisons. In NSR5T10, and NFR5T10, acute temperature change may have affected both the embryonic and larval periods to the same extent as there were no significant differences in metabolic exponents between the periods (t–test; $t = 0.71$, $p > 0.05$, for NF) and (t–test; $t = 0.35$, $p > 0.05$, for NS).

In contrast, during the larval period, acute temperature change significantly increased the metabolic exponents (i.e. 0.93 and 1.33 (NSR5T10 and NFR5T10 respectively), compared to those in the non–stressed treatments, (i.e. 0.79, 0.59 and 0.60 (NSR10T10, NFR5T5 and NFR10T10 respectively) (t–test; $t = 18.39$, $p < 0.001$, for NS) and (t–test; $t = 10.57$ and $t = 16.63$, $p < 0.05$, for NF) (Figure 5.6 (b–f)). In the NF population, no significant differences existed between the metabolic mass exponents of larvae raised and tested at 5°C, and those raised and tested at 10°C (t–test; $t = 0.05$, $p > 0.05$ for NF). Note that the relationships between oxygen uptake and dry weight in the larval period were not significant for NSR5T5 and could not be used in the comparisons.

Metabolic mass exponents determined from Figures 5.6 (a–f) are representative of the relationship between respiration and growth for the entire embryonic and larval periods. However, partitioning of total energy to growth and metabolism may change with development and growth throughout each period. Therefore, the larval period was divided into three periods: (1) yolk–sac, (2) transition (mixed feeding) stage and (3) the exogenous feeding stage that correspond to the periods defined in Ch. 4. Data were too few to further divide the embryonic period. Table 5.2 shows the energy equivalents for total growth and total metabolism in Joules for each developmental period; and the

Figure 5.6 Regressions of log total oxygen uptake (mgO_2/hr) vs log dry weight (μg) for three different temperature treatments, R5T5 (raised at 5°C and tested at 5°C), R5T10 (raised at 5°C and tested at 10°C) and R10T10 (raised at 10°C and tested at 10°C), for the Newfoundland (Nf) and Scotian Shelf (NS), populations of Atlantic cod.

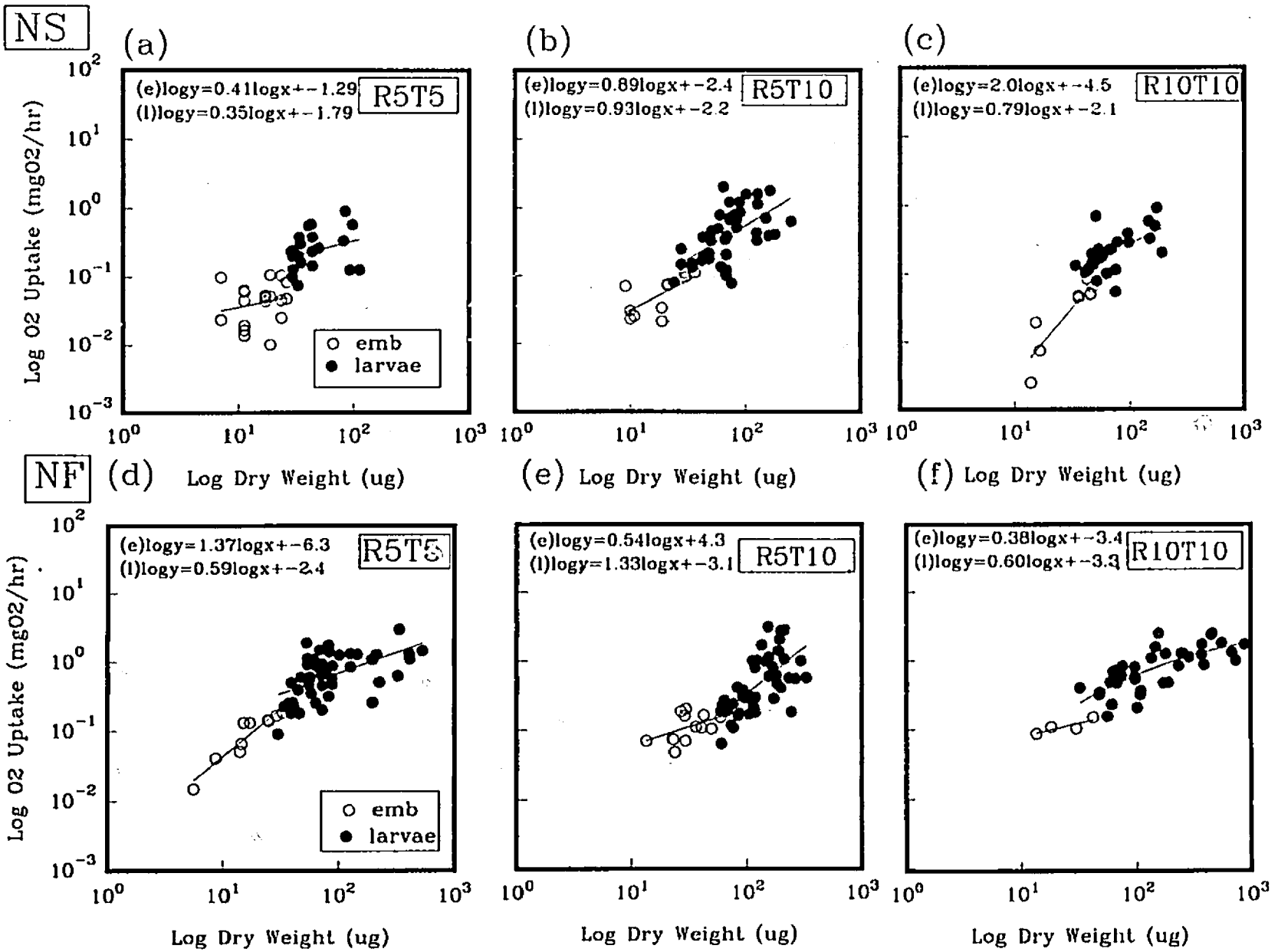

NS
(a)
(b)
(c)
(e)logy=0.41logx+-1.29
(l)logy=0.35logx+-1.79
R5T5
(e)logy=0.89logx+-2.4
(l)logy=0.93logx+-2.2
R5T10
(e)logy=2.0logx+-4.5
(l)logy=0.79logx+-2.1
R10T10
emb
larvae
Log 02 Uptake (mg02/hr)
NF
(d)
(e)
(f)
Log Dry Weight (ug)
(e)logy=1.37logx+-6.3
(l)logy=0.59logx+-2.4
R5T5
(e)logy=0.54logx+4.3
(l)logy=1.33logx+-3.1
R5T10
(e)logy=0.38logx+-3.4
(l)logy=0.60logx+-3.3
R10T10

Figure 5.6

percent of total metabolism allocated between growth and other metabolic activities in both populations and in all three treatments.

In general, the percent of total energy budget allocated to growth in the yolk-sac period was high (with the exception of NSR5T5) and ranged from 21% to 74%, suggesting that growth is a priority at this stage (Table 5.4). In the mixed feeding period, the proportion of energy allocated to growth decreased in all cases (with the exception of NSR10T10) and the proportion of energy diverted to other metabolic activities such as locomotion and feeding increased (again with the exception of NSR10T10; Table 5.3). During the exogenous feeding period, the percent of total oxygen uptake associated with growth increased over that of the mixed feeding period and reached approximately 50% in NFR10T10 and NFR5T5 and approximately 30–40% in NFR5T10, NSR10T10 and NSR5T5 (Table 5.4). In NSR5T10, percent of oxygen uptake associated with growth was extremely low in the mixed feeding stage (4%) and was at least 10% lower in the exogenous feeding period than in all other cases.

Acute temperature increases resulted in the reduction of energy available for growth (Table 5.4). This was evident in the transition and exogenous feeding stages of NSR5T10 and NFR5T10, in which 69% (NFR5T10) and 83% (NSR5T10) of total energy was diverted away from growth to other metabolic activities. In the non-stressed treatments (NFR5T5 and NFR10T10) the NF population allocated higher proportions of total energy to growth compared to the NS population (NSR5T5 and NSR10T10). This corresponds to the higher growth rates found in the NF population in Figures 5.3(a–b) and Table 5.1.

Table 5.4 : Growth efficiences G(/G+R) and metabolism R(/R+G) of three developmental stages: YS (yolk-sac), MF (mixed feeding stage) and Ex (exogenous feeding stage), in two populations of Atlantic cod, Gadus morhua. NF=Newfoundland, NS=Scotian Shelf

NF

	R5T10		R10T10		R5T5	
Stage	G/(G+R)	R/(R+G)	G/(G+R)	R/(R+G)	G/(G+R)	R/(R+G)
YS	46%	54%	74%	26%	22%	78%
MF	25%	75%	44%	56%	22%	78%
EX	31%	69%	45%	55%	53%	48%

NS

YS	49%	51%	30%	70%	21%	79%
MF	4%	96%	50%	50%	13%	87%
Ex	20%	83%	40%	60%	36%	64%

DISCUSSION

In this study, I described the characteristics of growth and metabolism of two populations (Newfoundland and Scotian Shelf), considered to be genetically discreet (Pogson 1993). In addition, I investigated differences in growth and metabolism between developmental periods (e.g embryonic and larval) and within developmental periods (e.g. yolk–sac, mixed feeding, and exogenous feeding stage). The results of these experiments advance a presently small body of research on: (1) energetic differences between populations, (2) the effects of environmental factors on energetic balances and (3) the stages during development which are most susceptible to changes in environmental factors.

Growth

Estimates of larval growth are often used as an index of the general condition or health of a cohort. Decreasing growth rates usually indicate imminent starvation, during which time fish larvae burn body tissue for energy (Kamler 1992). To date, most studies have investigated growth of fish larvae up to and just beyond the end of yolk–sac utilization and hence during starvation. In this study, growth was investigated throughout exogenous feeding in two populations (Newfoundland and Scotian Shelf), during which dry weight increased and no symptoms of starvation were evident. Under these conditions growth rates of the Newfoundland population were significantly higher than those of the Scotian shelf (Figure 5.3). High growth rates in the Newfoundland population began in the embryonic period and were maintained, albeit at a reduced level,

during the larval period. Thus, faster growing embryos hatch at larger sizes and maintain this size advantage throughout larval life (Shirota 1970). Faster growth and larger size endow larvae with three potential advantages: (1) faster growth allows quick passage through the periods of high vunerability to predators (Calow 1989), (2) larger individuals can swim faster and hence are more likely to capture prey and evade predators (Hunter 1981) and (3) larger, faster growing larvae may utilize their food supplies more efficiently for growth than smaller ones (Blaxter and Hempel 1966, Solberg and Tilseth 1988).

Over the entire larval life, the highest specific daily growth rates were found in the embryonic and yolk-sac stages (Table 5.2). Since it is during these periods that mortality is the highest (Hewitt et al. 1985.), growing faster and reducing the time over which mortality operates will likely increase survivorship (Calow 1989). In contrast, the lowest specific daily growth rates were found in the exogenous feeding stage. During this period, deficiencies in the energy budget occur, as endogenous resources are exhausted and exogenous feeding rates dictate growth (Solberg and Tilseth 1984). Energy deficits may have occurred in the Scotian Shelf larvae, as they did not develop or grow as well as those of the Newfoundland population (see also Ch. 1). Such differences during the exogenous feeding period may have been due to food availability or type (see Ch. 1). Although food concentrations in the tanks were kept high throughout all the experiments, variations in the type of wild caught zooplankton fed to the larvae probably occurred across seasons. This may have resulted in a growth advantage to the Newfoundland larvae which were raised in the late spring when zooplankton diversity was at its highest (I. McLaren personal communication). Alternatively, inter-population differences in

growth rates may reflect inherent genetic differences between the populations.

Growth rates of fish larvae are also affected by temperature (Blaxter 1992). In this study growth rates in both populations increased with temperature (Figure 5.3). Specific daily growth rates at 5°C were approximately half of those at 10°C (Table 5.1). These rates agreed quite closely with previously published estimates for Atlantic cod larvae (i.e. 6.67 to 8.75% per day at 7°C and 10°C respectively; Laurence 1978). However, Laurence (1978) found that both haddock and cod had depressed rates of growth at 4°C and that successful metamorphosis did not occur at this temperature. Thus sensitivity to temperature may also be a reflection of inherent population differences. Laurence (1978) studied cod and haddock larvae spawned by adults from Georges Bank. Sea water temperatures are higher on average on Georges Bank (5–15°C; Flagg 1987) than those on the Scotian Shelf (3–12°C; Griffin and Lochmann 1992) or off the coast of Newfoundland (1–5°C; Lear and Green 1984). Therefore, it is likely that cod and haddock larvae from Georges Bank are not well adapted for growth at low temperatures. However, both Scotian Shelf and Newfoundland cod larvae often experience 5°C and grow well at this temperature. Hence these findings suggest that preferred temperatures (sensu Fry 1947, 1971, i.e. the temperature chosen by a fish when it is placed in temperature gradient) exist for larval cod from different populations, and that temperature sensitivity reflects adaptation of populations to specific environmental regimes.

Metabolism

Changes in metabolism with development

Comparison of the mass-specific growth rates removed the effect of body size, so that patterns of metabolism throughout larval life could be observed. The present study is the first to track changes in mass specific metabolic rates over time (from the embryo to metamorphosis), linking changes in metabolism with development and behaviour. This has been made possible by a detailed study of form and function of Atlantic cod larvae at 5 and 10° C (see Ch. 1). In addition, activity patterns from hatching to exogenous feeding have been determined (see Ch. 4), and energy allocated to activity throughout larval development can be accounted for.

Results from the present study show that small amounts of energy are spent on the process of hatching, but that cost of hatching is independent of temperature (Table 5.2). Larvae from the Newfoundland population were more efficient at hatching and spent less energy on this critical, but potentially costly, process (Holliday et al. 1964, Eldridge et al. 1977). Since energy for hatching is obtained from the combustion of the yolk, excessive utilization would reduce the amount of energy available for growth and activity in later stages. Davenport and Lonning's (1980) estimates of the mass specific oxygen uptake of hatching (1.2 mgO_2/hr/gdwt) closely approximated those of the present study (mean for NS = 3.18 mgO_2/hr/gdwt and for NF = 1.4 mgO_2/hr/gdwt). Therefore, estimates of the energy consumed at hatching determined from the present study along with those from Davenport and Lonning (1980), confirm that Atlantic cod spend much less energy on hatching than other species such as plaice and herring (Holliday et al 1964,

Eldridge et al 1977, Davenport and Lonning 1980). Differences in cost may be partly due to the thinner chorion of Atlantic cod eggs (7μm; Davenport and Lonning 1980) versus the thicker and presumably tougher chorion of other species (Lonning 1972). As a result, for Atlantic cod, cost of hatching probably does not represent a significant proportion of the energy budget.

Following hatching, mass specific metabolic rates increased rapidly during the yolk-sac period (Figure 5.4 (a-c) and Figure 5.5 (a-b). This increase is probably due to energy expended for growth, as growth rates are still high at this stage (Table 5.1). In addition, some energy may also be expended in differentiation and development of organs and structures for feeding (see Ch.2). Activity during the yolk-sac period comprises only a small proportion of the total oxygen consumption (Ch.4) and, therefore, cannot explain increases in mass specific metabolic rate during the yolk-sac period.

Patterns of metabolic rate change in the yolk-sac period, described in the present study, generally agree with results from previous studies (Davenport and Lonning 1980, Solberg and Tilseth 1984, 1988). However, results differ as to the patterns of metabolic rates at yolk-sac extinction. In the present study, metabolic rates decreased at yolk-sac extinction (Figures 5.4 and 5.5 (a-c)). In contrast, Solberg and Tilseth (1988) found that the highest metabolic rates occurred at yolk-sac extinction in unfed cod larvae. Wieser et al. 1988 suggested that high metabolic rates in poorly fed cyprinid larvae may reflect a higher motivation for food searching activity. In the present study, food was introduced to the larvae shortly after hatching, and mixed feeding (i.e. yolk-sac plus exogenous food) supported growth during the yolk-sac and mixed feeding stages. In other studies in

which food was introduced to yolk–sac larvae, oxygen consumption also accelerated after a short–term depression during the transition from endogenous to exogenous feeding (Eldridge et al. 1982, De Silva et al. 1986, Kamler 1992). Although supplying exogenous food to larvae prior to first–feeding may increase activity (Solberg and Tilseth 1988) (i.e. reducing energy for growth), many would agree that introducing food well before yolk–sac absorption usually significantly enhances larval survival (Eldridge et al. 1977, MacCrimmon and Twongo 1980, Heming et al. 1982, Pedersen et al. 1987, Appelbaum 1989). Although, effects of pre–feeding may also differ between species, energy balances during early larval life are likely influenced by food availability and feeding success. Evidently, larvae may enter the exogenous feeding stage with either an energy deficit or surplus, depending on their feeding success and energy efficiency during the yolk–sac and mixed feeding stages (also see Solberg and Tilseth 1984).

The highest metabolic rates observed in this study occurred in the exogenous feeding period in all temperature treatments and in both populations (Figures 5.4 and 5.5 (a–c)). High rates of metabolism are probably a reflection of the combined cost of growth (including development) and activity associated with locomotion and feeding. The less than twofold increase in metabolic rates found in the Newfoundland population versus the 3–to–6 fold increase in metabolic rates in the NS population suggests that the NS population is much less energy efficient (Table 5.3). As the extra energy was not reflected in increased growth in the NS population, energy must have been spent on other metabolic activities such as locomotion. This is also supported by evidence from Ch. 4, which showed that swimming was more energetically expensive for Scotian Shelf larvae

than for Newfoundland larvae (Ch. 4, Figure 4.3).

Maximal rates of mass specific oxygen uptake occurred at around the same developmental stage (i.e stage 7–9), and did not differ significantly between populations or temperature treatments (Table 5.3). Development between stages 7 – 9 is characterized by the proliferation of gills and secondary lamellae and increases in complexity of the alimentary tract (see Ch. 1). In addition, high levels of activity (>40% of total energy expenditure, Ch.4) occur, as larvae increase their rates of locomotion and foraging for exogenous food supplies (see Ch. 4). Therefore, high metabolic rates are associated with procurement and assimilation of exogenous food. Moreover, the development of gills and secondary lamellae may accelerate in response to higher energy demands. These demands, coupled with decreasing surface area–to–volume ratios (due to growth), necessitate the transition from cutaneous to branchial respiration (see Chs. 3 & 4). Therefore, during the exogenous feeding period, maintenance of an energy surplus, in the form of growth, relies upon the capacity to capture food and combust it for energy. Some studies (Eldridge et al. 1981, Bergot et al. 1989, Kamler et al. 1990) have shown that high temperatures and high prey abundance (i.e. suitable prey), accelerate differentiation of both morphological structures and internal organs of fish larvae, enabling them to pass through this vulnerable period of life more rapidly.

Changes in metabolism with growth

Effects of growth on metabolism of eggs and fish larvae have been extensively studied since the 1960's (Holliday et al. 1964, DeSilva and Tytler 1973, Laurence 1978, Cetta and

Capuzzo 1982, Houde and Schekter 1983, Almatar 1984, Theilacker 1987, Giguere et al. 1988). Despite the improvements in methodologies to accurately measure oxygen uptake, disagreements over whether metabolism scales allometrically ($b \neq 1$) or isometrically ($b = 1$) with growth, still abound in the literature (see Giguere et al. 1988). In the present study, the metabolic mass exponents for the embryonic period in Atlantic cod ranged from 0.54 to 2.00, with a mean of 1.2. Since it is difficult to isolate embryonic tissue from yolk-sac tissue in unpreserved specimens, underestimates of embryonic weights could have resulted in the very high metabolic exponents. Eggs are regarded as closed systems in which activity is considered to be minimal (Rombough 1988b). Despite this, only a few estimates of metabolic mass exponents have been made for embryos. These estimates range from below 1.0 (Kiorboe and Mohlenberg 1987) to significantly above 1.0 (Kamler 1986, Rombough 1988a). However, in the study by Kiorboe and Mohlenberg (1987), yolk-sacs were not separated from the embryonic tissue. Since the yolk is considered to be non-metabolizing tissue (Rombough 1988b), the embryo must be isolated in order to correctly determine mass- specific metabolic rates. If only embryonic mass is used in calculations of mass-specific oxygen uptake, mass exponents are high (usually above one) compared to those of larval stages (Rombough 1988b).

Estimates of the mass exponents for the larval stage are numerous and range from 0.65 (DeSilva and Tytler 1973) to equal or greater than one (see Giguere et al. 1988). In the present study, metabolic mass exponents ranged from 0.59 to 0.79 in the non-stressed temperature treatments, were independent of long-term or chronic temperature changes, and were not significantly different between populations (Figure 5.6). However, mass

exponents approximated one and were greater than one in those treatments in which larvae were exposed to an acute 5°C temperature increase (Figure 5.6). Elevated metabolic rates in these experiments probably resulted from increases in activity, which may have reflected a stress–related response.

Giguere et al. (1988) advocate that routine metabolic rates scale isometrically (i.e. b=1) in fish larvae. Through extensive recalculation of previous work and through further experimentation of their own, Giguere et al (1988) arrived at the conclusion that the metabolic mass exponent (b) equalled one. However, on close examination of papers cited therein, it became apparent that the high exponents used in their evaluations were probably due to stress (e.g. Laurence 1978). In addition, their own techniques may have resulted in higher stress related metabolic rates than herein. For example, their chambers were static, boundary layer conditions were undetermined, and the respirometers were stirred using a glass bead only once prior to measurement. Although they checked for stress related to stirring shortly after the experiment had begun, stress – induced metabolic rates can be maintained at high levels for over an hour, as was discovered in the present study. Utilization of long–term respirometric techniques such as the twin–flow respirometer developed by Gnaiger (1983), may help resolve the dispute. Note that Kauffman (1990) using a modified version of the twin–flow respirometer found that mass exponents for routine metabolism in cyprinid larvae approximated 0.8.

Energy Budgets

The above relationships between growth and metabolism are further complicated by

the existence of separate developmental stages within the larval period and the relative importance of activity to these stages. Construction of energy budgets for three stages: yolk-sac, mixed feeding stage and exogenous feeding stage took these factors into consideration (Table 5.4). Results show that in most instances the contribution of activity increased with stage, while contribution to growth was variable between stages (Table 5.4). High net growth efficiencies in the yolk-sac period reflect the high specific daily growth rates and the low contribution of activity to total oxygen uptake (Ch. 4). High proportions of total energy expended on metabolism (R / (R + G)), evident in the exogenous feeding stage, reflect the high proportion of total oxygen uptake associated with increasing activity (see Ch. 4). Net growth efficiencies ranged from 30 to 50 % in the exogenous feeding period, and approximate net growth efficiences estimated in other studies (Solberg and Tilseth 1984, Cetta and Capuzzo 1982, Houde and Schekter 1983, Rombough 1988b).

The greater allocation of energy to metabolism in acutely stressed larvae of both populations support the result that acute temperature change elevates levels of metabolism, probably resulting from increases in activity. The temperature-independence of net growth efficiencies suggest that both NF and NS larvae can grow well at 5 and 10°C. However, overall net energy budgets confirm that the Newfoundland larvae are significantly more energy-efficient (i.e. devote a greater proportion of energy budget to growth) than those of the Scotian Shelf. One of the reasons for this may be that Newfoundland larvae seem to be more cost-effective swimmers (see Ch. 4). In addition, inherently faster growth rates may give them advantages that they carry throughout the

rest of larval life.

CONCLUSIONS AND FUTURE DIRECTIONS

In this thesis I have gathered and integrated information from the fields of functional morphology, physiology and ecology, in order to better understand the way in which cod larvae interact with their environment. I believe that utilizing such an interdisciplinary approach will more fully elucidate the factors that determine cod larval survival.

Two major objectives were accomplished in the present study. The first was to stage and describe the morphology and functional morphology of feeding and respiratory structures throughout cod early life history, in order to document structures and functions related to feeding and respiration in cod larvae, two processes that enable the animal to acquire energy. The results were presented in Chapters 1 through 3.

The second major objective was to determine how the energy obtained through feeding and respiration was allocated to growth and metabolism (primarily metabolism related to feeding and locomotory activity), and was to determine if energy allocation varied (1) as a function of temperature and/or (2) among genetically discrete populations (i.e. Newfoundland and Nova Scotia). The results were presented in Chapters 4 and 5.

The staging sequence for cod larvae described in Chapter 1 extends earlier work by providing a complete sequence for cod larvae. Results of staging at two different temperatures showed that staging is a more accurate way of determining developmental state than days post hatch or degree days. In addition, staging of structures such as the alimentary tract illustrated that variation in structural complexity may be influenced by interactions of intrinsic (genetic) and extrinsic (environmental) factors.

Increases in structural complexity have been linked to changes in function (Govoni et al 1986, Liem 1991) and to the potential for survival in fish larvae (O'Connell 1981, Theilacker 1986, Sieg 1992b). Therefore, one may expect that differences in structural complexity between (1) larvae of genetically different populations and (2) larvae raised at different temperatures, may be indicative of their relative probability for survival.

As a result of this study, changes in key structures or 'landmarks' have now been described for the larval period of cod. However detailed descriptions are still necessary for the transformation or metamorphic period in these animals. Transformation or metamorphosis is an important period in the early life of cod, as it is during this period that cod must adapt to a number of potentially stressful changes associated with the transition from a planktonic to a demersal environment. In addition, it has been suggested that survival through the transition from the larval to juvenile stage (in addition to survival through the larval period), may influence year class strength and adult population size (Hunter 1981, Leis 1991).

In the second chapter, description of cod larval stages was taken a step further by detailing the morphology of feeding and respiratory structures. Descriptions of the skeletal organization of the head showed that in cod yolk-sac larvae, viscerocranial structures are simple and non-integrated and that the yolk-sac serves as an endogenous food reserve. With growth, requirements for exogenous food are met by the progressive enlargement of the skeletal elements in addition to the development of structures such as the opercular apparatus. Together these changes allow an increase in jaw movement coordination and suction pressure generation necessary for prey capture. As fish size

increases, surface area to volume ratios decrease (Blaxter 1988), eventually necessitating a transition from cutaneous to branchial respiration. Thus, growth of the opercular apparatus and associated muscles and the integration of structural elements facilitates the transition from cutaneous to branchial respiration in order to meet metabolic requirements.

In Chapter 2, I focused on the changes in organization and composition of skeletal elements in the larval fish head throughout development. Some major muscles thought to be associated with the movement of the head were briefly described. However, further detail as to muscle cross-sectional area, muscle origin and insertion points would be invaluable. This would allow the derivation of product-moment relationships in the larval fish head, and as a result one could then estimate the theoretical forces that would be produced by viscerocranial structures during feeding and respiration.

In Chapter 3, kinematic mechanisms of feeding and respiration were described in detail. This was possible only after first describing the static anatomical make-up of the larval cod head in Chapter 2. Results reported in Chapter 3 showed that the 'hyoid period' described the primary mechanism responsible for mouth opening in the early part of larval life. As ontogeny progressed, new structures developed and become integrated or 'coupled' by the differentiation of ligaments or muscles. These changes characterized the 'opercular period' which was responsible for mouth opening in the latter part of the larval life, as well as in the juvenile and adult stages.

At present, mechanisms of feeding and respiration in larval fish can only be studied using reconstructive morphology in concert with video image analysis. In juvenile and adult fish, feeding and respiratory kinetics can be determined through invasive techniques

such as electromyography. In the future, development of micro-transducers and micro-pressure probes may aid in determining the sequence of musculoskeletal pathways that produce feeding and respiratory movements in larval fish. In so doing we may be able to deduce whether differences in feeding and respiratory mechanisms found between species in the present study reflect phylogenetic and/or environmental differences.

Chapters 4 and 5 describe how energy acquired through feeding and respiration is allocated to growth and activity metabolism throughout development. Experiments in Chapter 4 reveal that the proportion of total metabolism allocated to activity increased with growth and corresponded to the increasing energy demand in larval cod throughout development. However, larvae from the Newfoundland populations were more cost-effective and spent less energy on equivalent activities than larvae from Scotian Shelf populations. In addition, Newfoundland larvae appeared to be 'cold-adapted' and cost of transport was high when they were chronically exposed to temperatures much higher than they would normally encounter. In contrast, larvae from the Scotian Shelf population appeared to be 'warm-adapted', and they were less likely to suffer mortality if temperatures are high and remain stable throughout larval life. Scotian Shelf larvae may, however, be particulary sensitive to acute temperature increases. Such inter-population differences in energy allocation may be genetically determined. Additional support for this hypothesis originates from the results in Chapter 5.

In Chapter 5, in addition to inter-population differences in growth rates (also shown in Chapter 1), overall net energy budgets confirmed that the Newfoundland larvae are more energy efficient than those of the Scotian Shelf. Newfoundland larvae allocated a

higher proportion of total energy to growth while also being more cost–effective swimmers. In addition, the greater allocation of energy to activity metabolism in acutely stressed larvae of both populations supported the result that acute temperature change elevates rates of metabolism, probably as a result of increased activity as larvae search for their environmental preferendem (Fry 1971).

In summary, the energy budgets determined above, in concert with detailed knowledge of development, lay the foundation for the development of a future multivariate bioenergetic model. In addition, the differences in energy allocation throughout development, between temperature treatments and among populations, will likely require that future bioenergetic models accommodate these differences.

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