Individual variability in the behaviour and morphology of larval Atlantic cod (*Gadus morhua*, L.)

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Candidate's Declaration

I declare that the work recorded in this thesis is entirely my own work and that it is my own composition. No part of this work has been submitted for any other degree.

Felicity Huntingford commented at length on all chapters, Christopher Cutts commented on chapters detailing experiments carried out at SAMS Ardtoe and Øyvind Aas-Hansen and Borge Damsgård commented on the chapter detailing the experiment carried out at the Aquaculture Research Station, Tromsø.

Heather Forbes, December 2007

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Thesis Summary

Individuals both within and between populations can vary markedly in a number of traits, including behaviour, life-history patterns and morphology. These differences do not simply reflect noise around a mean, but reflect real and important variability that can have a number of important implications. Such individual variability is especially prevalent among larval fish, which undergo significant changes in size, anatomy, physiology and morphology as they develop into adults.

The potential for fish to develop differing behaviours and morphologies has important implications in the aquaculture environment. For example, some fish may be aggressive and/or cannibalistic while others are not, or some fish may have a propensity to take risks while others shy away from risk. Elucidation of the mechanisms underlying these differences could enable the farmer to mitigate the development of behaviours and morphologies that are not conducive with welfare and production. Such information would be especially useful in the rearing of species such as Atlantic cod, which are highly cannibalistic in the larval and early juvenile period of development.

The purpose of the work outlined in this thesis was to elucidate certain aspects of individual variability in predominantly larval cod that relate to the culture of this species. An introduction to the subject area is provided in Chapter 1. Fish husbandry techniques followed the standard procedure employed at the two study sites and are described in detail in chapter 2. This chapter also describes the morphometric technique used to analyse morphology, principal component analysis of linear measurements, and discusses the reasons for adopting this technique.

Chapters 3 and 4 examine morphological development in larval cod reared under standard culture conditions and using common commercial feeds, for the purpose of elucidating developments in trophic morphology that could potentially relate to the development of cannibalism in this species. Chapter 3 specifically examines patterns of change in head shape in larval Atlantic cod and the extent to which head development varies within a cohort, while chapter 4 examines the effect of diet on the development of head morphology in larval cod.

The former of these studies identified clear and consistent patterns of growth in various measures of head structure (and especially eye diameter). This was with the exception of jaw width, which developed in opposition to these measures. Periods of rapid change in head morphology coincided with points at which the larval diet changed and may have been caused by this change. Growth of the head and the post cranium was highly variable, especially in the latter stages of larval development and investment in head growth relative to post-cranial growth increased over the first two thirds of larval development, remaining constant thereafter.

The second of these studies found that fish fed different prey types developed different head morphology. Specifically, fish fed small prey developed more fragile heads and larger eyes relative to jaw width than fish fed larger prey. Analysis of the head morphology of dead fish indicated that at least some of these differences resulted, not from the death of certain morphotypes, but from a phenotypically plastic response to the different diets. The morphology of a small number of cannibalistic larvae analysed during the study indicated that fish fed the larger prey developed morphology comparable with that of cannibalistic morphs.

In the study detailed in chapter 5, aggressive interactions in larval cod were quantified in order to determine whether these interactions represented an early form of cannibalism or a battle for resources. Attacks where characterised by brief, one-way, nips by an attacker to a victim. Fish also commonly exhibited a pattern of burst swimming (darts) that appeared to reflect a generalised escape response. This darting behaviour was not affected by the presence of food, but was more common in fish fed the higher prey densities. Conversely, overall levels of prey did not affect the incidence of aggressive attacks, although analysis was confounded by a decline in levels of aggression with increasing fish density. The frequency of nips was highest when food was absent and nips were preferentially directed at the tail of victims, to victims of a smaller or similar size than the attacker and to victims that showed abnormal body posture. These findings indicated that at least some attacks by larval cod represented an early attempt at cannibalism.

Chapter 6 details a study in which differences in the risk taking behaviour of one-year old cod of different stock and/or family origin were examined. Fish of North-eastern Arctic stock origin were found to be more prone to take risks than fish of Norwegian coastal stock origin. Furthermore, although there were no significant differences in risk-taking between families of

North-eastern Arctic stock origin, a weakly significant difference existed between families of fish originating from coastal stock. The weight and condition of fish was significantly smaller in fish that emerged to escape than in fish that avoided risk and these factors may have contributed to the observed behavioural differences between stocks and families. Cortisol levels did not vary between risk avoiders or risk takers, but were significantly higher in control fish of North-eastern Arctic stock origin compared to control fish of coastal origin. These results provided evidence for a heritable component to risk-taking in cod.

The results of the aforementioned studies have important implications, particularly for the culture of cod and these implications are discussed in Chapter 7, together with a summary of the objectives and findings of each study. The future studies that are prompted by these findings are also considered.

Contents

Chapter 1

General Introduction

1.1 Larval development of fish

Many teleost fish adopt high-risk life histories that balance a high reproductive output with a high mortality of the young (Wieser 1991). Atlantic cod (*Gadus morhua*), for example, can produce around 5-6 million eggs each year (Kjesbu 1989), but as little as 3 % of these eggs will produce larvae that survive beyond three weeks of age (Zhao et al. 2001). Such a high mortality rate results primarily from the small size of the larvae, which are extremely vulnerable to predation (Bailey and Houde 1989) and consequently rapid larval growth is vital to young fish if they are to survive beyond the early developmental stages (Wieser 1991). A newly hatched cod larva, for example, can undergo a 200-fold increase in weight in just 40 days (Folkvord 2005).

In addition to this increase in size, many fish species undergo extreme anatomical, physiological and morphological changes during the larval period of development (Hunt von Herbing 2001). Cichlids, for example, can develop from a yolk-sac larva to a pelagic juvenile form in as little as 2 weeks (Otten 1982). Such rapid change is essential for the development of those structures required for such functions as external feeding, swimming and respiration (Osse and van den Boogaart 1995) and for the development of body morphology suited to the increasing ratio of viscous to inertial forces that occurs with increasing size (Hunt von Herbing et al. 1996a). Frequently such changes do not develop isometrically but result from differential relative growth or what is commonly termed allometry (Fuiman 1983).

1.2 Individual variability in development

Until recently, much classic ecological theory was based on the premise that populations/species contained identical individuals that differed only in sex and age (Kingsland 1995). However, it is now widely accepted that individuals within populations can differ in a number of traits, including behaviour (Wilson et al. 1994), life history patterns (Vøllestad and Lillehammer 2000), physiology (Cutts et al. 1998) and morphology (Bourke et al. 1997). For example, zebrafish (*Danio rerio*) exhibit consistent individual differences in their propensity to inspect a predator (Dugatkin et al. 2005). In this case, the authors forward inherited differences as a potential underlying cause, but in other cases, variability may be a manifestation of maternal effects (Lindholm et al. 2006) or represent a response to environmental differences (Wright et al. 2003). For example, differences in temperature have

been shown to influence the age at which yellowtail flounder (*Pleuronectes ferrugineus*) larvae undergo metamorphosis (Benoît and Pepin 1999).

The potential for individuals to differ both between and within populations of the same species can have a number of important implications. For example, variability in life history traits may affect recruitment variability (Benoît and Pepin 1999) and consequently individual differences should be considered when modelling population dynamics (Grimm and Uchmanski 2002). Individual variability may also have important evolutionary implications. For example, individuals that develop differences in phenotype as a result of a plastic response to features of the environment may eventually diverge to such an extent that they form new species (West-Eberhard 1989). Moreover, the potential for individuals to develop differing phenotypes can have important implications for the culture of species if, for example, certain phenotypes are more or less suited to the rearing process. This topic is discussed in greater detail in subsequent sections.

1.21 Trophic polymorphisms

One aspect of individual variability that has been well documented is that of varying feeding morphology (trophic polymorphism) (Skúlason and Smith 1995). Different populations of red-backed salamanders (*Plethodon cinereus*) (Maerz et al. 2006), Arctic charr (*Salvelinus alpinus*) (Skúlason et al. 1989; Adams et al. 1998a), Eurasian perch (*Perca fluviatilis*) (Svanbäck and Eklöv 2002), cichlids (Meyer 1990; Streelman et al. 2007) and three-spined sticklebacks (*Gasterosteus aculeatus*) (Schluter and McPhail 1992), for example, have been shown to differ in trophic morphology, apparently as a result of adaptations to local prey types. In a large number of these studies, littoral (benthic) morphs feeding predominantly on macroinvertebrates have been shown to possess a deeper body and a larger head and mouth compared to limnetic (pelagic) morphs feeding predominantly on zooplankton (Ehlinger and Wilson 1988; Skúlason et al. 1989; Schluter and McPhail 1992; Svanback and Eklov 2002). Such trophic polymorphisms are particularly interesting to science because they have the potential to result in genetic divergence between morphs and eventually speciation (West-Eberhard 1989).

While many of these polymorphisms appear to result from selective processes acting over many generations (Skúlason and Smith 1995), there is increasing evidence that trophic

variation can also occur within the same population and within an animal's lifetime (Thompson 1992; Wimberger 1992; Walls et al. 1993; Mittelbach et al 1999; Robinson and Wilson 1996; Hegrenes 2001; Hjelm et al. 2001). Long-toed salamander (*Ambystoma macrodactylum columbianum*), for example, have been shown to develop broader, longer and deeper heads when fed tadpoles and brine shrimp over brine shrimp alone (Walls et al. 1993) while Meyer (1987) found that the cichlid, *Cichlasoma managuense*, developed more pointed heads when reared on brine shrimp than those fed flake food and nematode worms. The mechanisms underlying these rapid changes in morphology are not always clear, but frequently appear to involve a type of developmental plasticity in which individuals respond to variations in the texture or nutritional content of prey (Wimberger 1992). The phenotypic differences that result from this plasticity are often distinguished from genetically induced polymorphisms by the term 'polyphenism' (West-Eberhard 1989).

1.22 Cannibalism

In the same way that fish can exhibit marked differences in prey preference (Bryan and Larkin 1972), fish can also exhibit marked differences in the extent to which they consume conspecifics. Different stocks of Arctic charr, for example, have been shown to vary in the extent to which they will feed cannibalistically, presumably as a result of some heritable cannibalistic tendency (Amundsen et al. 1999). However, extensive individual variations in cannibalism may also result from varying environmental conditions, such as the relative size differences of predator and prey and the density of conspecifics and alternative prey (Svenning and Borgstrom 2005). Moreover, in some species certain features of the environment may encourage the development of a distinct cannibalistic morph, such as has been observed in the plains spadefoot toad tadpole (*Spea bombifrons*) (Frankino and Pfennig, 2001).

During periods of low prey availability, cannibalism can be viewed as a form of optimal foraging (Dong and Polis 1992), since it provides the cannibal with highly appropriate food, while minimising the risks associated with foraging. Fish, for example, have a high content of digestible nutrients (Meffe and Crump 1987; Kubitza and Lovshin 1999), so fish feeding totally or partly on conspecifics generally grow faster than those feeding exclusively on other types of prey such as plankton (Baras et al. 2003). Varying levels of cannibalism can also have marked effects on recruitment (Neuenfeldt and Koster 2000) and play an important role

in regulating population densities (Persson et al. 2000). Furthermore, some modelling studies (e.g. Kohlmeier and Ebenhöh 1995; Nishimura and Hoshino, 1999) suggest that cannibalism can be considered an evolutionary stable strategy, since large animals sustain growth by eating smaller conspecifics that in turn feed on different levels of the trophic system.

Cleary there are many benefits to consuming conspecifics. However, many species do not cannibalise and this most likely reflects the number of disadvantages that relate to this behaviour. For example, Pfennig et al. (1998) showed that cannibalistic tiger salamanders (*Ambystoma tigrinum*) fed diseased conspecifics were less likely to survive to metamorphosis than those that ate diseased heterospecifics. This increased risk of pathogen transfer may at least partly explain why this species will preferentially prey on heterospecifics over conspecifics (Pfennig et al. 1998). Eating conspecifics that are related to you, such as siblings, also confers a genetic disadvantage since genes shared with the consumed prey are lost from the gene pool (Pfennig 1997). Consequently, some species will preferentially choose to consume non-kin over kin conspecifics (e.g. plains spadefoot toad tadpoles, *S. bombifrons* and *S. multiplicata*, Pfennig 1999).

Despite these disadvantages, cannibalism appears to be particularly common in fishes and especially prevalent in those species that exhibit carnivory or piscivory (Hunter and Kimbrell 1980). In the wild, inter-cohort cannibalism tends to predominate, especially in the larval and juvenile stages of fish. For example, up to 40% of the annual mortality of 0-group cod can result from inter-cohort cannibalism at around the time of settling (Daan 1975). In cultured fish, losses to intra-cohort cannibalism can be similarly high and, as in the natural environment, especially so during the larval and early juvenile stages of rearing (Baras 1998). Unfortunately, the primary objective of aquaculture, i.e. to maximise production, appears to create features of the rearing environment that trigger and/or increase the potential for individuals to consume conspecifics (see Baras and Jobling 2002 for review). These features can include high stocking densities (walleye, *Stizostedion vitreum*: Li and Mathias 1982; dorada, *Brycon moorei*: Baras et al. 2000), high temperatures (Atlantic cod: Bjornstad et al. 1999) or temporal variations in food availability (European seabass, *Dicentrarchus labrax*: Katavich et al. 1989).

1.23 Behavioural variation

It is widely recognised that, as in humans, animals can exhibit consistent individual differences in behaviour (Wilson 1998; Sih et al. 2004). For example, some individuals are consistently aggressive while others are consistently non-aggressive (McLaughlin et al. 1999). Such differing behaviours reflect differing personality traits and where a suite of behaviours is consistently expressed across different situations are frequently termed "behavioural syndromes" (Gosling 2001). Behavioural variation is particularly interesting, since a propensity to exhibit one type of behaviour, e.g. aggression, may be appropriate in some situations but not in others and thus can limit behavioural plasticity and result in non-optimal behaviour (Sih et al. 2004).

One aspect of behavioural variation that is well documented in many fish species is that of risk taking (Huntingford and Coyle 2007). Fish that are risk takers tend to explore a novel environment more readily (*Brachyraphis episcopi*, Brown and Braithwaite 2004), to spend longer out of cover (rainbow trout, *Oncorhynchus mykiss*, Sneddon 2003) or to inspect a model predator more readily (*Nannacara anomala*, Brick and Jakobsson 2002), than those that avoid risk. Consequently, risk taking individuals are commonly referred to as 'bold' and their non risk-taking counterparts as 'shy' or 'timid' (Wilson et al. 1994).

Many studies of variable risk taking in fish describe interpopulation differences in this behaviour. For example, Fraser and Gilliam (1987) found that Hart's rivulus (*Rivulus harti*) and guppies (*Poecilia reticulata*) from high predation sites foraged proportionately more in the presence of a predator than fish from low predation sites. It is not always clear what underpins these differences in risk taking. However, studies of consistent population differences in the behaviour of both wild-caught and laboratory-reared three-spined sticklebacks (Bell and Stamps 2004; Bell 2005) and zebrafish (Wright et al. 2003) indicate that genetics may play an important role.

1.3 The Atlantic cod

A fish species of enormous importance, both ecologically and culturally, is the Atlantic cod. This species has been the dominant piscivore in many marine ecosystems and supported significant fisheries for many centuries (Kurlansky 1997). Cod are a temperate species and

distributed throughout the northern Atlantic, the Baltic Sea and the Barents Sea (Cohen et al. 1990). Although predominantly demersal in nature, they occupy a variety of habitats, from the shoreline down to the continental shelf (Cohen et al. 1990). Typically, cod mature at 2-4 years old and thereafter spawn once a year. The larval stage spends approximately three weeks in the upper ocean and then descends to the sea bottom, when the young fish begin their life in the demersal zone. Cod feed predominantly on crustaceans, such as copepods, mysids, shrimps, amphipods and crabs and increasingly as they develop, fish (Palsson 1994; Link and Garrison 2002).

Over-fishing since the 1950s, and particularly over-fishing of immature individuals, has led to a dramatic decline in cod stocks and presently the Atlantic cod is classed as endangered (FAO 2007). As a consequence of the reduced availability of this species and the resulting high marker price (Tilseth 1990), there has been an increased interest in the aquaculture of this species and presently cod farms exist in Norway, Scotland, Canada and the U.S.A (Brown et al. 2003). Worldwide farmed cod production is expected to increase from 8000 tonnes per annum in 2005 (FAO 2007) to 400,000 tons per annum in 2020 (Solsletten 2001). Initially protocols for the intensive culture of cod were dependent on 'borrowing' techniques from those developed for other species. However, this approach has not always been effective and there is now a move towards the research and development of cod-specific production protocols.

One of the greatest challenges facing cod aquaculture is the reduction of levels of mortality in the early rearing stages. Of the initial yolk-sac larvae, only 5-7% may survive to day 72 (Howell 1984). In the early larval stages the greatest losses occur due to the failure of many larvae to commence feeding. However, during the mid- to late larval and early juvenile stages, cannibalism is frequently cited as the main cause of mortality (e.g. Howell 1984; Folkvord 1989), with attacks subsiding once juveniles grow beyond 40mm (0.6 g) (Folkvord 1993 in Ottera 1994; Rosenlund et al. 1993; Ottera and Lie 1991). Consequently, mitigation of cannibalistic behaviour during these developmental stages is considered paramount to the successful culture of cod.

1.4 The objectives of the thesis

The objectives of the work described in this thesis fall broadly into two categories: firstly, to examine aspects of the morphological and behavioural development of larval cod, particularly in relation to the feeding regimes used in aquaculture and the development of cannibalism, and secondly, to examine differences in risk-taking between different stocks and families of one-year old cod.

The now well-documented existence of trophic polymorphisms in some species of fish raises the possibility that head shape in cultured species may also develop in response feed type and so may result in the development of head morphology that is undesirable. For example, the prey offered may enhance a fish's ability to feed cannibalistically, by promoting the development of large jaws early in ontogeny. Given the importance of mitigating cannibalism during larval cod rearing, it would therefore be useful to know more about ontogenetic trends and variability in the development of larval cod morphology, and whether the development of trophic morphology is influenced by prey type. With this in mind, the aim of chapter 3 was to describe patterns of change in larval cod head shape and the extent to which these patterns varied within a cohort reared on standard culture feeds. Chapter 4 follows on from this study and examines the effect of varying prey type and size on the development of larval cod head shape.

Cannibalism is now well-documented in larval cod (Howell 1984; Folkvord 1989) but little is known about the aggressive behaviour of cod prior to the onset of cannibalism and the extent to which such behaviour is indicative of cannibalism later in development. This is despite the fact that non-cannibalistic aggressive behaviour has been shown to slow growth in both the attackers (African catfish, *Clarias gariepinus*: Hecht and Uys 1997) and the victims of attacks (cichlid, *Tilapia zillii*: Koebele 1985) and result in significant losses as fish are injured and subsequently diseased (African catfish: Kaiser et al. 1995). The aim of the study outlined in chapter 5 was therefore to describe aggressive interactions in larval cod and to establish the extent to which aggressive attacks were representative of an early form of cannibalism and/or the result of a battle for resources. Since variations in prey density are known to influence predatory and competitive aggression in different ways the study also aimed to establish the effect of prey availability on the incidence of aggressive attacks.

The potential for different populations of fish to behave in contrasting ways could have important implications for commercial aquaculture. For example, timid fish appear not to respond to the process of domestication as favourably as their more bold counterparts (Huntingford 2004) and consequently, understanding how boldness varies in farmed species could have implications for the welfare of these fish (Huntingford and Adams 2005). This is particularly the case for new, as yet undomesticated aquaculture species, such as cod. During the course of this research, the opportunity arose to conduct a behavioural study of individual cod of different stock and different family origin, produced as part of the breeding programme at the Norwegian Institute of Fisheries and Aquaculture Research. The objectives of the study outlined in chapter 6 were to characterise variation in the risk taking behaviour of these cod, as reflected by exploration of a novel, potentially dangerous environment and to determine whether the propensity to risk take varied between cod of different stock and family origin. Since individual differences in risk-taking are frequently associated with differences in stress physiology (Korte et al. 2005), an additional aim was to relate any observed behavioural differences to plasma cortisol levels.

Chapter 2

Fish Husbandry and Morphometric Analysis

2.1 Fish husbandry

The following provides a generalised summary of the cod larval rearing practices employed in the studies described in this thesis. Experimental manipulations such as those relating to feed type and quantity are described in greater detail in the relevant chapters. The rearing practices described reflect the standard techniques employed at the main study site, the Scottish Association for Marine Science Ardtoe Ltd., Ardtoe Marine Laboratory, Argyll and are largely based on the work of Cutts and Shields (2001). A brief description of the rearing practices employed at the Tromsø Aquaculture Research Station is also provided at the end of this section.

2.11 Broodstock

The cod broodstock employed in the following experiments were 3 years old and therefore most likely had been sexually mature for one year. Broodstock employed in the studies described in chapter 3 and chapter 4, were reared at Ardtoe and originated from fish local to the area. Broodstock employed in the study described in chapter 5 were of wild Shetland Islands origin and their eggs obtained directly from the Nufish hatchery, Shetland Islands.

Cod raised at ambient temperatures and under a natural lighting regime spawn between February and April of each year, for a period of approximately 2 weeks, with 2 to 3 days between each batch. However, since maturation is triggered by the seasonal light regime, spawning can be delayed in any given year by providing fish with continuous light from their first summer solstice. Two of the four experiments carried out as part of this research employed eggs from broodstock for whom spawning had been delayed (chapter 3 and chapter 4, experiment 1), while two experiments employed eggs from broodstock reared under normal ambient conditions (chapter 4, experiment 2 and chapter 5).

2.12 Egg collection and incubation

Cod spawn naturally in captivity and eggs were easily collected from the outflow of the holding tank using a plankton net suspended in water. The eggs were then carefully transferred to a bucket and allowed to stand for several minutes, during which viable eggs floated to the surface. A sample of these floating eggs was then placed under a microscope

and fertilisation and developmental stage assessed. Assuming most eggs were successfully fertilised, they were jugged into a fine mesh and placed in a solution of the disinfectant 'Kickstart' for approximately 45 seconds. This disinfection procedure was carried out in order to remove pathogens such as bacteria. Following disinfection, the mesh and eggs were rinsed with UV-sterilised seawater (hereafter referred to as 'water') and weighed (the mesh weight having been already recorded). An estimate of the number of eggs in each batch was based on previous work that found that each gram contains approximately 500 eggs (Gara and Shields 1997).

Eggs were then transferred to 70 L cylindro-conical incubation tanks at a density of 1 million eggs per tank. These tanks contain a central standpipe surrounded by a nylon mesh with an air collar situated at the base of the mesh for the purposes of providing gentle aeration. Water flow rates were maintained at 2ml/min and water temperature was maintained at 8 to 10 °C by means of air chilling units. Air was supplied to each tank via an air collar situated at the bottom of the tank. Lighting was continuous but very dim, at around 5 to 10 lux. One day following egg incubation and every other day after that, air and water supplies were briefly cut off in order to allow dead eggs to sink to the bottom of the tank. These eggs were then removed using a siphon and weighed in order to give an estimate of numbers lost. In addition, eggs were regularly sampled and placed under the microscope so that developmental stage could be assessed. Upon reaching the final stage of pre-hatching development, eggs were transferred to rearing tanks.

2.13 Stocking of rearing tanks

Prior to transferring eggs to rearing tanks, air and water supplies were again switched off in order to permit live eggs to float the surface and dead eggs to sink to the bottom. Live eggs were then transferred to a fine mesh net immersed in UV-sterilised seawater. In order to kill any prematurely hatched eggs, disinfection was again carried out by briefly immersing the eggs in a 4% solution of 'Kickstart'. The eggs were then rinsed, transferred to a container containing water and the dead larvae removed with a siphon. In order to estimate the number of eggs in any given volume, a known sample volume was taken and further diluted to ease counting. The numbers of eggs in three sub samples of this original sample were then counted by placing the eggs over a rigid mesh. It was then possible to estimate the total number of

eggs in the original volume of water, by taking the average of these three samples and back calculating.

The eggs were then moved to the rearing rooms and a volume of water containing the appropriate number of eggs placed in each rearing tank. The tanks in to which eggs were stocked varied between experiments but were identical within each experiment. They consisted of black 1300 L production tanks, black 100 L production tanks or clear 10 L aquaria. The largest tanks contained a longitudinal central standpipe surrounded by a nylon mesh that permitted debris to escape but retained the larvae. The 100 L tanks contained a transverse wire mesh centrally located at the bottom of the tank and connected to an externally located standpipe. The 10 L aquaria contained a perforated plastic tube, centrally located on the bottom of one end of the tank, connected to an externally located standpipe. Gentle aeration was supplied to all tanks by means of an airline. Each tank had been cleaned and filled with water on the day prior to stocking and water temperature checked to make sure it was comparable with that in the incubation tanks.

2.14 Larval rearing

Newly stocked eggs were kept in static water and under darkness until hatching was complete, this absence of light promoting synchronous hatching (Cutts and Shields 2001). At approximately 100-degree days (daily temperature x number of days), eggs hatched (0 days post hatch or 0 dph). At this point, lights were switched on and thereafter periodically increased in intensity. Water temperature was maintained at 10 to 12 °C through the use of air chilling units. Water salinity was checked each day and ranged from 33 to 34 ppt.

Tank hygiene was maintained by periodically increasing flow rates and siphoning debris and dead fish from the bottom of tanks. However, siphoning was not possible before 25 dph, due to the danger of removing live fish. In addition, ‘skimmers’ were fitted from 5 dph in order to remove the oily surface film associated with live feed that, if left, could prohibit larvae from inflating their swimbladder. These skimmers were composed of synthetic scouring pads and were partially suspended in the tank water. A larger skimmer was used in the 1300 L tanks, composed of a rectangular floating polystyrene trap containing an airline into which surface water was drawn. Around 35 dph, the mesh surrounding the standpipe in the 1300 L tank was replaced with mesh of a larger pore size in order to prevent the build up of debris.

During the rotifer-feeding period (from approximately 0 dph to 32 dph, see below) microalgae were added to tanks each day. One, or a combination of two, types of algae was used, either *Nannochloris* sp. or *Pavlova* sp. The addition of algae appears to benefit larval rearing by continuing to enrich rotifers that are not eaten, while also altering aspects of the physical environment such as light attenuation and dissolved oxygen content (Cutts et al. 2003). Algae may also aid the development of the larval digestive system and thus promote first feeding (Cutts et al. 2003).

2.15 Feed

Two types of live prey were used in experiments: rotifers (*Brachionus plicatilis*) and brine shrimp (*Artemia salina,* hereafter referred to as *Artemia*). Rotifers are microscopic aquatic animals of the phylum Rotifera, while *Artemia* are small branchiopod crustaceans. Both these species are widely used in aquaculture due to their nutritional content, small body size and relatively slow motility, making them easy to capture. They are also easily reared due to a high reproduction rate in the case of rotifers and the formation of dormant embryos or 'cysts' in the case of *Artemia* (Lavens and Sorgeloos 1996).

Larvae were fed rotifers from 1 dph for a period of approximately 4-5 weeks and *Artemia* from around 27 dph for a period of approximately 4-5 weeks. Both feeds were either offered once in the morning or the feed split between a morning and afternoon feed. A one-week period of cofeeding both rotifers and *Artemia* was required in order to prevent starvation of those individuals that did not switch to *Artemia* immediately. During the period of feeding live prey, a small sample of water was removed each day from each tank and the number of prey items recorded. When prey were found to be absent or present in only low numbers, feeding levels were increased. Where more than one tank was used, increases (or % increases, chapter 5) in the level of feed were uniform for all tanks receiving that prey type. Gradually increasing sizes of the inert feed, AgloNorse (EWOS Ltd), were then offered from approximately 50 dph to the end of the experiment, at least four times each day. This diet is an agglomerated microbound feed composed of a high dietary protein content (60%) and a low carbohydrate content. It is highly palatable, with attractants added to stimulate feeding and is readily digested and absorbed by the larval intestinal system. It is currently used to rear cod, halibut, sole, sea bass and sea bream, among others (www.ewos.com/uk).

Neither rotifers nor *Artemia* contain sufficient nutrients to sustain cod larvae and consequently they are enriched with commercially available supplements. In the following experiments, the enrichments employed were those used at the study site at the time for commercial production. This is with the exception of the Prolon-enriched *Artemia* in Chapter 4, which were prepared specifically for that study. Of the enrichments used, Algamac 2000 (Aquafauna Biomarine) is a spray-dried single cell protist, while Selco (self-emulsified liquid concentrate), DHA (Docosahexaenoic) Acid Protein Selco and DC (disinfecting continuously) Selco (INVE Aquaculture) are lipid emulsions containing marine oils, antioxidants, emulsifiers and vitamins (Cutts et al. 2006). Prolon (INVE Aquaculture) is also a lipid emulsion, but is administered to *Artemia* following enrichment with any standard enrichment, such as those described above. The purpose of Prolon enrichment is to increase the size, and thus the nutritional content of the *Artemia*. All these enrichments are adsorbed and ingested by the prey and result in an increase the levels of essential fatty acids, particularly the highly unsaturated fatty acids (Sorgeloos et al. 1991). More detailed information on the biology, production and enrichment of rotifers and *Artemia* is provided in Lavens and Sorgeloos (1996).

2.16 Ongrowing

At the end of each experiment, cod that were not killed were returned to production tanks or, if older, moved to the nursery for ongrowing. Under standard practices, cod are transferred to the nursery at 75 dph (0.5 g or 50 mm standard length), when they are robust enough to survive the move. They are counted, graded either subjectively with the eye or with grading bars and placed in nursery tanks with similar sized fish. At approximately 12 to 18 months old or a weight of 500-1000 g, cod are transferred to broodstock tanks or alternatively sold for harvesting.

2.17 Fish health

Cod are susceptible to bacterial diseases, most commonly *Vibriosis*, and parasites, including some species of ciliate. Consequently, throughout each experiment, fish were frequently checked for any signs of disease and water was checked for the presence of pathogens. On occasion, ciliates were found to be present in the water, and if present in large numbers, were

killed by administering tanks with formalin at a concentration of 100 ppm. Small numbers of ciliates did not appear to affect larval health.

2.18 Fish husbandry at Tromsø

The rearing of cod at the other study site, Tromsø Aquaculture Research Station, Kårvik, Tromsø, Norway, followed a protocol similar to that employed at Ardtoe. The fish used were from a large group of age 0+, first-generation offspring of wild-caught Northeast Arctic cod and Norwegian coastal cod produced at the Norwegian National Cod Breeding Programme. Parental cod had been reared under natural temperature and light conditions until breeding. Offspring were fed rotifers (*Brachionus plicatilis*) from 2 – 25 dph and brine shrimp (*Artemia salina*) from 25 – 45 dph. Fish were subsequently weaned on to AgloNorse at 45 dph and Dana feed at 80 dph. During start-feeding, lighting was continuous and water temperature was maintained at 8 °C. From the point of weaning onwards, the fish were subjected to natural water temperatures (unheated, filtered seawater from the fjord) and natural light conditions (transparent roof, 70 ° northern latitude).

2.2 Morphometric analysis of head morphology

Two of the studies undertaken as part of this research involved assessment of differences in the morphology of cod and thus the selection of appropriate methods to achieve this. A variety of techniques can be used both for the measurement of animal morphology and for the subsequent analysis of those measurements, including identification of the major patterns of variation in morphology. The method used in this thesis reflects a traditional, but still widely used, method in which linear measurements (sometimes termed “trusses”, Strauss and Bookstein 1982) of morphology features are recorded and then entered into a multivariate analysis, in this case, principal component analysis (PCA). The PCA identifies those aspects of the dataset that account for the greatest amount of variation in the dataset (Pearson 1901).

A more recent method of analysing morphology, frequently referred to as geometric morphometrics, involves analysis of the relative geometric positions of body parts (Rohlf and Marcus 1993). For each individual, corresponding body landmarks are placed on a two-dimensional Cartesian grid and variations in shape determined by analysis of differences in

the coordinates of corresponding landmarks among individuals (Bookstein 1991). Although such analysis is especially useful when attempting to identify and visualise overall changes in body shape, traditional truss methods have been shown to be as accurate as geometric analysis at interpreting differences in morphology (Parson et al. 2003). It is for this reason, and because of the essential simplicity of the method, that linear morphometrics are employed in this thesis.

Chapter 3

Head morphology in larval cod: ontogenetic trends and individual variability

This chapter has been submitted for publication in Journal of Fish Biology

3.1 Summary

We examined patterns of change in head shape in larval Atlantic cod and the extent to which head development varied within a cohort. Cod were reared on standard aquaculture feeds (rotifers, then brine shrimp, then formulated feed) from 1 to 78 days post hatch. They were periodically sampled and photographed for analysis of head shape. Investment in head growth relative to post-cranial growth increased over the first two thirds of the study period and remained constant thereafter. Most of the measures of head structure (and especially eye diameter) exhibited comparable patterns of growth, increasing in relative size from week 4 onwards and then falling from week 9. However, jaw width followed an opposite trend, decreasing in relative size from week 4 and increasing again from week 9. Periods of rapid change in head morphology coincided with points at which the larval diet was changed. Whatever processes are involved in determining these patterns of development, they influence individual larvae differentially and, as a consequence, marked individual variation in head morphology is apparent by the end of the larval/early juvenile period.

3.2 Introduction

Studies of the morphological and functional development of cod larvae have increased in recent years, partly due to the realisation that such information is required for the successful culture of this species. Attention has focused primarily on the anatomical development of individual structures (Kjørsvik et al. 1991; Pedersen and Falk-Petersen 1992; Morrison 1993), with particular emphasis on the development of the head and feeding apparatus (Hunt von Herbing et al. 1996a, b; Hunt von Herbing 2001).

Frequently, studies relate cod larval development to size or age and to the functional requirements of the growing larvae (e.g. Hunt von Herbing 2001). In this respect, the developmental sequence is assumed to be fixed, having evolved over many generations and genetically controlled. Increasingly, however, studies of other fish species have highlighted that development, especially morphological development, may also be influenced by features of the rearing environment, such as habitat or prey type (Meyer 1990, Mittelbach et al. 1999; Andersson et al. 2005). For example, Arctic charr reared in structurally-complex habitats, where macroinvertebrates predominate, develop deeper bodies and blunter snouts than charr reared in structurally simple habitats, where zooplankton predominate (Andersson et al. 2005). The mechanisms underlying such differences are not always clear, but probably result either from variations in the nutritional content of prey or reflect bone remodelling in response to different sizes of prey (Wimberger 1992). If head shape in larval cod also develops in response to prey type or features of the rearing environment, farmed fish may be rapidly forced to develop undesirable head morphology. For example, the prey offered may enhance a fish's ability to cannibalise, by promoting the development of large jaws early in ontogeny.

One of the greatest challenges facing cod aquaculture is the reduction of levels of mortality in the early rearing stages. Of the initial yolk-sac larvae, only 5-7 % may survive to day 72 (Howell 1984) and cannibalism is frequently cited as the main cause of mortality (Howell 1984; Folkvord 1989). Given the importance of mitigating this behaviour during larval cod rearing, it would be useful to know more about ontogenetic trends in the development of larval cod morphology, especially trophic morphology. The aim of this study was to describe patterns of change in larval cod head shape and to establish the extent to which these patterns of change varied within a cohort. I used hatchery-reared larval cod and employed the standard

larval feeding regime in cod culture, namely rotifers, followed by brine shrimp (*Artemia salina*) and formulated feed, due to the relevance of results for the culture of this species.

3.3 Materials and Methods

3.31 Rearing of fish

The study was carried out at the Scottish Association for Marine Science Ardtoe Ltd., Ardtoe Marine Laboratory, Argyll, Scotland in May to July 2004. Larval rearing during the study followed the standard procedure employed at this site (Shields et al. 2003). Fertilised cod eggs were obtained from broodstock reared at the study site under ambient temperature and photoperiod conditions and originating from fish local to the area. One day prior to hatching, 65,000 eggs were transferred to a standard 1300 L production tank and kept in static water and under darkness until hatching was complete. Only one tank was available at the site for use in this experiment.

From 1-day post hatch (dph) to 32 dph, larvae were fed Algamac or Docosahexaenoic Acid Protein Selco (DHAPS) enriched rotifers (*Brachionus plicatilis*) at a density sufficient to provide approximately 5 rotifers mL^{-1} (based on residual counts). Algamac or Disinfecting continuously self-emulsified liquid concentrate (DC Selco) enriched brine shrimp (*Artemia*) were offered from 27-60 dph at a density sufficient to provide approximately 0.25 *Artemia* mL^{-1}. Gradually increasing sizes of the formulated feed, AgloNorse (EWOS), were offered from 51-78 dph at a density sufficient to create a thin covering over the water surface. Microalgae (*Nannochloris* sp. and *Pavlova* sp.) were added to tanks from 0-32 dph at a density of 500,000 cells mL^{-1} day^{-1} as a feed for the rotifers. Water temperature was maintained at between 10-12 °C. Lighting was continuous and ranged from 50 lux at 0 dph to 500 lux at the end of the experiment (78 dph). Tank hygiene was maintained by gradually increasing water flow rates from 500 mL^{-1} min^{-1} at 0 dph to 3 L^{-1} min^{-1} at 78 dph and by frequently siphoning debris from the bottom of the tanks. In addition, rectangular floating polystyrene ‘skimmers’ were fitted from 1 dph to improve swim bladder formation by removing the oily surface film associated with live feed.

3.32 Morphological analysis

Each week, 20 larvae were randomly removed from the tank for morphometric analysis, killed using an overdose of anaesthetic (MS222) and photographed using a Nikon Coolpix 4500 digital camera fitted to a dissecting microscope. Photographs consisted of a lateral view of the whole larva and lateral, dorsal and ventral views of the head. The larvae were then measured using the image analysis programme, Image Pro Express (Media Cybernetics). From the lateral view (Fig. 3.1a), the following features were recorded: total length (measured from the lateral view of the whole larva); horizontal eye diameter, length of the premaxilla (once formed, therefore only in larvae older than 28 dph), length of the jaw (Meckel's cartilage), head depth at the posterior edge of the eye, length of the snout (from the anterior tip of the snout to the intersect with the line defining head depth) and angle of the snout. A large snout angle reflected a pointed (acutorostral) snout, while a small snout angle reflected a blunt (obtusorostral) snout. From the dorsal view (Fig. 3.1b), the following additional features were measured: medulla width, jaw width (maximum distance between maxillae) and head length (from the anterior tip of the snout to the point midway between the operculae). The maximum distance between the gills was measured from the ventral view of the head from 35 dph onwards, when it became possible to manipulate the larvae so that the ventral side was uppermost (Fig. 3.1c). Total length (distance from the tip of the snout to the tip of the longest lobe of the caudal fin) rather than standard length (distance from the tip of the snout to the caudal peduncle) was measured since the caudal fin was more clearly defined in images than the caudal peduncle. When a larva was too large to be photographed in its entirety, callipers were used to measure total length. The caudal fin was never so badly nipped that measurements were inaccurate. Post-cranial length (distance from the edge of the operculum to the end of the caudal fin) was determined by subtracting the length of the head from the total length of the larvae.

At the end of the experiment, all larvae were killed with an overdose of anaesthetic and total length recorded. Survival and growth rates did not differ significantly between this rearing tank and any other tank used to rear cod during this period.

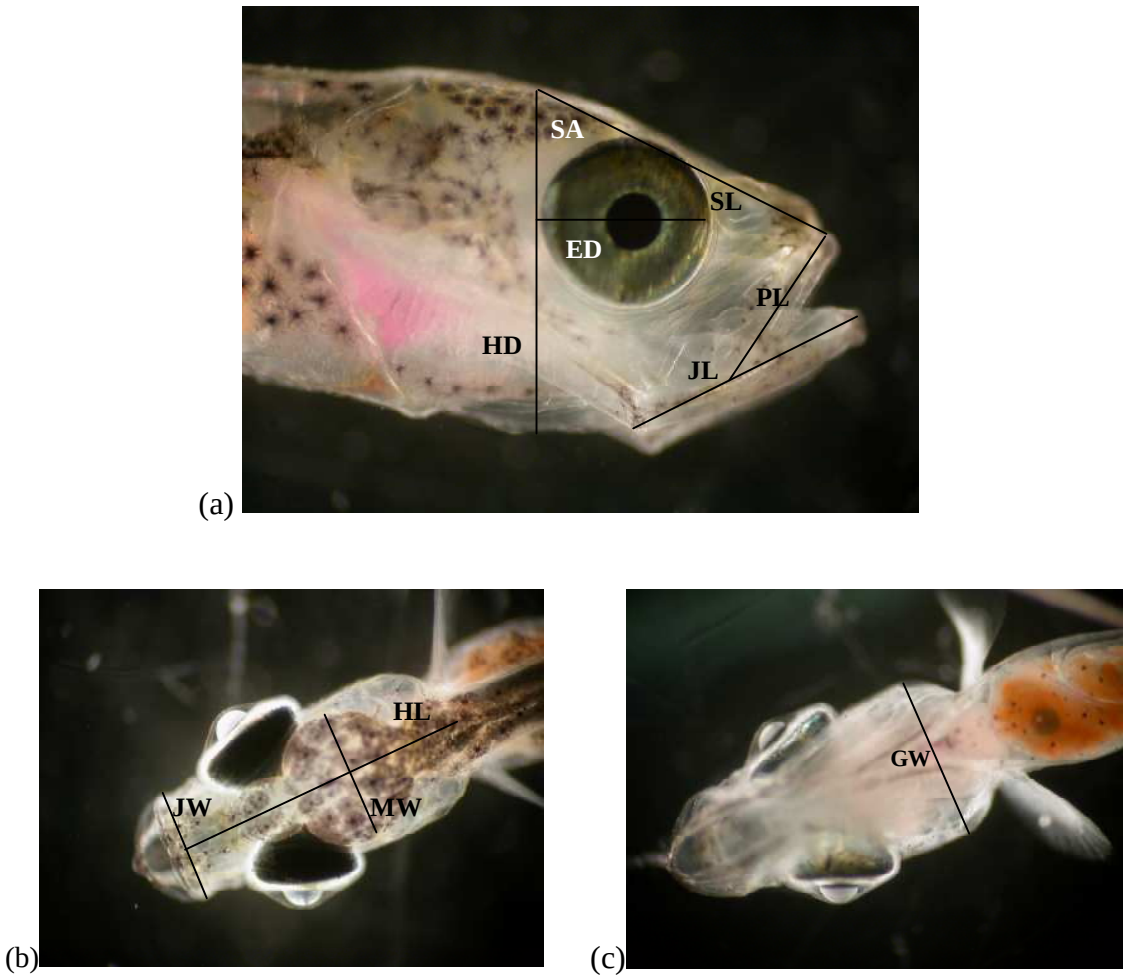

Fig. 3.1. Photographs of a 36 dph cod larva showing morphometric head features recorded: (a) lateral view, ED = eye diameter, PL = premaxilla length, JL = jaw length, HD = head depth, SL = snout length, SA = snout angle; (b) dorsal view, JW = jaw width, MW = medulla width, HL = head length; (c) ventral view, GW = gill width.

3.33 Statistical analysis

To summarise patterns of head growth in relation to post-cranial growth, I used the ratio of head length to post-cranial length. In order to correct head morphometric data for an effect of size, residual scores were obtained from regression analysis of each head measure against head length. These residual scores are referred to in the following text as length-corrected measures. To investigate the relationships among these length-corrected measures and to identify the aspects of morphology that accounted for the greatest amount of variation in the dataset, Principal Component Analysis (PCA) was carried out on some of the length-corrected measures. Premaxilla length and gill width were omitted since these features were not

recorded in all weeks of the experiment. Snout angle was also omitted since there was a large degree of variation of this measure in all weeks of the study. Measures were not normally distributed, so were ranked prior to PCA. Table 3.1 summarises the results of the PCA. There were two PCs accounting for 63.8 % of variance. The loadings for the first PC were all positive and thus this PC reflected the size of all head measures relative to head length and is hereafter referred to as head 'robustness' (as opposed to head 'fragility': see chapter 4). The second PC opposed eye diameter to jaw width, medulla width and snout length. In order to summarise this aspect of head shape in an intuitively simple way, rather than using the PC2 scores, I created an index reflecting the development of eye diameter relative to jaw width by dividing eye diameter by jaw width. Changes with time/age of morphological features and the two derived indices were examined using Kruskal-Wallis one-way analysis of variance. Changes with time/age in the variability of morphometric measures were examined by regression analysis of the coefficient of variation of each measure across weeks.

Table 3.1. PC1 and 2 loadings for PCA of 6 length-corrected head measurements.

Measure	PC1	PC2
Eye diameter	0.553	0.226
Jaw length	0.486	0.025
Head depth	0.542	0.005
Snout length	0.386	-0.372
Jaw width	-0.091	-0.609
Medulla width	0.078	-0.662
Eigenvalue	2.392	1.437
% of variance	39.9	23.9

3.4 Results

3.41 Head growth in relation to post-cranial growth

There was a significant increase in total length, head length and post-cranial length over the duration of the study (Fig. 3.2a, b and c). From week 1 to week 7 this increase was very gradual, with median increases of 29.58, 54.79 and 24.15 % per week respectively. From week 7 to the end of the study, total length and post-cranial length, increased more sharply; in

this period increases were of the order of 42.50, 42.68 and 42.58 % per week. The coefficients of variation for each of these measures were highest in weeks 8 and 9 (Fig. 3.2a, b and c: Total length: $R^2_{adj} = 0.520$, $F_{2,9} = 6.96$, $P = 0.015$, Head length: $R^2_{adj} = 0.643$, $F_{2,9} = 10.91$, $P = 0.004$, Post-cranial length: $R^2_{adj} = 0.439$, $F_{2,9} = 5.31$, $P = 0.030$).

The index of head length relative to post-cranial length also changed significantly over the period of the study (Fig. 3.2d). In the first 8 weeks, the index increased from 0.20 to 0.34. Thus, fish were increasing investment in head growth over post-cranial growth during this period. From week 8 to week 12, the index remained constant at c. 0.34. The coefficient of variation for this index did not change significantly over time (Fig. 3.2d: $R^2_{adj} = 0.062$, $F_{1,10} = 1.73$, $P = 0.218$).

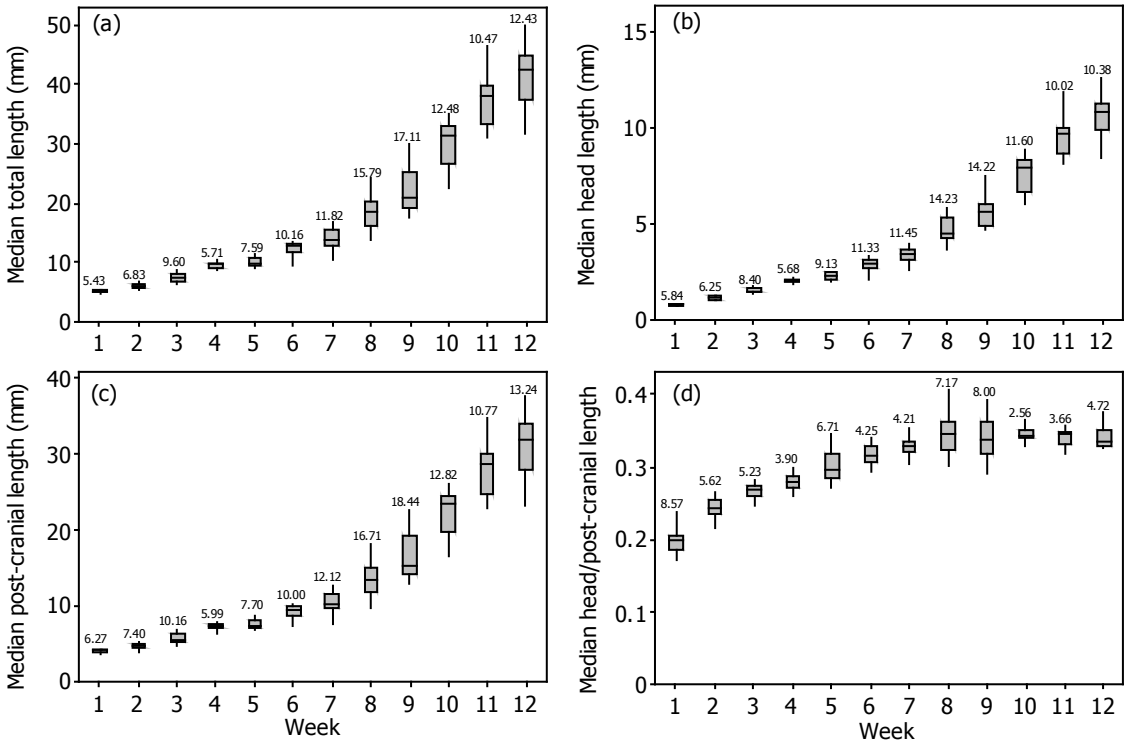

Fig. 3.2. Box plots showing the total length (a), head length (b), post-cranial length (c) and index of head length to post-cranial length (d) of larval cod in weeks 1 (1-2 dph), 2 (8-9 dph), 3 (15-16 dph), 4 (22-23 dph), 5 (29 dph), 6 (36-37 dph), 7 (43-44 dph), 8 (50-51 dph), 9 (57-58 dph), 10 (65 dph), 11 (71-72 dph) and 12 (78 dph) of the study. Each box shows the median, 25 (Q1) and 75 (Q3) percentiles; upper whisker = Q3 + 1.5 (Q3 - Q1), lower whisker = Q1 - 1.5 (Q3 - Q1). The coefficient of variation for each week is shown above each box. Each measure varied significantly with week (total length: $H = 232.88$, $DF = 11$, $P < 0.001$; head length: $H = 234.36$, $DF = 11$, $P < 0.001$; post-cranial length: $H = 231.84$, $DF = 11$, $P < 0.001$; head/post-cranial length: $H = 195.10$, $DF = 11$, $P < 0.001$).

3.42 Development of head shape

Analysis of length-corrected head measurements identified significant changes in patterns of growth during the study period (Fig. 3.3). In the first 4 weeks, length-corrected eye diameter, jaw length, head depth and snout length were slightly negative or showed negative allometric growth (Fig. 3.3a, c, d and e). At week 4 or 5, this trend was reversed and these length-corrected measures, with the addition of premaxilla length, began to increase, reaching zero at week 6 or 7 and continuing to rise until week 9 (Fig. 3.3a-e). Thus, from weeks 6-7 to week 9 there was positive allometric growth of these features. Finally, from week 9 to week 12, these length-corrected measures again decreased, reaching zero at week 11, beyond which point relative growth once again was negative. With the exception of length-corrected jaw length and length-corrected snout length, there was a general trend for coefficients of variation to increase for these head measures over the duration of the study (Eye diameter: $R^2_{adj} = 0.360$, $F_{2,9} = 4.09$, $P = 0.054$, Premaxilla length: $R^2_{adj} = 0.739$, $F_{2,5} = 10.91$, $P = 0.015$, Jaw length: $R^2_{adj} = 0.170$, $F_{2,9} = 2.13$, $P = 0.175$, Head depth: $R^2_{adj} = 0.342$, $F_{2,9} = 3.86$, $P = 0.062$, Snout length: $R^2_{adj} = 0.0$, $F_{2,9} = 0.06$, $P = 0.943$). Periods of negative allometric growth corresponded with periods when the snout was more pointed (acutorostral), while periods of positive allometric growth corresponded with periods when the snout was more blunt (obtusorostral) (Fig. 3.3f). The coefficient of variation for length-corrected snout angle decreased between weeks 1 to 9, before increasing slightly between weeks 9 to 12 ($R^2_{adj} = 0.775$, $F_{2,9} = 19.98$, $P < 0.001$).

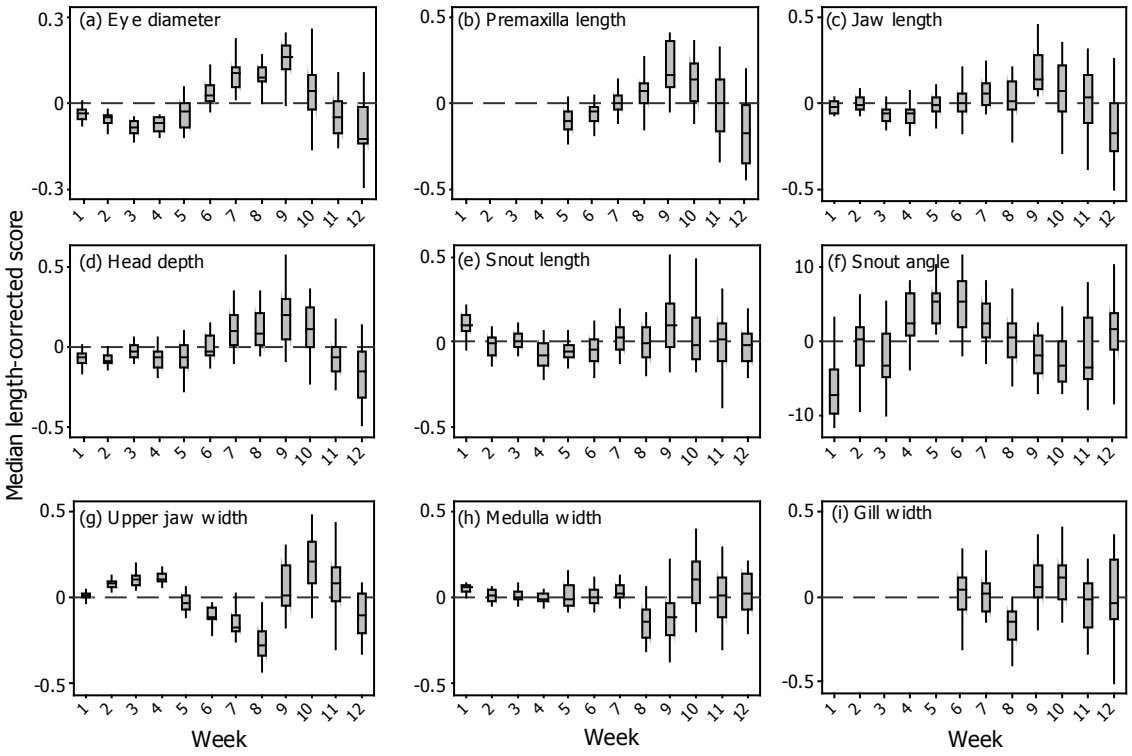

Fig. 3.3. Boxplots showing length-corrected scores for nine head measures (a-i) in larval cod for weeks 1 (1-2 dph), 2 (8-9 dph), 3 (15-16 dph), 4 (22-23 dph), 5 (29 dph), 6 (36-37 dph), 7 (43-44 dph), 8 (50-51 dph), 9 (57-58 dph), 10 (65 dph), 11 (71-72 dph) and 12 (78 dph). Each box shows the median, 25 (Q1) and 75 (Q3) percentiles; upper whisker = Q3 + 1.5 (Q3 - Q1), lower whisker = Q1 - 1.5 (Q3 - Q1). Each measure varied significantly with week ($P<0.001$).

Length-corrected jaw width also changed significantly over the duration of the study, following a pattern of growth almost diametrically opposed to that of the aforementioned measures (Fig. 3.3g). From week 1 to week 4, length-corrected jaw width increased, indicating increasing positive allometric growth. From week 4 to week 8, this trend was reversed and increasingly negative allometric growth took place. Between week 8 and week 10, length-corrected jaw width increased sharply, such that positive allometric growth took place from week 9 onwards. Finally, from week 9 to week 12, this measure fell once more, reaching just below zero in week 12. Length-corrected medulla width and gill width did not exhibit any consistent pattern of growth (Fig. 3.3h and i). The coefficients of variation significantly increased for length-corrected jaw width and length-corrected medulla width over the duration of the study, but not for length-corrected gill width (Jaw width: $R^2_{adj} = 0.485$, $F_{2,9} = 6.18$, $P = 0.020$, Medulla width: $R^2_{adj} = 0.522$, $F_{2,9} = 7.02$, $P = 0.015$, Gill width: $R^2_{adj} = 0.420$, $F_{2,4} = 3.17$, $P = 0.150$).

Analysis of the ratio of eye diameter to jaw width identified a significant change in this index over the 12 weeks of the study (Fig. 3.4). In the first 4 weeks, the index fell from 1.01 to c. 0.8, indicating that jaw width became larger in relation to eye width during this period; it also became more uniform. From week 4 to weeks 7-8, this trend was reversed and the index increased from c. –0.8 to c. 1.35; it also became more variable. Thus between week 5 and week 8, eye diameter was increasing in size relative to jaw width. Finally, between week 8 and week 10 the index fell back to, and stayed at, a median of 1, such that by the end of the study period, eye diameter and jaw width were in balance again; the eye-jaw ratio also became more uniform during this period. The ratio of eye diameter to jaw width changed significantly with total fish length (R^2_{adj} 0.373, $F_{3,236} = 48.35$, $P<0.001$), following a pattern of development similar to that which existed between this index and time (week of study). Since week of study and length cannot be differentiated, it is unclear whether this relationship is dependent on age or size.

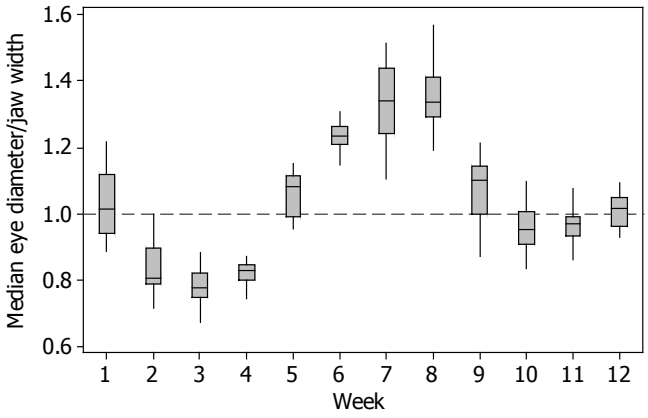

Fig. 3.4. Boxplot showing the eye diameter to jaw width index of larval cod for weeks 1 (1-2 dph), 2 (8-9 dph), 3 (15-16 dph), 4 (22-23 dph), 5 (29 dph), 6 (36-37 dph), 7 (43-44 dph), 8 (50-51 dph), 9 (57-58 dph), 10 (65 dph), 11 (71-72 dph) and 12 (78 dph). Each box shows the median, 25 (Q1) and 75 (Q3) percentiles; upper whisker = Q3 + 1.5 (Q3 - Q1), lower whisker = Q1 - 1.5 (Q3 - Q1). This index varied significantly with week ($H = 201.13$, $DF = 11$, $P < 0.001$).

3. 5 Discussion

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Results of this study identified clear patterns of change in various measures of larval cod head morphology. In most cases, these patterns were consistent between measures, although some morphological features were shown to develop in opposition to each other, such as eye diameter and jaw width. Growth of the head and the post cranium was highly variable, especially in the latter stages of larval development and investment in head growth relative to post-cranial growth increased over the first two thirds of larval development, remaining constant thereafter. As described in the Methods section, for logistical reasons this experiment was unreplicated and this raises the possibility that these patterns of morphological change may have been due to tank effects. This is unlikely since both the rearing conditions and survival levels of fish in this tank were identical to those in other tanks. However, the possibility of tank effects must be borne in mind when interpreting the results

The observed changes in cod morphology could result from one of two mechanisms, or a combination of both. Firstly, allometric growth may have occurred within the lifetime of individual fish as a consequence of environmental factors such as feeding or a genetically predetermined ontogenetic pathway. Alternatively, selective mortality may have removed certain morphotypes, with the remaining fish possessing the morphotypes most adapted to the environment. Unfortunately, I could not identify which of these two processes were operating, since it was not possible to follow individual fish for the duration of the study. In this discussion, the data are explored as if observed changes in measures resulted as a consequence of developmental growth. However, it is important to emphasise that these changes may have resulted from/been additionally influenced by selective mortality.

Many fish larvae have been shown to exhibit a Gompertz-like growth pattern, with a period of slow growth followed by a period of growth at an exponential rate (Bolz and Lough 1988). Results from this study suggest that larval cod may also grow in this way and that the period of exponential growth occurs around the commencement of metamorphosis, approximately 43 dph (week 7), when larvae undergo a number of rapid changes in morphology, physiology and anatomy (Pederson and Falk-Petersen, 1992). These findings are in accordance with other studies of growth in larval cod (Bolz and Lough 1988; Puvanendran and Brown 1999; Hunt von Herbing 2001; Puvanendran and Brown 2002). In cod, the exponential growth phase occurs at a point in development when cod switch from using a simple hyoid coupling to open

the mouth to a more complex secondary mouth opening mechanism (Hunt von Herbing et al. 1996b). This mechanism, facilitated by the differentiation of new structures, involves contraction of the muscles in the operculum, resulting in transmission of a force along the interoperculomandibular ligament to the lower jaw, which is then opened (Hunt von Herbing et al. 1996b). This switch most likely facilitates the consumption of larger prey items and may occur around the time that larval cod switch their prey choice (Hunt von Herbing 2001).

Investment in head growth relative to post-cranial growth increased over the 8 weeks of the study, after which head growth remained at around one third of post-cranial growth. Fuiman (1983) showed that nine different fish species exhibited a U-shaped growth profile during the larval stage, with the head and caudal regions growing faster than the middle regions. I did not measure the caudal region individually and therefore cannot rule out the possibility that such a U-shaped growth profile also existed in my fish. The priority given to development of the head in larval fish most likely reflects the requirement for early completion of the feeding and respiratory apparatus that facilitate consumption of planktonic food (Fuiman 1983; Osse and van den Boogaart 1995). Upon entering the juvenile stage of development, cod appear to develop isometrically (Bolz and Lough 1988) as do many other fish species (e.g. *Catostomus commersoni*, *Couesius plumbeus*, *Osmerus mordax*, Fuiman 1983).

Variations in total length, head length and post-cranial length were highest in weeks 8 and 9, when cod increased their rate of growth. Since the development of larvae varies within a cohort, there is a differential onset of the exponential growth phase, resulting in an increase in size variation (Folkvord et al. 1994). Beyond weeks 8 and 9, size heterogeneity declined once more, perhaps as smaller fish succumbed to cannibalism by larger conspecifics (Folkvord and Otterå 1993). Cod exhibit high levels of predatory aggression during this period of development (see chapter 5) when gape height relative to body length is at its greatest (Otterå and Folkvord 1993; Folkvord 1997).

Analysis of length-corrected measurements revealed that the pattern of growth for most morphological measures (excluding medulla width and gill width) changed on two occasions, week 3 to 4 and week 9 to 10 (with an additional switch occurring in jaw width growth at week 8). These changes in patterns of allometric growth occurred at the same time as feed changed, and it its possible that fish were exhibiting a phenotypically plastic response to new prey. For example, several species of cichlid fish have been shown to exhibit a phenotypically

plastic response to different prey types, developing a blunter snout when fed on worms than when fed on *Artemia* (Meyer 1987; Wimberger 1992; Hegrenes 2001). Moreover, results of a companion study (see chapter 4) provide evidence that the development of trophic morphology in larval cod may also be influenced by prey type. The mechanisms underlying this plasticity, if its exists, may relate to the remodelling that occurs when different prey sizes and textures produce different stresses and strains on cartilage or bone (Wimberger 1992).

Providing fish with different feed types may also affect morphological development by altering levels of nutrition. Most studies of feeding in fish have highlighted the improved growth or reduction in deformities that occur when copepods are provided as feed over *Artemia*, rotifers or a formulated diet (Hamre et al. 2002; Cutts 2003; Imsland et al. 2006). However, differences in the development of fish fed on rotifers, *Artemia* or inert feed are less clear. Larval fish require higher levels of vitamin C than adults (Merchie et al. 1997). However, Hamre (2006) showed that Rotimac enriched rotifers (an algal enrichment similar to the Algamac and DHAPS enrichments used here) contained less than half the vitamin C contained in *Artemia* and around one third that contained in copepods. In addition, vitamin A concentrations were below detection levels in these rotifers, which, unlike *Artemia*, have only low levels of carotenoids to convert to vitamin A in the event of a shortage (Hamre 2006). I cannot rule out the possibility that the observed decline in the growth of most head measures during the rotifer feeding period and subsequent increase in growth that occurred upon the introduction of *Artemia*, was at least partly attributable to these or other deficiencies of a rotifer diet.

Upon the introduction of formulated feed, the relative growth of length-corrected head measurements (including jaw width) decreased and again I cannot rule out an effect of diet on this change in the pattern of head growth. Problems with formulated feeds can include protein leakage, poor palatability and digestibility and inappropriate nutritional content for the species/age of larvae (Hamre 2006). With regard to AgloNorse, Ostaszewska et al. (2005) showed that AgloNorse-fed pike-perch (*Sander lucioperca*) larvae survived in similar numbers, grew at similar rates and developed similar digestive tracts to those larvae fed *Artemia*. Similarly, in this experiment, the increase in total length of fish remained constant through the *Artemia* and formulated feed stages, suggesting that growth was not compromised by the introduction of AgloNorse. Nevertheless, the effect of formulated feeds on the development of larval and juvenile cod morphology requires further analysis.

Length-corrected jaw width varied in the opposite direction to length-corrected eye diameter. This observation could be interpreted as a trade off between the need for visual acuity (perhaps necessary for locating small prey) on the one hand and large gape on the other (necessary for eating large prey, Otterå and Folkvord, 1993). Alternatively, since other measures of head length (including premaxilla length and jaw length) also varied in opposition to jaw width, the relationship between jaw width and eye diameter may be a consequence of a general trend for fast growth in length to be incompatible with fast growth in width.

This study has shown that the larval and juvenile cod undergo complex patterns of development, both with respect to the development of different areas of the head and of the head relative to the post cranium. The processes involved in determining these patterns of development (either differential mortality or differential growth of body parts), influence individual larvae differentially and generate marked individual variation in head morphology by the end of the larval/early juvenile period. Furthermore, since periods of change in the direction of head development coincided with periods when feed changed, development may be influenced by changes in the diet. This latter possibility has been tested and will be reported in chapter 4.

Chapter 4

The effect of diet on the development of head morphology in larval cod: possible implications for the development of cannibals

This chapter has been submitted for publication in Canadian Journal of Fisheries and Aquatic Sciences

4.1 Summary

Two related experiments were carried out to examine the effect of prey size on the development of head morphology in larval cod. In the first experiment, larval cod were fed on either rotifers or one of three different types of brine shrimp (*Artemia*) from 26 to 51- 52 days post hatch; head morphology was analysed at the end of this period. In the second experiment, larval cod were fed either rotifers or enriched *Artemia* from 27 to 55-57 days post hatch. Head morphology of both live and dead fish was analysed at weekly intervals, using Principal Components Analysis to characterise patterns of variation. In both experiments, fish fed on smaller prey had more fragile heads, although this difference was not significant for experiment 2. In both experiments, eye diameter was significantly larger relative to jaw width in fish fed smaller prey sizes. Analysis of the head morphology of dead fish ruled out differential mortality by head morphology as an explanation for these results and suggested that some of observed differences in morphology between fish fed the different prey types was a result of developmental plasticity. Development of clear diet-induced differences in morphology in less than 30 days represents an extremely fast rate of change compared to other studies of changes in trophic morphology in fish.

4.2 Introduction

Trophic polymorphism has been identified in a number of fish species, most notably Arctic charr (Skulason et al. 1989; Adams et al. 1998a; Jonsson and Jonsson 2001), perch (Svanbäck and Eklöv 2002), cichlids (Meyer 1990; Streelman et al. 2007) and sticklebacks (Schluter and McPhail 1992). Typically such studies have focused on fish in the natural environment and compared littoral (benthic) morphs with limnetic (pelagic) morphs, the former having a deeper body, and larger head and mouth (Ehlinger and Wilson 1988; Skúlason et al. 1989; Schluter and McPhail 1992; Schluter 1993, 1995; Hjelm et al. 2000, 2001; Svanbäck and Eklöv 2002). While intraspecific differences in morphology can result from genetic differences between morphotypes (McPhail 1984; Skúlason et al. 1989), there is increasing evidence that morphology can vary as a result of a phenotypically plastic response to a feature of the environment, most commonly habitat or availability of prey. For example, several species of cichlid fish have been shown to exhibit a phenotypically plastic response to different prey types, developing a blunter snout when fed on worms than when fed on brine shrimp (Meyer, 1987; Wimberger 1992; Hegrenes 2001).

The potential for fish to develop morphotypes best suited to the available prey could have important implications for aquaculture. For example, the prey offered may enhance a fish's ability to act as a cannibal, by promoting the development of large jaws early in ontogeny. Cannibalism is a particularly prevalent problem in the culture of several fish species and in none more so than the Atlantic cod. Of the initial yolk-sac larvae, only 5-7 % may survive to day 72 (Howell 1984) and cannibalism is frequently cited as the main cause of deaths (Howell 1984; Folkvord 1989). Given the importance of mitigating this behaviour during larval cod rearing, it would be useful to explore the relationship between prey type and the development of larval cod head morphology, and particularly trophic morphology.

The purpose of this study was to examine the effects of feeding different prey types (and therefore different prey sizes) commonly used in culture on the development of larval cod head morphology and to examine the potential for feeds to induce morphology comparable with that of cannibalistic morphs. The development of larval cod head and feeding structures has been described in several studies (e.g. Kjørsvik et al. 1991; Morrison 1993; Hunt von Herbing et al. 1996a, b; Hunt von Herbing 2001) but no studies have assessed the influence of prey type on this development. Two experiments are described. In the first one I assessed the

morphology of fish at the end of a period in which they were fed four different prey types. However, during this experiment, survival rates were low and I was unable to establish whether variations in morphology resulted from selective mortality of certain morphotypes or developmental plasticity in response to the prey type offered. Therefore, in a second experiment I increased prey density (in an attempt to increase survival rates) and I examined the morphology of both live and dead fish. In this second experiment I offered only two prey types but analysed morphology at regular time periods, so as to provide an indication of the rate of any observed changes in shape.

4.3 Material and Methods

Both studies were carried out at the Scottish Association for Marine Science Ardtoe Ltd, Ardtoe Marine Laboratory, Argyll, Scotland in June to July 2004 (Experiment 1) and again in April to May 2006 (Experiment 2). Larval rearing followed the standard procedure employed at this site (Shields et al. 2003). The methodologies employed in each experiment were as follows:

4.3.1 Experiment 1

Fertilised cod eggs were obtained from broodstock reared at the study site under photoperiod conditions that delayed spawning. One day prior to hatching, eggs were stocked in twenty black 100 L tanks at a density of 3000 eggs $tank^{-1}$ and kept in static water and under darkness until hatching was complete. From one-day post hatch (dph) to 21 dph, larvae were fed Algamac or Docosahexaenoic Acid Protein Selco (DHAPS) enriched rotifers (*Brachionus plicatilis*) at a density of 400,000 rotifers day^{-1} $tank^{-1}$, increasing to 600,000 rotifers day^{-1} $tank^{-1}$ from 22-31 dph. Microalgae (*Nannochloris* sp. and *Pavlova* sp.) were added to tanks from 0-31 dph at a density of 500,000 cells mL^{-1} as a feed for the rotifers. Water temperature was maintained between 10-12 °C. Lighting was continuous and ranged from 50 lux at 0 dph to 500 lux at the end of the experiment (52 dph). Tank hygiene was maintained by gradually increasing water flow rates from 70 ml min^{-1} at 0 dph to 140 ml min^{-1} at 52 dph and by frequently siphoning debris from the bottom of the tanks. In addition, synthetic scouring pads or 'skimmers' were partly suspended in the water of each tank from 1 dph in order to improve swim bladder formation by removing the oily surface film associated with live feed.

From 26 dph to 52dph, larvae were fed one of four different diets, with four replicates per diet. These diets consisted of Algamac enriched rotifers, fresh-hatch brine shrimp (*Artemia salinus*), Algamac enriched *Artemia* or Prolon enriched *Artemia*. The enrichments are proprietary emulsions designed to improve the fatty acid profile of live feed. Algamac is widely used throughout marine fish culture, Prolon is specifically designed to increase the biomass of prey, and fresh-hatch is yolk-sac *Artemia* which have not undergone enrichment. Dry weight analysis of the different prey types confirmed that Prolon enriched *Artemia* weighed, on average, 43% more than Algamac enriched *Artemia*; Algamac enriched *Artemia* weighed, on average, 20% more than fresh-hatch *Artemia*; fresh-hatch *Artemia* weighed, on average, 400% more than Algamac or DHAPS enriched rotifers.

All prey items were offered to larvae in gradually increasing amounts and at densities that ensured prey biomass was consistent between treatments. Rotifers were fed to four tanks at a density ranging between 6-20 rotifers mL^{-1} with no other prey item offered to larvae in these tanks; Algamac enriched *Artemia* were offered to four tanks at a density ranging from 0.5-3.4 *Artemia* mL^{-1}; four tanks received fresh-hatch *Artemia* at a density ranging from 0.6-4 *Artemia* mL^{-1} and another four tanks received Prolon enriched *Artemia* at a density ranging from 0.35-2.4 *Artemia* $\cdot mL^{-1}$. Under standard rearing conditions, a new prey species is cofed with rotifers for a period of one week. Consequently, those larvae fed fresh-hatch *Artemia*, Algamac enriched *Artemia* or Prolon enriched *Artemia* were also cofed three rotifers $\cdot mL^{-1}$ from 26-31 dph.

At the end of the experiment, over a two-day period (51 and 52 dph), ten larvae from each tank were photographed for morphometric analysis. The total lengths of 20 different larvae from each tank were also measured. All remaining larvae were removed from the tanks and counted in order to obtain an estimate of survival.

4.3.2 Experiment 2

Fertilised eggs were obtained from broodstock reared at the study site under ambient temperature and photoperiod conditions. One day prior to hatching, these eggs were transferred to a standard 1300 L production tank and kept in static water and under darkness until hatching was complete. From 0–26 dph the resulting larvae were reared and fed in exactly the same way as described for the larvae in the first experiment.

Upon reaching 27 dph, larvae were stocked in eight black 100 L tanks at a density of 400 larvae per tank. Larvae were thereafter fed one of two diets, with four replicates per diet until the end of the experiment (55-57 dph). Larvae in the first four tanks were fed Algamac or DHAPS enriched rotifers at densities ranging from 6-20 rotifers mL^{-1}. Larvae in the remaining four tanks were fed equal quantities of both Algamac and self-emulsified liquid concentrate (Selco) enriched *Artemia* at total densities ranging from 0.5-9 Artemia mL^{-1}. Larvae in these four tanks were also cofed Algamac or DHAPS enriched rotifers from 28-32 dph at densities ranging from 1.5-3 rotifers mL^{-1}. Prey biomass was consistent between treatments (Selco enriched *Artemia* equal in weight to Algamac enriched *Artemia*). Microalgae (*Nannochloris* sp.) were added to tanks from 27-32 dph at a density of 500,000 cells mL^{-1}. Flow rates, water temperature, lighting and tank hygiene were maintained as in experiment 1.

Throughout this experiment mortalities were removed using a siphon, counted and, if intact, photographed for subsequent morphometric analysis (Week 5: 20 *Artemia*-fed, 20 rotifer-fed Week 6: 19 *Artemia*-fed, 20 rotifer-fed; Week 7: 3 *Artemia*-fed, 20 rotifer-fed; Week 8: 12 *Artemia*-fed, 20 rotifer-fed). Each week, five live larvae were also sampled from each tank and photographed. At the end of the experiment, over a three-day period (55-57 dph), 20 live larvae from each tank were photographed for morphometric analysis. All remaining larvae were removed from the tanks and counted. Unfortunately, the pictures of two live larvae in an *Artemia*-fed tank in week 7 and an *Artemia*-fed tank in week 8 were not of a good enough quality to permit analysis.

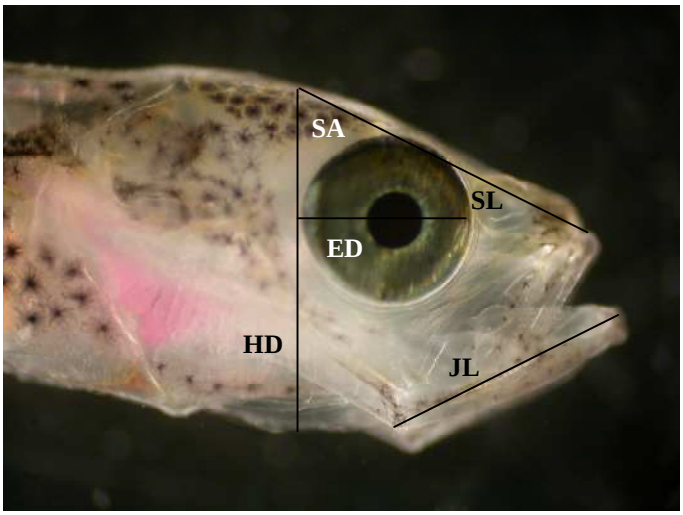
During both experiments, cannibalism was occasionally observed in rearing tanks other than those used in this study. In such instances (n = 6) the cannibal was removed from the tank and photographed for morphometric analysis. The cannibals analysed were all reared following similar protocols to those described above and aged between 47 and 64 dph.

4.3.3 Morphological Analysis

Larvae randomly sampled for morphometric analysis were killed using an overdose of anaesthetic (MS222) and photographed using a Nikon Coolpix 4500 digital camera fitted to a dissecting microscope. Photographs consisted of a lateral view of the whole larva and lateral and dorsal views of the head. The larvae were then measured using the image analysis

programme, Image Pro Express (Media Cybernetics). From the lateral view (Fig. 4.1a), the following variables were recorded: total length (measured from the lateral view of the whole larva); horizontal eye diameter, length of the jaw (Meckel's cartilage), head depth at the posterior edge of the eye and length of the snout (from the anterior tip of the snout to the intersect with the line defining head depth). Initially, the angle of the snout was also recorded. However, this dimension was difficult to measure consistently and consequently was not included in subsequent analyses. From the dorsal view (Fig. 4.1b) the following additional variables were measured: medulla width, jaw width (maximum distance between maxillae) and head length (from the anterior tip of the snout to the point midway between the operculae).

(a)



(b)

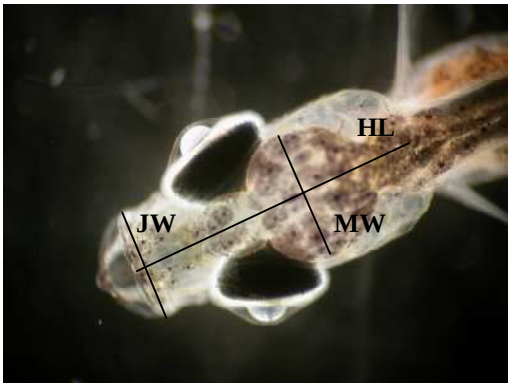


Fig. 4.1. Photographs of a 36 dph cod larva showing morphometric head measures recorded: lateral view (a) ED = eye diameter, JL = jaw length, HD = head depth, SL = snout length; dorsal view (b) JW = jaw width, MW = medulla width, HL = head length.

Total length, rather than standard length, was measured since the caudal fin was more clearly defined in images than the caudal peduncle. When a larva was too large to be photographed in its entirety callipers were used to measure total length. The caudal fin was never so badly nipped that measurements were inaccurate. Post-cranial length (distance from the edge of the operculum to the end of the caudal fin) was determined by subtracting the length of the head from the total length of the larvae.

4.3.4 Statistical analysis

In order to correct head morphometric data for an effect of size, residual scores were obtained from regression analysis of each head variable against head length. These variables are referred to in the following text as length-corrected measures. To investigate the relationships among the features measured and to identify those features of head morphology that accounted for the greatest amount of variation in the dataset, Principal Component Analysis (PCA) was carried out on these length-corrected measures. The results of the PCA informed subsequent analysis, the nature of which is described further in the Results section. A one-way ANOVA was used to examine changes in survival and length in both experiments, and differences in various features of head morphology among fish fed different prey items in experiment 1. A two-way ANOVA was used to examine whether features of head morphology varied among feeding treatments or weeks and the interactive effects of week and feeding regime in Experiment 2. A two-way ANOVA was also used to examine whether head morphology varied among live/dead fish or weeks and the interactive effects of week and live/dead in Experiment 2. In this latter analysis, in order to increase sample size, weeks 5 and 6 were grouped together, as were weeks 7 and 8. When a significant result was obtained Tukey's pairwise comparison was used to identify which means differed.

4.4 Results

4.4.1 Survival and overall growth

Estimated survival from egg to 51-52 dph was significantly affected by diet in Experiment 1 (Table 4.1), with fish fed rotifers surviving in significantly greater numbers than fish fed Algamac enriched *Artemia* or Prolon enriched *Artemia*. There was no effect of feeding regime on survival from 27 to 55-57 dph in Experiment 2 (Table 4.2). Total length varied significantly between fish fed different prey types at the end of both experiment 1 (Table 4.1) and experiment 2 (Table 4.2). In experiment 1, fish fed on Prolon enriched *Artemia* were significantly longer than fish fed fresh-hatch *Artemia* or Algamac enriched *Artemia*. Rotifer-fed fish were also significantly longer than fish fed Algamac enriched *Artemia*. In Experiment 2, fish fed *Artemia* were significantly longer than fish fed rotifers.

Table 4.1. Mean estimated % survival from egg to 51-52 dph and mean length of larval cod fed rotifers, fresh-hatch *Artemia* (Fr.-hatch *Artemia*), Algamac enriched *Artemia* or Prolon enriched *Artemia* at the end of Experiment 1.

	Prey type				Results of statistical analysis		
	Rotifers	Fr.-hatch *Artemia*	Algamac *Artemia*	Prolon *Artemia*	F	DF	P
Mean % survival from egg	27.32	20.00	9.40	2.44	18.55	3,12	<0.001
Mean length (mm)	14.21	13.50	12.47	15.45	13.30	3,156	<0.001

Table 4.2. Mean % survival from 27 to 55-57 dph and mean length of larval cod fed rotifers or Algamac and Selco enriched *Artemia* (Alg./Selco *Artemia*) at the end of Experiment 2.

	Prey type		Results of statistical analysis		
	Rotifers	Alg./Selco *Artemia*	T	DF	P
Mean % survival from 27 dph	67.50	71.50	0.67	4	0.541
Mean length (mm)	16.37	20.60	-8.90	1	<0.001

4.4.2 Identifying patterns of head shape

The first two principal components (PCs) resulting from PCA of the seven head measures accounted for 61.3 % of the total variance in experiment 1 and 63.7 % of the total variance in experiment 2 (Table 4.3). In both experiments, the loadings for the first PC were all negative and thus this PC loaded the size of all head measures against head length and is hereafter referred to as head 'fragility' (as opposed to head 'robustness': see chapter 3). Consequently, subsequent analyses of head fragility were carried out on the PC1 scores obtained from PCA of head measures, excluding snout angle. The second PC opposed eye diameter and snout length to jaw length and jaw width in experiment 1 and eye diameter to jaw width in experiment 2. In order to summarise this aspect of head shape in an intuitively simple way, rather than using the PC2 scores, I analysed eye diameter and jaw width individually and created an index reflecting the development of eye diameter relative to jaw width by dividing eye diameter by jaw width.

Table 4.3. PC1, 2 and 3 loadings for PCA of 6 length-corrected head measurements in larval cod.

Measure	Experiment 1		Experiment 2	
	PC1	PC2	PC1	PC2
Eye diameter	-0.312	0.612	-0.354	0.603
Jaw length	-0.314	-0.310	-0.323	-0.149
Head depth	-0.559	-0.191	-0.496	-0.316
Snout length	-0.451	0.447	-0.440	0.290
Jaw width	-0.252	-0.529	-0.265	-0.652
Medulla width	-0.474	-0.116	-0.510	0.071
Eigenvalue	2.121	1.557	2.415	1.412
% of variance	35.4	25.9	40.2	23.5

4.4.3 The effect of prey type/size on head shape: Experiment 1

Head fragility decreased significantly with increasing prey size (Fig. 4.2a: $F_{3,156} = 21.97$, $P < 0.001$). Fish fed rotifers had significantly more fragile heads than fish fed fresh-hatch or Algamac enriched *Artemia* which, in turn, had significantly more fragile heads than fish fed Prolon enriched *Artemia*. Length-corrected eye diameter also varied significantly between fish

fed the different prey types, with fish fed fresh-hatch *Artemia* having significantly larger eyes than fish fed Algamac or Prolon enriched *Artemia* (Fig. 4.2b: $F_{3,156} = 6.68$, $P < 0.001$). Length-corrected jaw width increased significantly with increasing prey size (Fig. 4.2c: $F_{3,156} = 18.82$, $P < 0.001$). Rotifer-fed fish had significantly smaller jaws than fish fed fresh-hatch *Artemia* which, in turn, had significantly smaller jaws than fish fed Algamac or Prolon enriched *Artemia*. As a consequence of these differences in eye diameter and jaw width, the eye diameter/jaw width index decreased significantly with increasing prey size (Fig. 4.2d: $F_{3,156} = 17.64$, $P<0.001$). Fish fed rotifers or fresh-hatch *Artemia* had a significantly larger eye diameter/jaw width index than fish fed Algamac or Prolon enriched *Artemia*. The head/post cranial length index also varied significantly between fish fed the different prey types, although the significant difference existed only between rotifer and Prolon enriched *Artemia* fed fish (Fig. 4.2e: $F_{3,156} = 3.04$, $P < 0.001$).

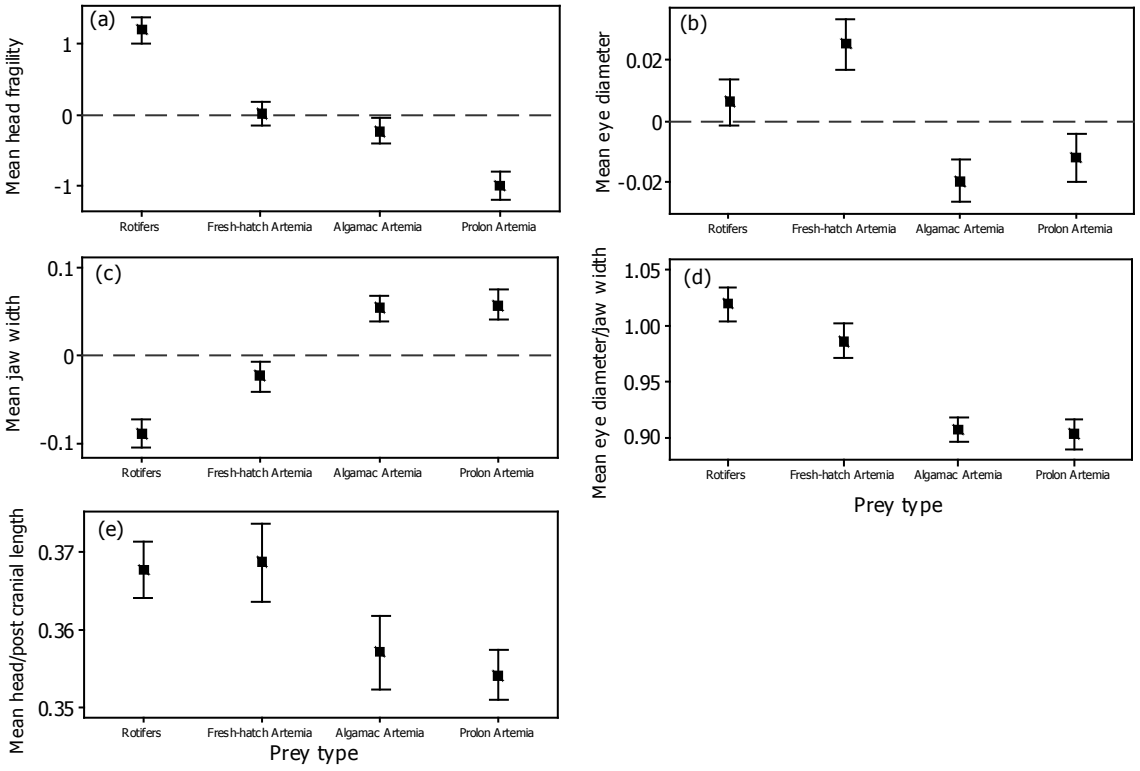


Fig. 4.2: Changes in head fragility (a), length-corrected eye diameter (b), length-corrected jaw width (c), eye diameter/jaw width index (d), and head/post cranial length index (e) of 51-52 dph larval cod fed rotifers, fresh-hatch *Artemia*, Algamac enriched *Artemia* or Prolon enriched *Artemia* in Experiment 1. Error bars are ±1 SE.

4.4.4 The effect of prey type/size on head shape: Experiment 2

Two-way ANOVA indicated a significant effect of week on head fragility, although pairwise comparisons failed to identify the nature of specific differences (Table 4.4 and Fig. 4.3a). Head fragility did not vary significantly between fish fed the different prey items. Length-corrected eye diameter increased significantly across weeks in rotifer-fed but not *Artemia*-fed fish (Table 4.4 and Fig. 4.3b). Consequently, by weeks 7 and 8, length-corrected eye diameter was significantly greater in rotifer-fed than *Artemia*-fed fish. Length-corrected jaw width decreased significantly across weeks in rotifer-fed fish but not *Artemia*-fed fish (Table 4.4 and Fig. 4.3). Consequently, by week 8, length-corrected jaw width was significantly smaller in rotifer-fed than *Artemia*-fed fish. The eye diameter/jaw width index increased significantly across weeks in rotifer-fed fish but not in *Artemia*-fed fish (Table 4.4 and Fig. 4.3d). Consequently, by weeks 7 and 8, this index was significantly greater in rotifer-fed than *Artemia*-fed fish. The head/post-cranial length index increased significantly across weeks in both rotifer-fed and *Artemia*-fed fish (Table 4.4 and Fig. 4.3e). There were no significant differences in the head/post-cranial length index between rotifer-fed and *Artemia*-fed fish in any corresponding week. Cannibals were found to have neither robust nor fragile heads, but an intermediate overall head type, small length-corrected eye diameter, large length corrected jaw width and small eye diameter to jaw width index.

Table 4.4. Results of two-way ANOVA testing the effects of week, feeding regime or the interaction between week and feeding regime on various features of larval cod morphology in Experiment 2 (eye diameter and jaw width are length-corrected).

Morphological feature	Week			Feeding regime			Week*Feeding regime		
	F	DF	P	F	DF	P	F	DF	P
Fragility	2.93	3,270	0.034	0.00	1,270	0.979	1.74	3,270	0.158
Eye diameter	17.55	3,270	<0.001	21.17	1,270	<0.001	11.40	3,270	<0.001
Jaw width	5.94	3,270	0.001	26.95	1,270	<0.001	4.56	3,270	0.004
Eye/jaw width index	34.18	3,270	<0.001	57.27	1,270	<0.001	14.79	3,270	<0.001
Head/post-cranial length	51.77	3,270	<0.001	0.55	1,270	0.458	4.12	3,270	0.007

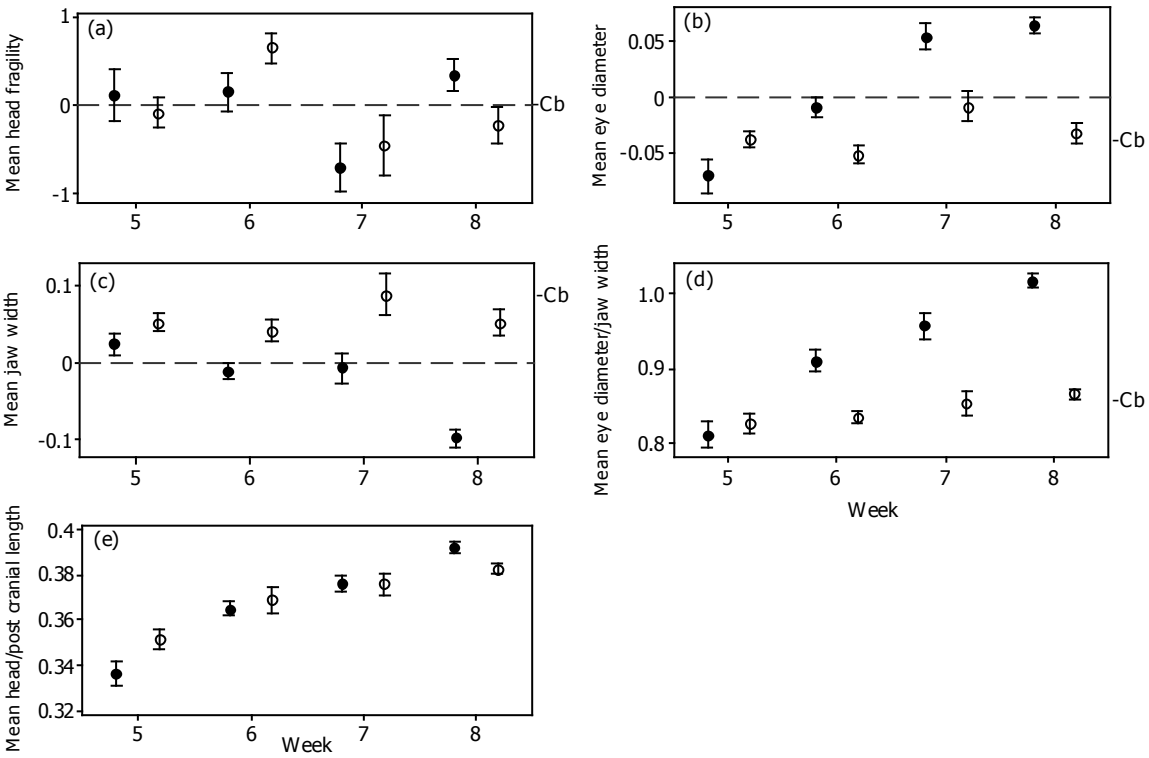


Fig. 4.3: Changes in head fragility (a), length-corrected eye diameter (b), length-corrected jaw width (c), eye diameter/jaw width index (d), and head/post cranial length index (e) of larval cod fed rotifers (black circles) or Algamac and Selco enriched *Artemia* (white circles) over the four weeks of Experiment 2 (week 5: 33-35 dph, week 6: 40-42 dph, week 7: 48-50 dph, week 8: 55-57 dph). The mean values for the first four of these measures in 6 cannibalistic larval cod are indicated on the y-axis as 'Cb'. Error bars are ±1 SE.

4.4.5 Differences in the morphology of live and dead fish

For both rotifer-fed and *Artemia*-fed fish, length-corrected eye diameter was significantly smaller in live than in dead fish in both weeks 5 and 6 and weeks 7 and 8 (Table 4.5 and Fig. 4.4a). Length-corrected jaw width did not differ significantly in rotifer-fed fish in weeks 5 and 6, but in weeks 7 and 8 this measure was significantly smaller in live than in dead fish (Table 4.5 and Fig. 4b). There were no significant differences in the length-corrected jaw width of live and dead *Artemia*-fed fish in either two-week period (Table 4.5 and Fig. 4.4b). There were no significant differences in the eye diameter/jaw width index in either two-week period for rotifer-fed fish, but in *Artemia*-fed fish a significant difference did emerge in weeks 7 and 8, with a smaller index in live than in dead fish (Table 4.5 and Fig. 4.4c). Consequently, live *Artemia*-fed live fish possessed relatively larger jaws relative to eye size than dead *Artemia*-fed fish in these two weeks. The head/post cranial length index did not differ significantly in

Artemia-fed fish in either 2-week period, but this index did differ significantly in weeks 5 and 6 in rotifer-fed fish, with a larger head relative to the post-cranium in live fish compared to dead fish (Table 4.5 and Fig. 4.4d).

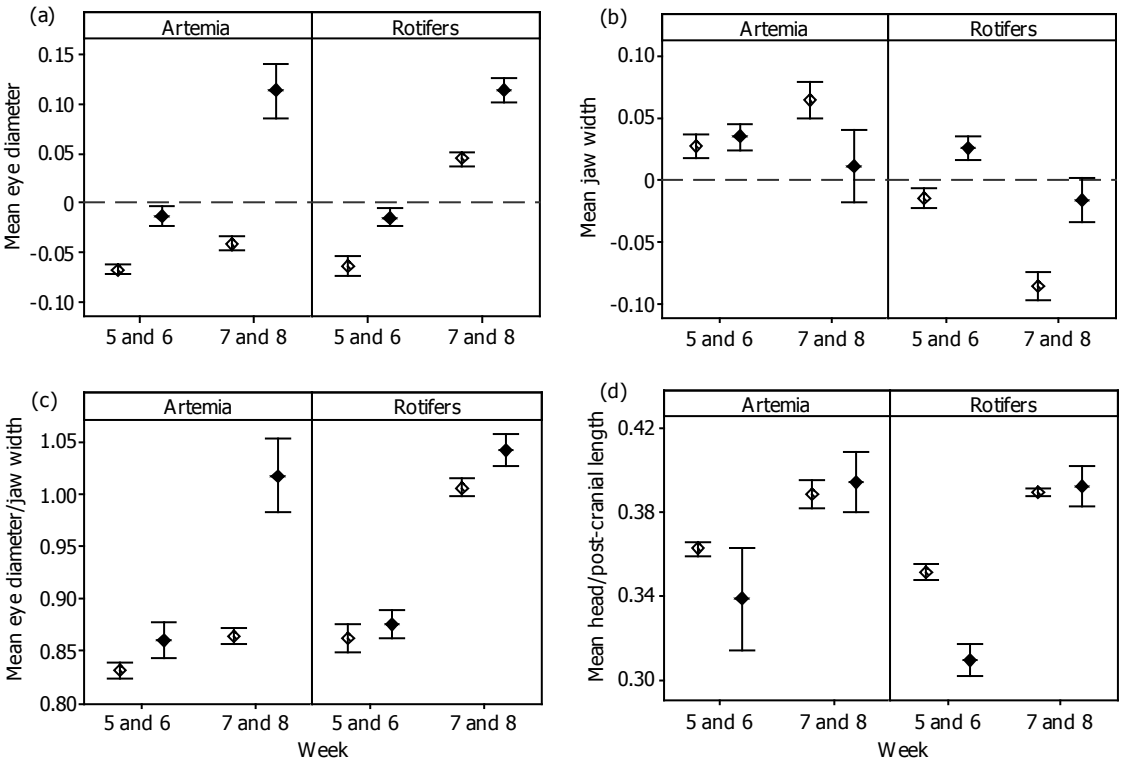


Fig. 4.4: Length-corrected eye diameter (a), length-corrected jaw width (b) eye diameter/jaw width index (c), total length (d) of live (white diamonds) or dead (black diamonds) larval cod in weeks 5 and 6 (33-42 dph) and weeks 7 and 8 (48-57 dph) in Experiment 2. Error bars are ±1 SE.

Table 4.5. Results of two-way ANOVA testing the effects of week, alive/dead or the interaction between week and alive/dead on various features of larval cod morphology in Experiment 2 (eye diameter and jaw width are length corrected).

Morphological feature	Week			Alive/dead			Week*Alive/dead		
	F	DF	P	F	DF	P	F	DF	P
Rotifer-fed fish									
Eye diameter	161.22	1,188	<0.001	40.16	1,188	<0.001	1.26	1,188	0.262
Jaw width	17.20	1,188	<0.001	16.61	1,188	<0.001	1.12	1,188	0.292
Eye/jaw width index	143.21	1,188	<0.001	3.50	1,188	0.063	0.70	1,188	0.404
Head/post-cranial length	125.89	1,188	<0.001	13.25	1,188	<0.001	17.14	1,188	<0.001
Artemia-fed fish									
Eye diameter	38.59	1,188	<0.001	71.32	1,188	<0.001	16.47	1,188	<0.001
Jaw width	0.10	1,188	0.751	1.24	1,188	0.268	2.12	1,188	0.147
Eye/jaw width index	42.27	1,188	<0.001	39.45	1,188	<0.001	18.24	1,188	<0.001
Head/post-cranial length	7.80	1,188	0.006	0.37	1,188	0.543	1.06	1,188	0.305

4.5. Discussion

The results of both studies identified clear and consistent patterns of variation in larval morphology. In both experiments a large amount of variation centered around head fragility, or in other words, the overall size of morphological features relative to the length of the head, while a second component opposed eye diameter to jaw width. To varying degrees, both these measures varied among fish fed the different diets and these differences were broadly consistent between the two experiments. The observed differences in morphology occurred very quickly: under 30 days in both experiments. As far as I am aware, this is one of the fastest morphological changes documented in fish and comparable with the Arctic charr studied by Andersson et al. (2005) that underwent morphological changes in just 30 days.

In Experiment 1 fragility of the head increased with decreasing prey size, while by the end of Experiment 2 head fragility was also greatest in fish fed the smallest prey item, although this difference was not significant. Furthermore, in both experiments eye diameter relative to jaw width was greater in fish fed smaller prey sizes. Further analysis of the patterns of change of both eye diameter and jaw width in Experiment 2 indicate that this difference is attributable to both an increase in eye diameter and a decrease in jaw width over time in rotifer-fed fish, with

Artemia-fed fish exhibiting no changes in these features across weeks. It is possible, therefore, that rotifer-fed fish developed the larger eyes suited to searching for small prey and that this compromised growth of the jaws. Alternatively, it is also possible that such an increase in eye size relative to jaw size reflects a predetermined ontogenetic pathway in larval cod. *Artemia*-fed fish, conversely, must and did maintain a relatively large jaw size in order to consume larger prey. Regardless of the ontogenetic pathway that exists in the wild, each pathway demonstrates a diet-mediated physical adaptation.

While a large number of studies have identified trophic polymorphisms within species located at different sites, the mechanisms underlying the polymorphisms frequently relate to genetic differences and/or reflect many generations of adaptive selection (Lavin and McPhail 1986; Mittlebach et al. 1992; Robinson et al. 1993; Skulason et al. 1996; Hjelm et al. 2000). However, an increasing number of studies have confirmed the potential for differing prey types to produce differing morphologies within a fish's lifetime (Meyer 1987; Thompson 1992; Wimberger 1992; Mittelbach et al. 1999; Robinson and Wilson 1996; Hegrenes 2001; Hjelm et al. 2001). For example, in a study of the cichlid, *Cichlasoma managuense*, Meyer (1987) found that fish reared on *Artemia* developed more pointed heads than fish fed flake food and nematode worms. In many of these studies the prey are different in both texture and size (Mittelbach et al. 1999; Wainwright et al. 1991) and/or require different methods of acquisition (Robinson and Wilson 1995; Hegrenes 2001; Wintzer and Motta 2005). Consequently, differences in morphology are frequently cited as resulting from the differing stresses and strains that act on the bone (Wimberger 1992). In this study, all the prey employed were planktonic, soft-bodied and acquired via a type of 'ram feeding' (Hegrenes 2001) in which the predator simultaneously moved the head forward, while opening the mouth to ingest static prey. Consequently, these results suggest that differing prey sizes alone may be sufficient to induce differences in morphology, either as a result of the adaptive bone remodeling that develops for macrophagy or the increased visual acuity that develops to locate smaller prey items.

Providing fish with different feed types alters levels of nutrition, and thus growth rates, and these factors may also contribute to differences in morphological development. Meyer (1987), for example, attributed the phenotypic changes in the morphology of *Cichlasoma managuense* to heterochrony resulting from the retarded development of fish in one prey group. However, although I observed some differences in the growth rates of fish fed the different prey types,

in Experiment 1, rotifer-fed fish and Prolon enriched *Artemia*-fed fish did not differ in final total length, yet exhibited differing morphologies. This finding suggests that mechanisms other than heterochrony contributed to the observed results.

With regard to nutrition, although much is known about the improved growth or reduction in deformities that occur when copepods are provided as feed rather than *Artemia*, rotifers or an inert diet (Hamre et al. 2002; Cutts 2003; Imsland et al. 2006), differences in the development of fish fed on rotifers or differently enriched *Artemia* are less well documented. However, Hamre (2006) showed that Rotimac enriched rotifers contained less than half the vitamin C contained in *Artemia* and undetectable amounts of vitamin A, unlike *Artemia* that have sufficient levels of carotenoids to convert to vitamin A in the event of a shortage. In addition to micronutrients, studies of nutrition in farmed fish have also highlighted the importance of providing larvae with the optimal fatty acid and lipid concentrations (Koven 2003). For example, Hamre et al. (2002) showed that Atlantic halibut (*Hippoglossus hippoglossus*) larvae fed DHA Selco enriched *Artemia* were significantly more prone to malpigmentation and impaired eye migration compared with those fed copepods. In these experiments fatty acid and lipid compositions varied on account of both prey type and the enrichment method used. Consequently, I cannot rule out the possibility that the observed differences in eye size and jaw size observed in these fish were at least partly attributable to these or other differences in the nutritional content of the different diets.

The poor survival rates of many fish in Experiment 1 raised the possibility that the observed variations in morphology may have resulted, at least in part, from the selective mortality of certain morphotypes. In Experiment 1 the survival rate from egg to 51-52 dph was highly variable between fish fed the different prey types. However, these figures were derived from an estimate of egg numbers stocked and it is highly probable that many of these eggs did not hatch and/or that a large number of larvae were lost in the first few days and not during the period that prey type was manipulated. This conclusion is supported by the high survival rates of fish after 27 dph in Experiment 2.

Comparison of head morphology in live and dead fish in Experiment 2 provides some insight into the question of selective mortality. Since rotifer-fed fish were found to have larger eyes than their *Artemia*-fed counterparts, an effect of selective mortality on the observed results would involve the death of small-eyed morphotypes. However, in rotifer-fed fish, length-

corrected eye diameter was larger in dead fish compared with live fish and there was no difference in length-corrected jaw width between live or dead *Artemia*-fed fish. This rules out simple selective morphology as the cause of the differences in head morphology in surviving fish. However, evidence to suggest an effect of selective mortality is provided by the significantly greater eye size in *Artemia*-fed mortalities and the significantly greater jaw width in rotifer-fed mortalities. Therefore, these results seem to indicate that trophic plasticity definitely underpinned some of the differing effects of prey type on morphology, while the evidence for selective mortality of morphotypes not suited to the available prey is inconclusive.

An effect of prey type on the trophic development of farmed cod, irrespective of the underlying mechanism, raises the possibility that fish may be offered prey that encourages the development of morphology suited to cannibalism. This is particularly relevant to the culture of cod, since this species are known to be highly cannibalistic in the early stages of rearing (Howell 1984). In the six cannibals that I encountered in this study, eye diameter was relatively small and jaw width was relatively large and comparable with the morphology of fish fed the standard feeding protocol, i.e. enriched *Artemia*. Although based on a small sample size this larger jaw size presumably aids consumption of conspecifics in what are known to be gape-limited predators (Ottera and Folkvord 1993). The development of morphology in newly cannibalistic cod merits study.

This study has clearly shown that providing larval cod with differing prey types can induce significant differences in head morphology over a very short period of time. These differences are, at least in part, attributable to a plastic response to the size of prey. Furthermore, there is some indication that larvae fed the enriched *Artemia* so commonly used in aquaculture, develop morphology comparable with that of cannibalistic larvae. Current culture techniques may therefore encourage the development of cannibalistic morphs.

Chapter 5

Aggression in larval cod: competition or cannibalism?

This chapter has been submitted for publication in Canadian Journal of Fisheries and Aquatic Sciences

5.1 Summary

Aggressive interactions in larval cod were quantified in order to determine whether these might represent an early form of cannibalism rather than simply a battle for resources. We fed larvae one of four prey densities from 27 to 62 days post hatch and recorded the number of attacks that occurred in the absence of food and during feeds. Most attacks took the form of brief, one-way, nips by an attacker to a victim. The fish also commonly showed a pattern of burst swimming (darts) that appeared to reflect a generalised escape response. Darting was not affected by the presence of food, but was more common in fish fed the higher prey densities, possibly as a result of increased fish condition or size. Varying overall levels of prey did not affect levels of aggression, although analysis was confounded by a decline in levels of aggression with increasing fish density. The frequency of nips was highest when food was absent and nips were preferentially directed at the tail of victims, to victims of a smaller or similar size than the attacker and to victims that showed abnormal body posture. These findings are consistent with the suggestion that at least some attacks by larval cod represent early attempts at cannibalism.

5.2 Introduction

Aggression can be defined as behaviour that has the potential to inflict non-accidental injury on other animals (Huntingford and Turner 1987). It can occur between different species (interspecific), or between conspecifics (intraspecific) and may occur as individuals attempt to protect offspring (Colgan and Gross 1977), obtain fertilising opportunities (Colgan et al. 1981), compete for food (Adams et al. 1998b) or protect territories (Keenleyside and Yamamoto 1962). In such cases, aggression represents a form of social interaction resulting from competition with other individuals. However, aggression may also be predatory in origin and arise as an animal attempts to consume part, or all, of another animal. A lion, for example, attempting to kill an antelope for food is exhibiting a form of predatory aggression, whereas a lion that attacks a conspecific for access to a carcass is exhibiting a form of competitive aggression. Clearly, these behaviours are distinct in function and causation, but they are not mutually exclusive, and this is especially true with regard to cannibalistic species that eat smaller conspecifics. For example, Sakakura and Tsukamoto (1996) concluded that cannibalistic behaviour in yellow tail (*Seriola quinqueradiata*) resulted from aggressive tendencies towards victims and not because cannibals regarded victims as food.

One possible way to examine the nature of conspecific aggression in fish would be to examine the extent to which the availability of non-cannibalistic prey influences aggression. For example, aggression that is predatory/cannibalistic in nature increases with a decline in an alternative food source, as has been seen in cannibalistic Japanese flounder juveniles (*Paralichthys olivaceus*) (Dou et al. 2000), cannibalistic larval and juvenile sharptooth catfish (*Clarias gariepinus*) (Hecht and Appelbaum 1988) and cannibalistic juvenile snakehead (*Channa striatus*) (Qin and Fast 1996). Ultimately, the relationship between prey availability and aggression depends on the motivation behind aggressive attacks and various aspects related to the provision of food. Greaves and Tuene (2001), for example, showed that Atlantic halibut initiated aggressive attacks during feeding but not at any other time. In this study, food acts as a stimulus for resource competition. However, levels of feeding, and thus levels of hunger, may also influence aggression. Davis and Olla (1987), found that levels of aggression were highest in juvenile chum salmon (*Oncorhynchus keta*) fed the largest prey rations. In addition, the synchronicity and frequency of feeding may also influence levels of aggression as a result of variations in resource defensibility (Bryant and Grant 1995). Japanese medaka

(*Oryzias latipes*) for example, fed food only once every minute have been shown to exhibit higher levels of aggression than medaka fed once every 5 seconds (Bryant and Grant 1995).

Aggressive behaviour is a significant problem in aquaculture, resulting in significant losses, as fish are injured and subsequently diseased or cannibalised (Kaiser et al. 1995). Moreover, aggressive activity can result in slow growth in both the attackers (Hecht and Uys 1997) and the victims of attacks (Koebele 1985). A particularly aggressive culture species is the Atlantic cod. In the early rearing stages, only 5-7 % of initial yolk-sac larval cod may reach metamorphosis (Howell 1984) and cannibalism is frequently cited as the main cause of mortality during the mid- to late larval and early juvenile stages (Howell 1984; Folkvord 1989). Although starvation and type of prey have been shown to affect the incidence of cannibalistic attacks in juvenile cod (Folkvord 1991), I am not aware of any studies that have explicitly examined early forms of aggressive behaviour in cod, including the nature of that aggression (i.e. competitive and/or predatory). This is despite the fact that non-cannibalistic attacks can result in large numbers of mortalities in cod (Folkvord 1989).

The purpose of this study was therefore to describe aggressive interactions in larval cod, including the relative size of victim and attacker and the condition of the chosen victims. I hoped to establish the extent to which aggressive attacks were representative of an early form of cannibalism and/or the result of a battle for resources. In light of the above studies, I tested the hypothesis that predatory aggression would be infrequent during feeding. Since cod direct cannibalistic attacks at the tails of victims (Forbes personal observation) and the ability to cannibalise is governed by the predator to prey length ratio (Folkvord and Ottera 1993), I also predicted that predatory attacks would be directed at the tail of smaller individuals. Conversely, I expected competitive aggression to be more prevalent during periods of feeding and directed randomly at the body of individuals that varied widely in relative size to the attacker. Since prey availability is known to influence each type of aggression in different ways, I also examined how the overall density of food affected the incidence of aggressive attacks.

5.3 Materials and Methods

5.3.1 Rearing of fish and morphological screening

The study was carried out at the Scottish Association for Marine Science Ardtoe Ltd., Ardtoe Marine Laboratory, Argyll, Scotland in February and March 2005. Larval rearing prior to and during the study followed the standard procedure employed at this site (Shields et al. 2003). Fertilised Atlantic cod eggs were obtained from broodstock reared in the Shetland Islands under ambient temperature. One day prior to hatching, the eggs were transferred to three 1300 L production tanks and kept in static water and under darkness until hatching was complete. Following hatch (0 days post hatch or 0 dph), fish were fed Algamac or Docosahexaenoic Acid Protein Selco (DHAPS) enriched rotifers (*Brachionus plicatilis*) from 1-32 dph and Algamac or Disinfecting continuously self-emulsified liquid concentrate (DC Selco) enriched brine shrimp nauplii (*Artemia*) from 27-35 dph. Microalgae (*Nannochloris* sp. and *Pavlova* sp.) were also added to tanks from 0-32 dph at a density of 500,000 cells mL^{-1} day^{-1} as feed for the rotifers. Water temperature was maintained between 10-12˚C. Lighting was continuous and ranged from 50 lux at 0 dph to 500 lux from 10 dph onwards. Tank hygiene was maintained by gradually increasing water flow rates, ranging from 500 mL^{-1} min^{-1} at 0 dph to 3 L^{-1} min^{-1} at 35 dph and by frequently siphoning debris from the bottom of the tanks. In addition, rectangular floating polystyrene ‘skimmers’ were fitted from 1 dph to improve swim bladder formation by removing the oily surface film associated with live feed.

Upon reaching 35 dph, fish were randomly sampled from production tanks and placed in twelve 10 L aquaria at a density of 225 fish $tank^{-1}$. Due to the high number of mortalities that occurred during the transfer period, fish numbers per aquaria were maintained at 225 for a period of eight days by replacing the number of mortalities with a corresponding number of live fish from the production tank. At 36 dph, Algamac enriched *Artemia* were fed to fish at four different densities: 5, 10, 15 and 20 % of fish biomass, with three replicates per density. Fish biomass was estimated by sampling and weighing 100 other fish of the same age from the same production tanks. Feeds were thereafter increased in an attempt to maintain prey density within each regime at the same proportion of fish biomass, although the weight of fish and prey density were only directly compared at 36 dph. At the study site, fish are normally offered prey at a density of 10 % fish biomass. Consequently, the decision to offer prey at a density of 5 % was first discussed and approved by a government inspector. Daily feeds were

split into two and *Artemia* offered once in the morning and once in the afternoon until the end of the experiment (61-62 dph). Water temperature was maintained at 9-10.5 °C. Lighting was continuous and maintained at 300 lux. Tank hygiene was maintained by gradually increasing water flow rates from 2 mL^{-1} min^{-1} at 35 dph to 3 mL^{-1} min^{-1} from 53 dph onwards and by frequently siphoning debris from the bottom of the aquaria. Dead fish were removed from each tank every day and counted.

At the end of the experiment, all fish were killed with an overdose of anaesthetic (MS222), standard length recorded and weight recorded for 10 of these fish from each tank. Condition of fish was defined as:
Condition factor (K) = W / L^3,
where W is the weight (g) and L is the standard length (cm).

5.3.2 Experimental set-up, data collection and video recording

Tanks were observed daily until 45 dph, when aggressive encounters (largely comprising of nipping conspecifics) increased from zero or one incident per 10 min observation period, to 3 or more incidents in each 10 min period. Video recording commenced five days later at 50 dph for a period of 11 days. Each day, one tank from each food density was recorded for a period of 1 h, resulting in a total recording time of 4 h each day. Prey were introduced into each tank exactly halfway through this 1 h period of recording, using a pipette. This process took no longer than a few seconds and permitted observations of the behaviour of fish that had not been fed for several hours (pre-feed) as well as the behaviour of fish during the half-hour immediately following the introduction of feed (post-feed). Prey were available to fish throughout the post feed recording period at all prey densities. Recordings always took place in the morning and prior to the afternoon feed in order to maximise the time since the fish were last offered food.

5.3.3 Behavioural analysis

Since it was impossible to record accurately all fish movements, one quarter of the video screen was viewed, to the left of centre but vertically central. The rest of the screen was blocked from view using black cardboard. The first five minutes of filming were ignored in order to allow the fish to settle, as was the first five minutes post feed, when the addition of

prey often blurred the screen. For the remainder of each 1 h recording, I analysed the first two minutes of every five minutes of footage. Previous analysis had shown that this gave a representative picture of behaviour during the whole five minutes. The number of fish in view at the start and end of each two-minute observation period was noted, followed by the incidence of nips in each 2 min period and the location of each nip on the victim's body (head, post cranial region excluding caudal tail or caudal tail). A nip was characterised by a sudden movement of the head towards a victim, resulting in an attempt to bite that victim. Both a successful and unsuccessful attack would elicit a burst of rapid swimming by the victim away from the attacker. When possible (225 out of 343 nips) the relative on-screen length of both attacker and victim were noted. In addition, the orientation of 121 victims was recorded, plus the orientation of 100 randomly selected control fish. Three orientation positions were recorded: horizontal, head downwards at 45° or head downwards vertical.

Preliminary observations showed that a lot of bursts of fast swimming were taking place, hereafter defined as 'darts'. Analysis of such movements in 110 randomly selected fish (48-58 dph) identified a bimodal frequency distribution of swimming speeds, with darts constituting the second peak and ranging from 2-3.5 s body length^{-1} (Mean = 2.87, IQR = 0.78). I also counted the number of darts that took place in each 2 min observation period that were not associated with any obvious nip.

5.3.4 Statistical Analysis

There was no significant effect of aquarium on the incidence of nips (Prey density: 5 % fish biomass: $H = 2.67$, $DF = 2$, $P = 0.263$; 10% fish biomass: $H = 0.02$, $DF = 2$, $P = 0.988$; 15 % fish biomass: $H = 0.09$, $DF = 2$, $P = 0.956$; 20 % fish biomass: $H = 2.39$, $DF = 2$, $P = 0.303$) or darts (Prey density: 5 % fish biomass: $H = 1.31$, $DF = 2$, $P = 0.519$; 10 % fish biomass: $H = 0.49$, $DF = 2$, $P = 0.784$; 15% fish biomass: $H = 0.56$, $DF = 2$, $P = 0.755$; 20 % fish biomass: $H = 0.73$, $DF = 2$, $P = 0.694$). In order to derive a single figure for the rate of nips or darts for pre- and post-feed periods in each 1 h record, the number of nips or darts recorded in each 2 min period was divided by the average of the number of fish at the start and end of the period and halved to give the number of nips or darts per fish per minute. The median of these figures was used to assess temporal changes in the incidence of nipping or darting during the half-hour period prior to or post feeds.

There were no temporal changes in the incidence of nipping during the half-hour prior to (Table 5.2c) or during the half-hour following a feed at any prey density (Table 5.2d) so a single score was derived for all subsequent analyses. However, since there was an effect of pre/post feed (Table 5.2a), analyses of nips were carried out on pre- or post-feed scores. In most cases the frequency of darts was stable across the pre- or post-feed periods (Table 5.3c, d) so again a single score was derived for all subsequent analyses. However, since there was no effect of pre/post feed (Table 5.3a), analyses of darts were carried out on 1 h records, combining pre- and post-feeds together. For every half-hour or hourly record, the median of the nips, or mean of the darts, per fish per minute (hereafter referred to as the 'nip score' or 'dart score') was used to analyse the relationship between the number of nips or darts and prey density or fish density. (It is important to note that I did not initially set out to analyse the effect of fish density, but were prompted to do this as a result of differential survival between tanks.) The median of all the nip or dart scores was then used to assess the relationship between the number of nips or darts and pre/post feed or age.

I used ANOVA to assess the relationship between length, the coefficient of variation (CV) of length, weight, condition factor, survival and feeding regime. Univariate nonparametric statistical analyses (Kruskal-Wallis or Mann-Whitney as appropriate) were used to assess differences between the pre- and post-feed nip or dart scores (see above) and between the number of nips or darts and age. Significant results were followed by a parametric (Tukey) or nonparametric post-hoc multiple comparison test to identify differences among treatments. The relationship between the number of nips or darts and fish density was identified by regression analysis of these variables within each feeding regime. The relationship between the number of nips, darts or fish density and prey density was also identified by regression analysis of these variables. A Chi^2 test was used to assess if attackers favoured victims orientated in a specific direction, relative to the distribution of orientations of all fish during the study. The same test was used to determine whether the relative lengths of attackers to victims were non random. This was achieved by comparing results with the relative lengths of 80 randomly paired cod larvae (51-52 dph) from a separate study, reared under similar conditions. Chi^2 tests were also used to assess any bias in the location of nips on the body of victims, relative to fish body proportions (assessed in 20 randomly selected fish of the same age) or relative to the size differential between attacker and victim, or relative to the orientation of victims.

5.4 Results

5.4.1 Growth and survival

Fish density declined throughout the period of the study in all prey density treatments, although the decline was much steeper in fish fed the lower prey densities (Fig 5.1). Consequently, by the end of the experiment there was a significant difference in the survival rate of fish fed the different prey densities (Fig 5.2a: $F_{3,8} = 9.93$, $P = 0.005$). Fish fed the two highest prey densities survived in significantly greater numbers than fish fed the lowest prey density and consequently, there was a positive relationship between fish density and prey density ($R^2_{adj} = 53.2$ %, $F_{1,40} = 47.53$, $P < 0.001$). The length and weight of fish also varied significantly between prey densities (Fig 5.2b: Length: $F_{3,116} = 10.41$, $P < 0.001$; Fig 5.2c: Weight: $F_{3,116} = 10.10$, $P < 0.001$). Fish fed the lowest prey density were significantly shorter and lighter than fish fed any of the other prey densities. There was a general trend for variation in length (CV) to increase with increasing prey density (Fig. 5.2d: $F_{3,8} = 4.43$, $P = 0.041$), although pairwise comparisons did not identify the location of specific differences. There was no significant difference in the condition factor of fish fed the different prey densities ($F_{3,116} = 2.52$, $P = 0.062$).

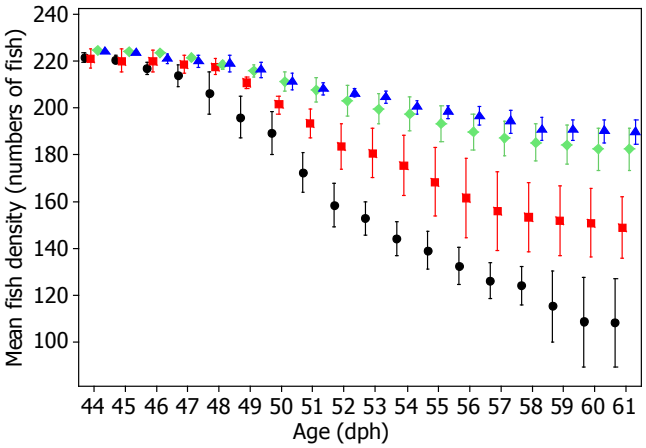


Fig. 5.1 Fish density versus age at each prey density (black circle: 5 % fish biomass; red square: 10 % fish biomass; green diamond: 15 % fish biomass; blue triangle: 20 % fish biomass). Error bars are ±1 SE.

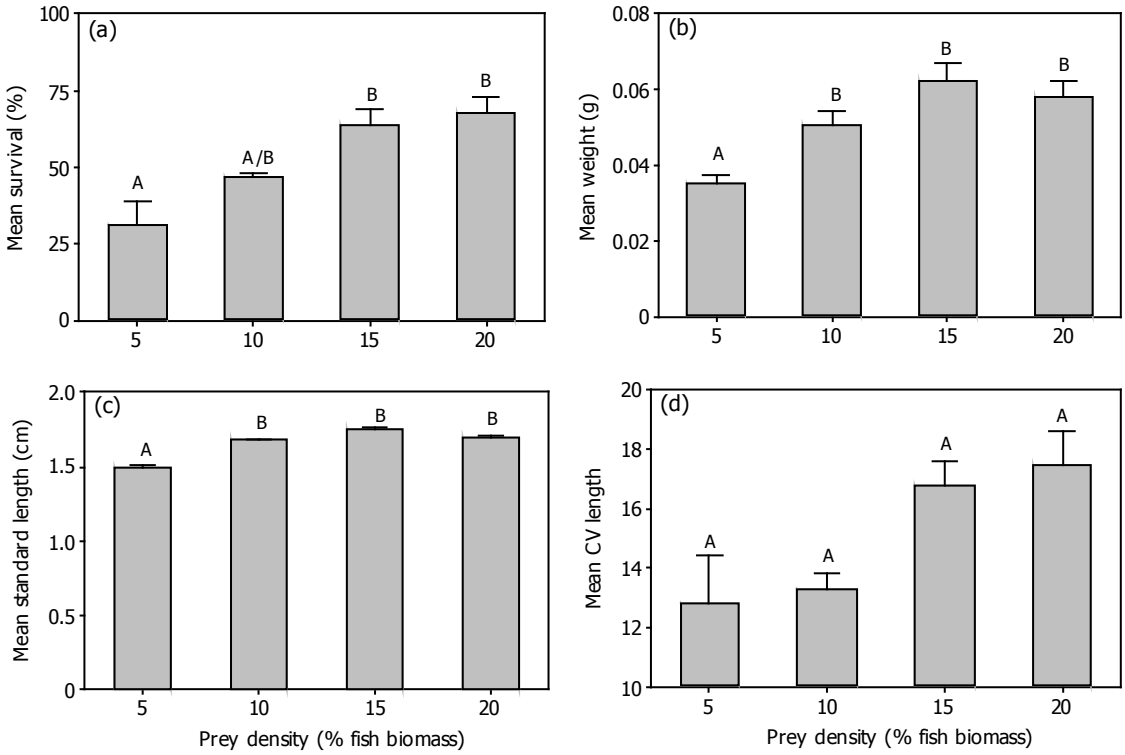


Fig. 5.2 Survival (a), length (b), weight (c) and coefficient of variation of length (d), of fish fed each prey density. Different letters indicate significantly different pairwise comparisons. Error bars are ±1 SE.

5.4.2 A general description of aggressive encounters: characteristics of attackers and victims

A total of 42 hours were studied, in which time a total of 343 nips were observed. The nip score ranged from 0-0.145 nips $fish^{-1}$ min^{-1} (Fig. 5.3: Median = 0.013 nips $fish^{-1}$ min^{-1}, IQR = 0.045). Nips were absent in 36 of the 84 half-hour periods prior to and following a feed. More nips were directed at a victim that was smaller than, or of the same size as, the attacker, than was to be expected relative to the size distribution of the population (Table 5.1). Furthermore, more nips were directed at fish that were orientated vertically, and less at fish that were horizontal, than was to be expected relative to the mean distribution of fish orientations (Table 5.1). Attackers also directed more nips to the tail of victims, and less to the body and head than was to be expected if attacks were randomly directed at the body of prey, taking into account fish body proportions (Table 5.1). The predominance of attacks towards the tail was seen regardless of the size of the attacker, but this effect was weaker in cases where the attacker was smaller than the victim (78.38 % as opposed to 90.91-94.74 %) (Chi^2 = 7.991, DF = 2, P = 0.018). Similarly, the predominance of attacks towards the tail was seen

regardless of the orientation of the victim, but this effect was weaker in cases where the victim was orientated horizontally (82.61 % as opposed to 96.30-97.92 %) (Chi^2 = 8.231, DF = 2, P = 0.016).

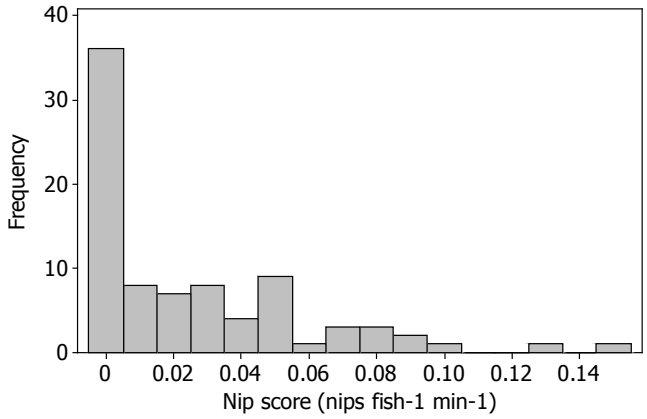


Fig. 5.3 Frequency distribution of nip score (nips $fish^{-1}$ $minute^{-1}$).

Table 5.1 Features of victims during attacks (nips): size relative to attacker (a), orientation when attacked (b) and the bodily location of nips (c). Refer to Material and Methods for further information on the derivation of control figures.

Victim feature	Study (%)	Control (%)	Chi^2	DF	P
a) Size relative to attacker					
Smaller than attacker	59.11	43.75	18.78	2	<0.001
Similar size to attacker	24.44	16.25			
Larger than attacker	16.44	40.00			
b) Orientation					
Horizontal	38.02	66.00	25.33	2	<0.001
Head downwards at 45°	22.31	23.00			
Head downwards vertical	39.67	11.00			
c) Location of nip					
Tail	90.38	20.64	338.99	2	<0.001
Body	5.25	53.49			
Head	4.37	25.87			

A total of 3961 darts were observed in the absence of an obvious nip during the 42 hours of the study. Darts were observed in every one of the half-hour observation periods prior to and following a feed. The dart score ranged from 0.06-0.58 darts per fish per minute (Fig. 5.4: Median = 0.23 darts per fish per minute, IQR = 0.17). Of these darts, 82.6 % could not be attributed to any obvious cause, 8.9 % occurred when the tails of two fish touched, 4.7 %

occurred when a fish met another fish face on and 3.8 % occurred when a fish was tracked by another fish.

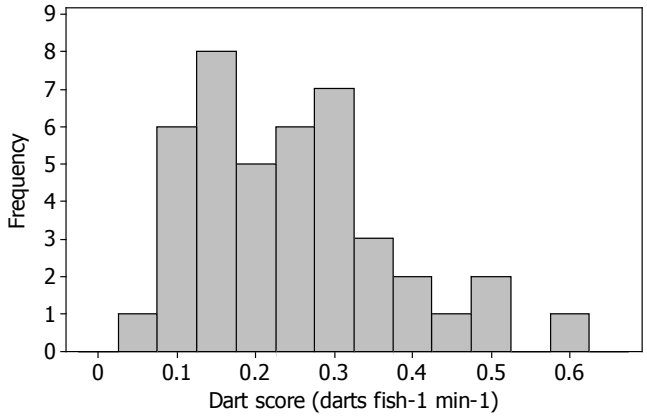


Fig. 5.4 Frequency distribution of dart score (darts $fish^{-1}$ min^{-1})

5.4.3 Factors that affected rates of nipping

Significantly more nips occurred pre-feed than post-feed (Table 5.2a and Fig. 5.5). In total, only 27 nips were recorded post-feed throughout the study. This difference in the incidence of nipping behaviour pre- and post-feed was consistent between feeding regimes (Table 5.2a). There was no temporal changes in the incidence of nipping during the half-hour prior to, or the half-hour following, a feed (Table 5.2c, d). This absence of an effect of time was consistent within feeding regimes (Table 5.2c, d). Due to the small number of nips that occurred post feed, all subsequent analyses of nipping behaviour include only nips that occurred prior to a feed.

Table 5.2 Median number of nips fish^{-1} min^{-1} at each prey density (represented as % fish biomass) pre- and post-feed (a), at each age (b) and over the half-hour period prior to (c) and since a feed (d).

Prey density	Median number of nips fish^{-1} min^{-1} (*pre-feed only)					Results of statistical analyses					
						H	DF	W	N_1	N_2	P
a) Effect of feeding											
	Pre-feed	Post-feed									
5	0.035	<0.001						144	10	10	0.0036
10	0.051	<0.001						181	11	11	0.0004
15	0.039	<0.001						144	10	10	0.0036
20	0.042	<0.001						163	11	11	0.0181
Overall	0.043	<0.001						2464	42	42	<0.0001
b) Effect of age											
	Age (dph)*										
	50-51	52-53	54-55	56-57	58+						
5	<0.001	0.092	0.035	0.023	0.045	6.68	4				0.154
10	0.012	0.048	0.042	0.090	0.069	7.52	4				0.111
15	0.020	0.037	<0.001	0.039	0.059	4.60	4				0.331
20	0.048	0.045	0.021	0.017	0.047	2.71	4				0.607
Overall	0.012	0.057	0.033	0.034	0.054	8.69	4				0.069
c) Effect of time prior to feed											
	Time prior to feed (min)										
	25	20	15	10	5						
5	0.036	0.032	0.031	0.028	0.039	0.24	4				0.993
10	0.067	0.050	0.045	0.077	0.049	2.30	4				0.680
15	0.048	0.039	0.037	0.039	0.034	0.79	4				0.940
20	0.036	0.045	0.047	0.029	0.023	2.88	4				0.579
Overall	0.040	0.042	0.037	0.039	0.030	0.77	4				0.943
d) Effect of time since feed											
	Time since feed (min)										
	5	10	15	20	25						
5	<0.001	<0.001	<0.001	<0.001	<0.001	3.33	4				0.504
10	<0.001	<0.001	<0.001	<0.001	<0.001	3.99	4				0.408
15	<0.001	<0.001	<0.001	<0.001	<0.001	6.51	4				0.164
20	<0.001	<0.001	<0.001	<0.001	<0.001	5.66	4				0.226
Overall	<0.001	<0.001	<0.001	<0.001	<0.001	4.78	4				0.310

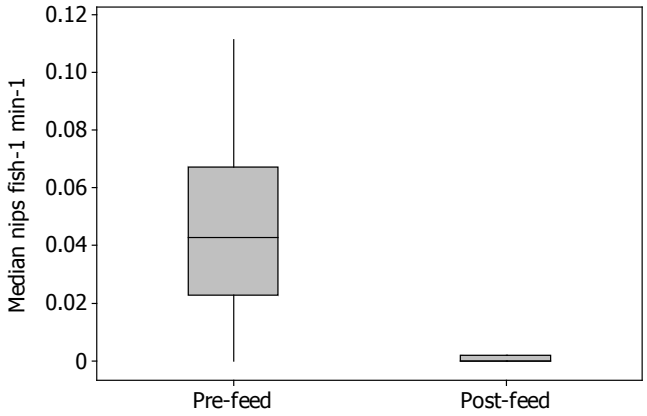


Fig. 5.5 Median number of nips fish^{-1} min^{-1} pre- and post-feed. Each box shows the median, 25 (Q1) and 75 (Q3) percentiles; upper whisker = Q3 + 1.5 (Q3 - Q1), lower whisker = Q1 - 1.5 (Q3 - Q1).

The age of the fish had no effect on the number of nips recorded over the 11 days that behaviour was recorded (Table 5.2b). The absence of such an effect was consistent within feeding regimes (Table 5.2b).

With regard to the effect of varying fish density over the duration of the study, for fish fed the second lowest prey density (10 % fish biomass) there was a significant negative correlation between the incidence of nipping behaviour and fish density (Fig 5.6: R^2_{adj} = 53.3 %, $F_{1,9}$ = 12.39, P = 0.007). Such a negative relationship between the incidence of nipping and fish density also existed for fish fed the other prey densities, although the relationship was not significant (Fig 5.6: Prey density: 5 % fish biomass: R^2_{adj} = 14.1%, $F_{1,8}$ = 2.48, P = 0.154; 15 % fish biomass: R^2_{adj} = 5.8%, $F_{1,8}$ = 1.55, P = 0.248; 20 % fish biomass: R^2_{adj} = 5.0 %, $F_{1,9}$ = 1.53, P = 0.248). No significant relationship existed between the number of nips and prey density (R^2_{adj} = 0.0 %, $F_{1,40}$ = 0.52, P = 0.476).

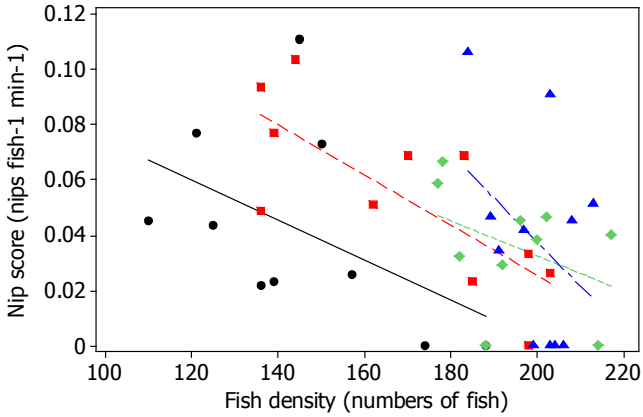


Fig. 5.6 Nip score (nips fish^{-1} per min^{-1}) (pre-feed) versus fish density at each prey density (black circle: 5 % fish biomass; red square: 10 % fish biomass; green diamond: 15 % fish biomass; blue triangle: 20 % fish biomass).

5.4.4 Factors that affected rates of darting

In contrast to the marked difference in the incidence of nipping behaviour prior to and following a feed, the number of darts did not differ between the pre- and post-feed periods (Table 5.3a). The absence of an effect was consistent within feeding regimes (Table 5.3a). In the half-hour prior to a feed, at both low (5 and 10 % fish biomass) and high prey densities (15 and 20 % fish biomass), the number of darts did not vary significantly with time (Low prey densities: H = 1.34, DF = 4, P = 0.854; High prey densities: H = 1.63, DF = 4, P = 0.804). Similarly, during the post-feed period, at high prey densities, the number of darts did not vary significantly with time (H = 4.83, DF = 4, P = 0.305). However, fish fed the low densities of prey showed a significant increase in darting behaviour as the time since the feed increased (Fig. 5.7: H = 12.05, DF = 4, P = 0.017).

Table 5.3 Median number of darts fish^{-1} min^{-1} at each prey density (represented as % fish biomass) pre- and post-feed (a), at each age (b) and over the half-hour period prior to (c) and since a feed (d).

Prey density	Median number of darts fish^{-1} min^{-1} *pre- and post-feed					Results of statistical analyses					
						H	DF	W	N_1	N_2	P
a) Effect of feeding											
	Pre-feed	Post-feed									
5	0.147	0.139						106	10	10	0.970
10	0.328	0.193						143	11	11	0.293
15	0.283	0.214						121	10	10	0.241
20	0.258	0.233						139	11	11	0.431
Overall	0.242	0.180						1953	42	42	0.134
b) Effect of age											
	Age (dph)*										
	50-51	52-53	54-55	56-57	58+						
5	0.095	0.117	0.118	0.219	0.300	6.98	4				0.137
10	0.146	0.212	0.166	0.290	0.361	9.00	4				0.061
15	0.120	0.241	0.168	0.284	0.429	7.89	4				0.096
20	0.139	0.215	0.190	0.365	0.489	9.03	4				0.060
Overall	0.129	0.215	0.168	0.289	0.364	32.69	4				<0.001
c) Effect of time prior to feed											
	Time prior to feed (min)										
	25	20	15	10	5						
5	0.175	0.163	0.145	0.137	0.141	1.52	4				0.823
10	0.290	0.211	0.282	0.303	0.241	1.69	4				0.792
15	0.181	0.218	0.302	0.252	0.316	3.35	4				0.501
20	0.250	0.261	0.276	0.269	0.308	0.05	4				1.000
Overall	0.233	0.226	0.265	0.266	0.242	0.14	4				0.998
d) Effect of time since feed											
	Time since feed (min)										
	5	10	15	20	25						
5	0.107	0.183	0.120	0.156	0.244	6.50	4				0.165
10	0.138	0.162	0.158	0.265	0.206	8.64	4				0.071
15	0.184	0.145	0.232	0.225	0.241	1.64	4				0.801
20	0.117	0.182	0.233	0.273	0.282	3.41	4				0.492
Overall	0.124	0.161	0.172	0.229	0.241	13.84	4				0.008

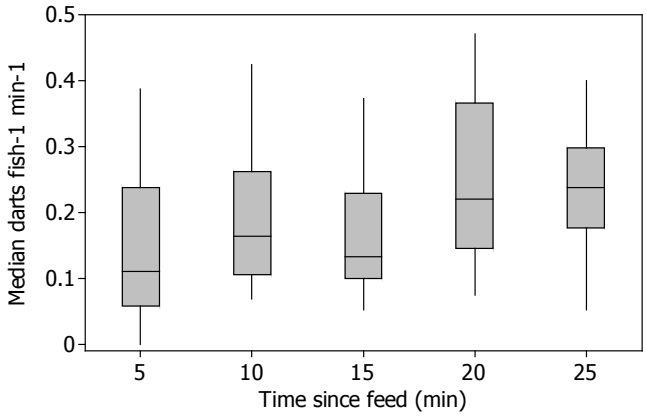


Fig. 5.7 Median number of darts fish^{-1} min^{-1} for fish fed the two lowest prey densities in the half-hour following a feed. Each box shows the median, 25 (Q1) and 75 (Q3) percentiles; upper whisker = Q3 + 1.5 (Q3 - Q1), lower whisker = Q1 - 1.5 (Q3 - Q1).

The number of darts increased with the age of the fish (Table 5.3b and Fig. 5.8). This relationship was consistent within each feeding regime (Table 5.3b).

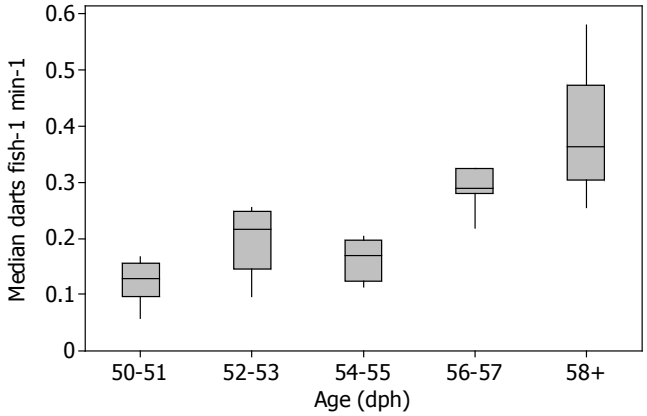


Fig. 5.8 Median number of darts fish^{-1} min^{-1} versus age. Each box shows the median, 25 (Q1) and 75 (Q3) percentiles; upper whisker = Q3 + 1.5 (Q3 - Q1), lower whisker = Q1 - 1.5 (Q3 - Q1).

With regard to the effect of varying fish density over the duration of the study, a significant negative correlation existed between the number of darts and fish density for fish fed each of the prey densities (Fig. 5.9: Prey density: 5 % fish biomass: R^2_{adj} = 39.1 %, $F_{1,8}$ = 6.79, P = 0.031; 10 % fish biomass: R^2_{adj} = 34.8 %, $F_{1,9}$ = 6.35, P = 0.033; 15 % fish biomass: R^2_{adj}= 59.5 %, $F_{1,8}$ = 14.20, P = 0.005; 20 % fish biomass: R^2_{adj} = 29.9 %, $F_{1,}$ = 5.27, P = 0.047). Darting behaviour therefore increased as the total number of fish decreased. The number of

darts increased significantly as prey density increased (Fig. 5.10: R^2_{ad} = 8.0 %, $F_{1,40}$ = 4.55, P = 0.039).

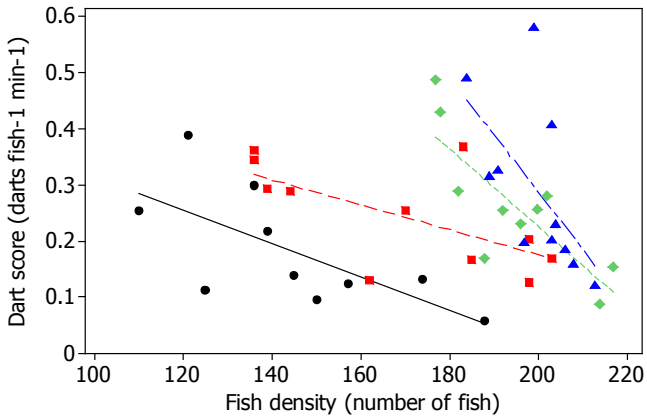


Fig. 5.9 Dart score (darts fish^{-1} min^{-1}) versus fish density at each prey density

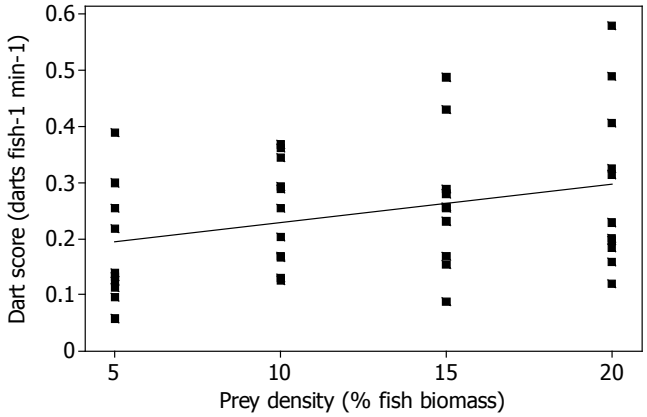


Fig. 5.10 Dart score (darts fish^{-1} min^{-1}) versus prey density.

5.5 Discussion

I examined aggression in larval cod because it was interesting as a behaviour in its own right, but also because I was interested to know whether such behaviour was indicative of cannibalism later in development and I discuss the results with the latter question particularly in mind. Due to the relevance of feed for the differentiation of predatory aggression from competitive aggression, we manipulated the amount of prey offered to cod in the hope that I could relate this variable to levels of aggression. However, during the study a number of

factors arose that I had not anticipated, primarily variations in survival and consequently fish density and I discuss this first.

Within all prey densities, there was a general decline in levels of aggression (number of nips) with increasing fish density. Although this finding is in line with cost/benefit models of resource competition (Grant 1993), aggression declined with increasing fish density during the pre-feed period, when no food was present. An effect of fish density on aggression, in the absence of food, suggests that fish were not motivated by the costs/benefits of competing for resources, but instead may have been exhibiting early signs of cannibalism. This conclusion is supported by the large number of studies that also report a decrease in cannibalism at high stocking densities in older fish (Baras and Jobling 2002).

One possible explanation for such a relationship is that prey selection by attackers is hampered at high fish densities. For example, Magurran and Seghers (1991) in a study of guppies, and Hecht and Uys (1997) in a study of sharptooth catfish, attributed a reduction in levels of aggression at high densities to the formation of schools above a certain threshold density. However, although cod will school in the wild (Methven et al. 2003) and in large production tanks (Forbes personal observation.), I did not observe the formation of schools in the small aquaria. Nevertheless, cod are visually orientated predators and consequently a perceptual confusion effect may have occurred at high fish densities and hampered prey selection by attackers. Baras (1999) and Kucharczyk et al. (1998) forwarded this as an explanation for decreases in cannibalism in juvenile vundu (*Heterobranchus longifilis*) at higher fish densities. It is also possible that, although the number of encounters between fish increased as fish density increased, the proportion of encounters that resulted in an attack declined. Jones (1983) reported the existence of such a relationship in a study of juvenile *Pseudolabrus celidotus*. In this study the number of darts declined as fish density increased, suggesting that the proportion of encounters resulting in an attack may also have declined with increasing fish density, and thus contributed to the overall decrease in levels of aggression.

Analysis of total fish density throughout the period of study identified a positive correlation between this variable and prey density. Furthermore, length, weight and the extent of variation in length (CV) differed between prey densities. Consequently, it was not possible to interpret the effects of overall prey density on levels of nipping or darting independently of

these other parameters. While I briefly discuss the observed relationship between prey density and levels of nipping and darting, I focus primarily on those results for which there were no confounding factors and which could be interpreted more clearly.

5.5.1 Darting

Darting was very prevalent among cod. Irrespective of the cause of these darts, they are not desirable in aquaculture since they use up large amounts of energy that could otherwise be used for growth (Krohn & Boisclair 1994). Analysis of the 17.4 % of darts that were attributable to an obvious cause clearly suggests that these darts reflected a response to a real or perceived threat from a conspecific. That at least some darts represented an escape response was supported by the fact that I also observed large numbers of darts upon the introduction of an adverse stimulus such as a cleaning siphon. It is also likely that darting is closely linked to the vigilance behaviour observed in a number of bird and mammal species and, if so, may explain why darting decreased at higher fish densities, since vigilance is well documented to decline with increasing group size (Elgar 1989). Darting was never observed as an attacking behaviour and hence was quite different to the short bursts of rapid swimming that propel fish forward in an attack (e.g. turbot, *Scophthalmus maximus* and sole, *Solea solea*, larvae, Knutsen 1992).

Burst swimming as part of an escape response has been reported in many fish species including northern anchovy (*Engraulis mordax*) larvae (Webb and Corolla 1981), herring (*Clupea harengus*) and plaice (*Peuronectes platessa*) larvae (Batty and Blaxter 1992) and more recently in larval cod (Williams et al. 1996). These studies describe three stages to the escape response: contraction of the musculature on one side of the body so that the larva forms a C shape, a strong beat of the tail in the opposite direction resulting in rapid propulsion forward and finally, a period of continuous high speed swimming. I hypothesise that some of the darts observed in this study represented the latter stage of such an escape response. However, many of the darts that I observed were not preceded by the C shape formation and in this regard were more comparable with some of the 'startle' responses reported in larval red drum (*Sciaenops ocellatus*) by Fuiman et al. (1999). For those fish for which there was no obvious reason for the dart to occur, the stimulus may have been off screen at the time. However, startle or escape responses can be triggered by a range of stimuli, including visual (Fuiman et al. 1999), acoustic (Fuiman et al. 1999), tactile (Blaxter and Batty 1985) and

mechanical (Blaxter and Fuiman 1990) stimuli and any one of these may have been present but not apparent to the observer.

In this study, I assessed two features of feed provision on aggression in cod, namely the presence or absence of food (i.e. food as a stimulus) and overall levels of food (i.e. ration level). Despite the increase in the incidence of nipping in the presence of feed, levels of darting were not affected by the presence or absence of feed. This observation raises the possibility that fish were constantly avoiding predators irrespective of the threat of attack. Conversely, while levels of nipping were not affected by overall prey density, levels of darting were higher in larvae fed higher prey densities. Chick and Van den Avyle (2000) found that larval striped bass (*Morone saxatilis*) reared at a low prey density, were less responsive to simulated predator attacks than those reared at two higher prey densities and attributed this finding to a decline in the condition of the low ration fish. In this study, condition factor did not vary between fish fed different prey densities, but both length and weight were lowest at the lowest prey densities. Consequently it is possible that size may have contributed to a reduction in the responsiveness of fish to real or perceived threats and thus the number of observed darts.

5.5.2 Nipping

Rosenlund et al. (1993) observed high levels of aggressive behaviour in juvenile cod (reared at similar temperatures) from 35 dph onwards and attributed this early occurrence to the initiation of weaning on to inert feed. The results of this study suggest that in cod fed only live feed in the early juvenile stages, aggressive behaviour becomes prevalent slightly later, at around 45 dph. The initiation and incidence of aggression most likely reflect the increase in growth that occurs around this time (see chapter 3) that not only increases appetite, but also results in an increase in size variation within the cohort (see chapter 3; Folkvord et al. 1994).

This study clearly demonstrates that juvenile cod are significantly less motivated to attack conspecifics during periods of feeding than prior to feeding, irrespective of the levels of prey offered. Similar, but less marked, results have been reported in sharptooth catfish juveniles (Kaiser et al. 1995; Almazán-Rueda et al. 2004) and chum salmon juveniles fed at high and intermediate rations (Davis and Olla 1987). Conversely, Magnuson (1962) observed an increase in aggressive activity in juvenile medaka when food was presented in food-limited

treatments (although not in excess food treatments), while Greaves and Tuene (2001) only observed aggressive interactions in adult halibut during feeding. Furthermore, Grant et al. (2002) observed a dome shaped relationship between levels of aggression and prey abundance in the juvenile convict cichlid, *Archocentrus nigrofasciatum*, in line with optimality models of aggression which highlight the defensibility of a resource at different prey densities.

Given the comparable pre feed levels of aggression at low and high prey densities and the near absence of aggression during feeding, the presence or absence of feed is most likely the main determinant of aggression in juvenile cod. Higher levels of aggression in sharptooth catfish prior to, than following, a feed have been attributed to increases in activity resulting from the expectation of that feed (Kaiser et al. 1995; Almazán-Rueda et al. 2004). However, it is unlikely that the marked differences in aggressive behaviour observed in this study could be attributed solely to such an effect. Instead, it seems more plausible that most fish were exhibiting early signs of cannibalistic behaviour and, since larval cod are known to increase their search for prey and become less selective when prey are scarce (MacKenzie and Kiørboe 1995), were most motivated to attack conspecifics in the absence of food. This conclusion is supported by the predominance of attacks to the tails of victims and of attackers of a similar or larger size than the victim. Cod are gape-limited predators (Otterå and Folkvord 1993) and therefore predatory cod would be expected to target smaller individuals. Similarly, attacks to the tail would facilitate easier consumption of individuals than attacks to the body or head and cannibalistic cod are known to direct attacks in this way (Forbes personal observation).

Further support for aggression of a predatory nature is provided by the predominance of attacks to vertically orientated and thus poor condition fish. Most larval cod inflate the swimbladder at around 7 dph (Cutts and Shields 2001), but inevitably some will fail to do so (Shields et al. 2003). Individuals that do not inflate the swimbladder may still live long enough to reach metamorphosis (Shields et al. 2003), but the excess weight renders the fish unable to swim in the normal horizontal position (as seen in perch, Elgloff 1996; cod, Shields et al. 2003). These and other diseased or damaged fish present an easy target for attackers since they are presumably unable to make a quick escape.

While the aforementioned observations provide strong support for aggression of a predatory nature, it is likely that some aggressive attacks were motivated by competition for resources. Since larger fish tend to be more dominant and exhibit higher rates of aggression when

competing for food, competitive aggression is also frequently focused at individuals of a smaller, or comparable size (Beeching 1992). In addition, it seems likely that the 16 % of attacks that were directed at victims larger than the attacker, represented competitive rather than predatory aggression. This conclusion is supported by the increase in the number of attacks directed at the head and body when the victim was larger than the attacker.

While the presence of prey clearly reduced the incidence of aggressive attacks, varying levels of prey had no overall effects on levels of pre-feed aggression, when a food stimulus was absent in all treatments. Studies of cannibalism frequently show an increase in aggressive behaviour as food density decreases and attribute these findings to a concomitant increase in growth depensation and hunger (e.g. European seabass, Katavich et al. 1989). However, in this study, size variation was lower at lower prey densities. Analysis of the length frequency distribution for fish fed the lower rations, confirmed that smaller fish were dying at the lower rations, either as a result of increased cannibalism or limited resources. Interpretation of the effect of prey density on levels of aggression is further complicated by the lower survival rates at the lower ration levels, since lower fish densities resulted in higher levels of aggression. I hypothesise that in this study, an increase in motivation to exhibit aggressive behaviour at low prey densities as a result of increased hunger and a low fish density, may have been offset by the concomitant decrease in size variation, resulting in no net difference in levels of aggression between treatments.

These results clearly demonstrate that aggression in larval cod is largely predatory in nature. The marked increase in the number of attacks in the absence of feed, the predominance of attacks to the tail of victims, to victims of a smaller or similar size and to victims in poor condition, all suggest that most attacks represent an attempt to consume part of the victim and are thus indicative of cannibalism later in development. In addition, darting was very prevalent and analysis of the darts for which the cause was apparent suggested that this behaviour represented an escape response to real or perceived threats. Irrespective of the underlying causes, both aggression and darting must be considered significant problems in aquaculture. Given the near absence of aggression in the presence of feed, it is possible that frequent feeding may reduce levels of aggression and possibly cannibalism, and I forward this as a potential subject for future studies.

Chapter 6

Variable Risk Taking in Atlantic Cod: effects of stock and family

6.1 Summary

I examined whether cod originating from different stock and/or family varied in their propensity to take risks, as reflected by exploration of a novel, potentially dangerous environment. One-year old cod derived from North-eastern Arctic or Norwegian coastal stock were placed in a covered area of an experimental arena that also contained an open area into which the fish were free to move. The movements of these fish were then recorded by the digital recording and analysis system, Ethovision, for a period of 25 minutes. After screening, blood samples were taken from a sub-set of fish and plasma cortisol concentrations determined. To provide an unstressed control, cortisol levels were measured in an additional 20 fish taken straight from their holding tanks. Using a discriminant function derived from movement patterns of a sub-sample of directly observed fish, cod were characterised as risk avoiders (those that failed to emerge from undercover or emerged only to attempt to escape from the arena) or as risk takers (those that emerged to explore the open arena). Fish of North-eastern Arctic stock origin were more prone to taking risks than fish of coastal stock origin. Furthermore, although there were no significant differences in risk-taking between families of North-eastern Arctic stock origin, a weakly significant family effect on boldness was found in fish originating from the coastal stock. The weight and condition of fish was significantly smaller in fish that emerged to escape than in fish that avoided risk and these factors may have contributed to the observed behavioural differences between stocks and families. After screening, plasma cortisol concentrations were strikingly elevated in all fish and there were no stock effects. Base line plasma cortisol levels were higher in cod of migratory origin than those of coastal origin. These results provide evidence for a heritable component to risk-taking in cod.

6.2 Introduction

Animals, including many fish species, frequently exhibit consistent individual differences in behaviour. For example, some individuals will more readily explore a novel environment (*Brachyraphis episcopi*, Brown and Braithwaite 2004), spend longer out of cover (Rainbow trout, Sneddon 2003) or more readily inspect a model predator (*Nannacara anomala*, Brick and Jakobsson 2002). In such cases, individuals are varying in their propensity to take risks, and reflecting the degree to which they are bold (risk takers) or shy (risk avoiders) (Wilson et al. 1994). In some such cases, individual differences in risk-taking are associated with differences in stress physiology (Korte et al. 2005).

A large number of the studies of behavioural variation among animals, including variation in risk taking behaviour, describe interpopulation differences in behaviour. For example, Brown et al. (2005) showed that populations of the tropical poeciliid, *Brachyraphis episcopi*, from high predation areas emerged from cover more quickly than those from low predation areas. Similarly, Fraser and Gilliam (1987) found that guppies and Hart's rivulus from high predation sites foraged proportionately more in the presence of a predator than fish from low predation sites. It is not always clear what underpins these differences in behaviour but consistent population differences in the behaviour of both wild-caught and laboratory-reared three-spined sticklebacks (Bell and Stamps 2004; Bell 2005) and zebrafish (Wright et al. 2003) indicate that genetics may play an important role.

The existence of inherited behavioural variation between populations of fish could have a number of important implications for commercial aquaculture. For example, timid fish that avoid risk may well have a hard time in intensive husbandry, especially if timidity is associated with a tendency to avoid fights. Certainly, the process of domestication in fish held in intensive husbandry systems favours bold over timid fish (Huntingford 2004). Consequently, selection of bold individuals for use in intensive systems could potentially improve welfare and growth and reduce mortality (Huntingford and Adams 2005). This is particularly the case for new, as yet undomesticated aquaculture species, such as Atlantic cod.

Along the coast of Northern Norway, at least two genetically distinct stocks of cod exist, Northeast Arctic cod and Norwegian coastal cod. The former stock mature at 7-8 years of age, migrate over long distances from feeding areas to spawning areas (Nordeide and Pettersen

1998) and are subject to a short growing season (Svåsand et al. 1996). Conversely, coastal cod mature at 3 to 5 years of age, remain largely in the same area from settlement (Nordeide and Pettersen 1998) and enjoy a relatively long growing season (Svåsand et al. 1996). Consequently, coastal cod are subject to a far lesser predation threat than their migratory counterparts. Presently, a breeding programme at the Norwegian Institute of Fisheries and Aquaculture Research, Tromso, has established families of cod derived from these two stocks and reared under identical conditions. These cod presented an excellent opportunity to examine the behavioural variations in this species in relation to the probable differences in the predation regime to which they are adapted.

The objectives of this study were to characterise behavioural variations in risk taking in cod, as reflected by exploration of a novel, potentially dangerous environment and to determine whether the propensity to risk take varied between cod of different stock or family origin. An additional aim was to relate any observed behavioural differences to plasma cortisol levels.

6.3 Materials and Methods

6.3.1 Origin of fish and experimental set-up

This study was conducted at the Aquaculture Research Station, Tromso, Norway in July 2005. The 162 cod used were bred at the station in Spring/Summer 2004 using eggs from wild North-eastern Arctic (hereafter referred to as migratory) or coastal cod. Fish were raised under identical conditions and held, mixed together, in identical tanks for 8 months prior to the experiment. Stock origin was determined by means of pantophysin-analysis (Sarvas and Fevolden 2005) while family relationships within each stock were based on the geographical location of forefathers. All cod contained PIT tags so that their family and stock origin could be identified.

Two adjacent experimental arenas (separated by a wall of perspex and a net) were used, situated in isolation at the research station. Each arena consisted of two equally-sized adjacent chambers (79.5 x 103 x 30 cm), one with a removable cover and one uncovered, with a sliding door linking the two chambers. This door was operated by a pulley system situated outside the arena. A flow through system operated in each chamber, with water (at ambient

temperature, ranging from 7.8 to 8.7 °C) passing from the nearside of a chamber to the farside (perpendicular to the door). Flow rate was regulated using a control valve fitted to each chamber. Light levels were the same in the two arenas and varied between 70 and 100 lux. A globe shaped tea strainer containing a single, defrosted capelin was suspended centrally at the farside of each uncovered chamber (near the outflow) to provide a scent cue. Two visual cues were also suspended adjacent to each scent cue in the form of a red lure and a 5 kroner coin. The coin created a small reflection in the water in the area around the lure, similar to that which might be created by prey.

Two digital cameras (Panasonic CP230/G) were fitted above each arena and linked through two VCRS to monitors and a PC that operated the EthoVision 3.0 programme (Noldus Information Technology). EthoVision is an integrated system that permits automatic detection, recording and analysis of animal behaviour (Nilsson et al. 1993). This system was set to record simultaneously the movements of fish in both arenas, relative to four predetermined 'zones'. These zones were the undercover zone, the door zone (an area just extending beyond the area occupied by the door, as viewed on screen), the food zone (35 x 35 cm, with the capelin in the centre) and the open zone (in the open but not in the food zone).

6.3.2 Experimental procedure

Prior to a trial commencing, water flow was set to 2 ml min^{-1} in each chamber, EthoVision was calibrated and pre-trial information was recorded. Two fish were then randomly sampled from a holding tank, transferred to individual buckets and carried to the experimental arena. Each fish was carefully released from the bucket into one of the two undercover chambers, the cover replaced and curtains surrounding the arenas closed. After 5 mins, the doors in each arena were opened remotely and fish movements thereafter recorded digitally by EthoVision.

After 25 minutes, the door in each arena was closed and recording ceased. Water flows were increased to maximum in order to flush out scents prior to commencement of the next set of trials. Each fish was then removed and anaesthetised by immersion in MS222 at a concentration of 0.06 g L^{-1}. Once sedated, the fish were identified using a PIT tag reader and weight and fork length (distance from the tip of the snout to the posterior end of the middle caudal rays) recorded. The fish were then released into a recovery tank.

To quantify physiological stress responses in the experimental fish, blood samples were collected from below the second dorsal fin in 20 fish immediately following a trial and plasma cortisol levels measured in diethyl-ether extracted plasma by radioimmunoassay (RIA) according to a standardised protocol (Shultz 1985; Jørgensen et al. 2002). To provide a control, cortisol levels were also measured in 10 migratory and 10 coastal, non-experimental fish contained in holding tanks.

6.3.3 Assessment of response to the novel environment

Preliminary observations of fish movements in the experimental arena identified three very distinct patterns of exploratory behaviour. Fish either remained in the undercover area for the duration of the experiment or emerged from cover into the arena. Those that emerged showed one of two clearly distinct patterns of movement or 'units of behaviour' (Martin and Bateson 1993). The first of these behaviours was characterised by an irregular, often fast, swimming speed and prolonged periods in the corner of the uncovered chamber, jumping at the water surface; fish showing this response were described as attempting to 'escape'. The second behaviour was characterised by a regular, slow swimming speed and a meandering path using most of the tank base. Fish showing this response were described as 'exploring'. It is important to note that I could not be sure what motivated this latter group of fish to swim in the open arena, and that this definition is merely meant to distinguish these fish from those that attempted to exit the arena.

In order to confirm the distinctness of these two patterns of movement, and to find discriminators for them in the data generated by Ethovision, 51 of the total of 140 fish that emerged from undercover were also observed directly via monitors and assessed as 'escaping' or 'exploring'. Discriminant function analysis (DFA) was then carried out on the movements of these 51 fish, relative to the behavioural groups to which each fish had already been assigned. This analysis assigns coefficients (loadings) to the movement variables, which are then arranged together in specific combinations (discriminant functions or factors) so as to maximally separate the groups from each other. The discriminant functions identified in this way were used on the Ethovision data for all unobserved fish in order to assign each of these fish to one of the behavioural groups. On this basis, fish were classified as exploring, attempting to escape, or non-emergent. In order to increase sample sizes and since both fish that remained undercover and fish that attempted to exit the arena were clearly not motivated

to reside in the open arena, fish in these latter two categories were grouped together to from a group defined as 'avoidance'.

DFA accurately assigned 91.3 % of fish to the correct behavioural group. The variables mean distance moved and velocity standard error most accurately discriminated between the two groups, based on DFA loadings. In accordance with preliminary observations, mean distance moved was markedly lower in fish that emerged to explore, while velocity standard error was markedly higher in fish that emerged to escape, due to the irregular swimming speed exhibited by these fish.

6.3.4 Other statistical analyses

Condition was calculated using the formula:
Condition factor (K) = W / L^3
where W is the weight (g) and L is the standard length (cm). Chi square tests were used to assess the relationship between stock or family origin and behavioural category defined as described above. Appropriate univariate parametric (ANOVA or T test) or nonparametric (Kruskal-Wallis or Mann-Whitney) statistical analyses were used to assess the relationship between length, weight, condition factor or cortisol level and patterns of exploration or stock/family origin. The same tests were also used to assess the relationship between various features of exploratory behaviour (e.g. time of first emergence) and stock/family origin.

6.4 Results

6.4.1 Stock and family differences in exploratory behaviour

There was marked variation in the behaviour of fish once placed in the experimental arena. Of all 162 fish, 56 (34.6 %) emerged to escape, 84 (51.9 %) emerged to explore, while 22 (13.6 %) fish did not exit the undercover chamber at all. There was a significant stock effect on the degree to which fish were prepared to exit the covered area of the arena and explore (Fig 6.1: $Chi^2 = 10.18$, DF = 1, P = 0.001). Of the migratory fish, 65 % emerged at least once to explore, while the comparable figure for the coastal fish was only 40 % (Table 6.1). All other fish either remained undercover or only emerged to attempt to escape from the arena. In total,

only 8% of the migratory fish remained undercover, while 19 % of the coastal fish remained undercover. Frequencies of exploratory and avoidance behaviour did not vary between families of migratory fish (Table 6.1: $Chi^2 = 13.18$, DF = 9, P = 0.154) or between families of coastal fish (Table 6.1: $Chi^2 = 15.5$, DF = 9, P = 0.078).

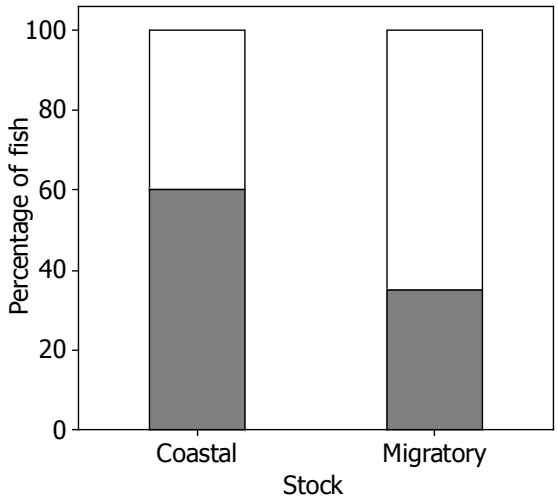


Fig. 6.1: Percentage of each stock that exhibited exploratory (white bar) or avoidance (grey bar) behaviour.

Table 6.1. Mean condition factor and the number of fish that exhibited exploratory or avoidance behaviour in coastal and migratory families.

Stock	Family	Exploring frequency (no. of fish)	Avoidance frequency (no. of fish)	Condition Factor	
				Mean	SE
Coastal	C1	3	4	1.07	0.03
	C2	6	3	1.02	0.02
	C3	4	3	0.99	0.03
	C4	2	8	1.04	0.03
	C5	3	6	1.05	0.01
	C6	1	7	1.08	0.03
	C7	2	8	1.09	0.03
	C8	5	2	1.10	0.03
	C9	2	6	0.97	0.02
	C10	6	4	1.06	0.02
Total (% of stock)		34 (40)	51 (60)		
Mean				1.05	
Migratory	M1	3	3	0.97	0.02
	M2	3	5	0.96	0.04
	M3	8	1	0.86	0.02
	M4	6	1	0.88	0.04
	M5	4	3	0.98	0.05
	M6	7	3	0.89	0.02
	M7	3	4	0.89	0.03
	M8	4	4	0.94	0.02
	M9	3	2	0.92	0.01
	M10	9	1	0.93	0.03
Total (% of stock)		50 (65)	27 (35)		
Mean				0.92	

6.4.2 Behaviour during exploration

Of fish that did emerge to explore, 83.3 % emerged in the first 5 mins, with all other exploring fish taking between 5.84 to 24.73 mins before first leaving the undercover chamber (Fig 6.2a: Median = 0.94 mins, IQR = 2.33). The total time spent exploring in both food and

open zones ranged from 0.03 to 10.95 mins (Fig 6.2b: Median = 0.78 mins, IQR = 2.66). Only 3 fish spent more than 51 % of their time, since emergence, exploring (Fig 6.2c: Median = 3.78 %, IQR = 11.62). The total distance moved by exploring fish ranged from 0.65 to 64.63 m (Fig 6.2d: Median = 7.16 m, IQR = 17.01).

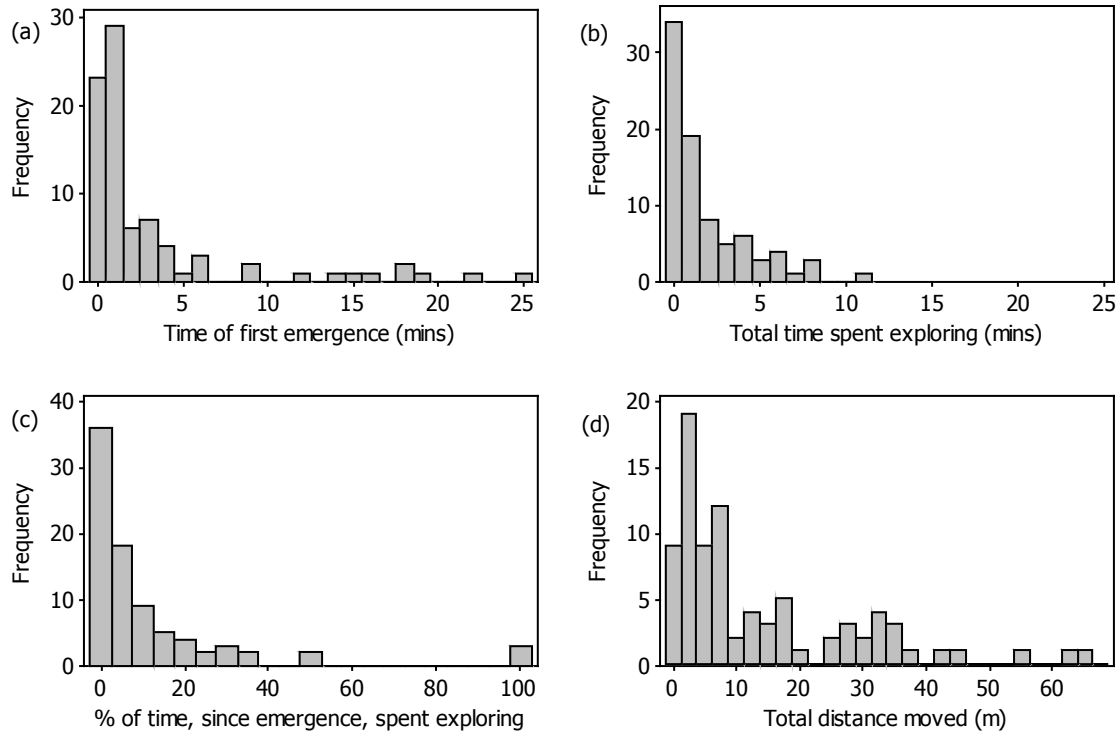


Fig. 6.2: Frequency distribution (numbers) of time of first emergence (a), total time spent exploring in food and open zones (b), percentage of time, since emergence, spent exploring in food and open zones food (c) and total distance moved in food and open zones (d) for cod that emerged to explore.

Behaviour during exploration did not vary significantly between stocks for any of the variables measured (e.g. mean time to emerge from undercover, mean total time spent exploring, mean total time in the food zone). However, several of these measures varied significantly between families of coastal cod; these include the total time spent exploring (in both open and food zones) (Fig. 6.3a: H = 20.40, DF = 9, P = 0.016), mean length of each visit to the open zone (H = 17.01, DF = 9, P = 0.049), mean length of each visit to the food zone (H = 17.98, DF = 9, P = 0.035) and number of visits to the food zone (H = 20.83, DF = 9, P = 0.013). There were no significant family effects on the behaviour of migratory fish while exploring for any of the variables measured (e.g. Fig. 6.3b: H = 9.82, DF = 9, P = 0.366).

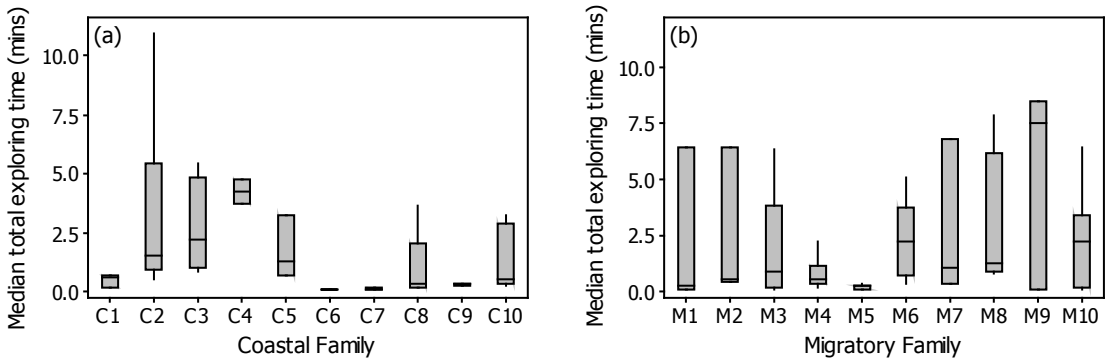


Fig. 6.3: Median total exploring time in food and open zones for each coastal family (a) and each migratory family (b). Each box shows the median, 25 (Q1) and 75 (Q3) percentiles; upper whisker = Q3 + 1.5 (Q3 - Q1), lower whisker = Q1 - 1.5 (Q3 - Q1).

6.4.3 Cortisol levels

Cortisol levels were significantly higher in fish that had undergone a trial compared with control fish (Table 6.2: Coastal fish: W = 45, N_1 = 9, N_2 = 4, P = 0.007; Migratory fish: W = 55, N_1 = 10, N_2 = 16, P < 0.0001). Stock differences in cortisol levels also existed for control fish, with higher levels observed in migratory fish than in coastal fish (Table 6.2: W = 45, N_1 = 9, N_2 = 10, P = 0.0003). Post trial cortisol levels did not differ between stocks (Table 6.2: W = 174, N_1 = 16, N_2 = 4, P = 0.633) or between the two behavioural categories (Median cortisol level of exploring fish: 89.70 ng ml^{-1}, Median cortisol level of risk avoiding fish: 138.40 ng ml^{-1}, W = 103, N_1 = 12, N_2 = 7, P = 0.163).

Table 6.2. Median cortisol levels in coastal and migratory, control and post-trial fish.

	Coastal (ng/ml)	Migratory (ng/ml)	P-value
Control fish	1.09	9.07	0.0003
Post trial fish	89.63	97.67	0.603
P-value	0.007	<0.0001	-

6.4.4 Length, weight and condition factor

Condition factor varied significantly between stocks, with a higher mean condition factor in coastal fish than in migratory fish (Table 6.1: Mean coastal = 1.05, Mean migratory = 0.92, T

= 9.85, DF = 156, P < 0.001). Condition factor also varied significantly between families of coastal fish (Table 6.1: $F_{9,75} = 2.52$, P = 0.014) and weakly between families of migratory fish (Table 6.2: $F_{9,67} = 2.01$, P = 0.051). There were no stock differences in length (Mean length of coastal fish = 29.48 cm, Mean length of migratory fish = 30.07 cm, T = -1.58, DF = 159, P = 0.116) and only a weak difference in weight (Median weight of coastal fish = 268 g, Median weight of migratory fish = 254 g, W = 7455, $N_1 = 85$, $N_2 = 77$, P = 0.077). However, length did vary significantly between families of coastal fish ($F_{9,75} = 2.79$, P = 0.007) and between families of migratory fish ($F_{9,67} = 4.72$, P < 0.001). Weight also varied significantly between families of migratory fish (H = 22.36, DF = 9, P = 0.008), but not between families of coastal fish (H = 15.92, DF = 9, P = 0.068).

The length of fish did not vary significantly between behavioural categories (Mean length of exploring fish = 29.58 cm, Mean length of risk avoiding fish = 29.95 cm, T = 1.01, DF = 159, P = 0.315). However, fish that exhibited exploratory behaviour weighed significantly less than did fish that exhibited avoidance behaviour (Median weight of exploring fish = 244.50 g, Median weight of risk avoiding fish = 273.50 g, W = 6236, $N_1 = 84$, $N_2 = 78$, P = 0.041). Fish that exhibited exploratory behaviour also had a lower condition factor than fish that exhibited avoidance behaviour (Mean condition factor of exploring fish = 0.96, Mean condition factor of risk avoiding fish = 1.01, T = 2.86, DF = 159, P = 0.005).

6.5 Discussion

The results of this study highlight the great individual variation in the propensity of cod to take risks once placed in an experimental arena. Fish that remained undercover were clearly showing less risk-prone behaviour than were fish that exited the arena to explore. Furthermore, within the group that emerged to explore, there were additional variations in behaviour patterns that have been used as indicators of boldness (for example, total foraging time; Sneddon 2003), although such variation was small, with no exploring fish spending more than 11 minutes in the open arena. In this study, assessing levels of risk-taking was complicated by those fish that emerged not to explore, but to attempt to escape the arena. Although these fish emerged from the shelter, their behaviour was clearly typical of fish motivated to escape from a dangerous environment and as such also reflects risk avoidance.

My results show that cod originating from migratory stock were somewhat more prone to take risks than cod from coastal stock. This result is somewhat surprising, since these migratory fish travel long distances, presumably under high risk of predation compared to that experienced by coastal fish. Several studies have shown that fish from populations exposed to high predation risk tend to show risk averse behaviour (for example, in sticklebacks, Huntingford et al. 1994, Bell and Stamps 2004; Bell 2005). However, other studies have documented high levels of risk-taking in fish that naturally experience high levels of predation. For example, in a study of the poeciliid, *Brachyraphis episcopi*, Brown et al. (2005) found that fish from high predation areas were bolder than those from low predation areas. Similarly, guppies and Hart's rivulus from high predation sites were more tenacious than fish from low predation sites (Fraser and Gilliam 1987). It is interesting to note that the offspring of wild caught Northeast Arctic cod have been shown to be more successful at obtaining sequentially offered food than the offspring of Norwegian coastal cod (Salvanes et al. 2004) and the proposed mechanisms underlying this finding may also be relevant here. For example, the authors suggest that migratory cod may be more active in the pursuit of food as a result of the limited growing period in which they have to obtain prey, the increased competition in Northern fjords and/or the uncertainty regarding pelagic food availability (Salvanes et al. 2004). It is possible that risk-taking in these fish may also have reflected not simply an adaptation related to predation threat, but an adaptation related to prey availability and features of life-history.

The numbers of fish in each family were small and thus the finding that families of coastal cod differed in their propensity to take risks must be treated with caution. However, this finding does provide further evidence that the observed differences in behaviour were at least partly attributable to genetic differences. Although evidence for a genetic component to personality traits comes largely from a number of quantitative genetic or selection studies of great tits (van Oers et al. 2004a, b; Dingemanse et al. 2002; Drent et al.; 2003) and mice (Sluyter et al. 1995; Turri et al. 2001), there is increasing evidence that there may also be a genetic basis to personality in fish. For example, Wright et al. (2003) found significant differences in the levels of boldness (defined as exploration of a novel object) exhibited by the offspring of four wild-caught populations of zebrafish. Furthermore, Bell (2005) and Bell and Stamps (2004) observed consistent population differences in boldness under risk of predation in both wild caught three-spined sticklebacks and their laboratory-reared offspring. The similarity of risk taking behaviour among families of migratory fish raises the possibility

that these families may have been less genetically distinct than families of coastal cod. Since family origin was based on geographical location and migratory cod travel over greater distances than coastal cod, the likelihood of genetic overlap arising between families of this stock was probably greater.

Accurately interpreting the underlying mechanisms driving variations in personality traits is complicated by the large number of biological factors that may additionally influence behaviour, such as gender (Brown et al. 2005; Johnsson et al. 2001), age (Brown et al. 2005), reproductive state (Sinn and Moltschaniwskyj 2005) and size (Krause et al. 1998). For example, Brown and Braithwaite (2004) in a study of the poeciliid, *Brachyraphis episcopi*, and Dowling and Godin (2002) in a study of the banded killifish (*Fundulus diaphanous*) observed strong negative relationships between length and the time to emerge from a shelter. Although there were no significant differences in the length of fish from each behavioural category, both weight and condition factor were smaller in migratory fish and in those fish that emerged from the shelter to explore. These observations raise the possibility that size and condition may have at least partly contributed to the observed differences in risk taking behaviour exhibited by migratory and coastal fish. Furthermore, Norwegian coastal cod are known to mature more early than Northeast Arctic cod (Berg and Albert 2003) and since boldness may decrease with increasing age (Brown et al. 2005), it is possible that differences between the stocks were influenced by ontogenetic processes. Ultimately, relationships between size, condition factor or maturity and risk-taking may be driven by the higher metabolic rate and hence higher levels of hunger, experienced by smaller, less fit or less mature fish, compelling them to forage for food more readily than their larger counterparts (Wootton 1994).

An individual's response to a new or threatening situation is at least partly determined by a wide range of neuroendocrine changes (Moberg 1985). Moreover, there is increasing evidence that in teleost fish, as in mammals, individuals that exhibit a withdrawal response react to stress in an unfamiliar environment with a greater increase in plasma glucocorticoid levels compared to individuals that exhibit a fight/flight response (Schjolden et al. 2005). While we were not able to monitor changes in the levels of plasma cortisol during an experiment, cortisol levels were found to be significantly higher in control fish of migratory origin compared to their coastal counterparts. Consequently it is possible that the behaviour of experimental fish may have been affected by underlying differences in neuroendocrine

physiology. Not surprisingly, cortisol levels were significantly greater in post trial fish than in control fish and although no differences in cortisol levels existed between stocks of post trial fish, stress levels were presumably so high by this stage that any differences between stocks were masked.

6.5.1 Conclusion

In this study hatchery-reared migratory cod were found to exhibit a greater propensity to take risks than did their coastal counterparts. This agrees with some studies of population differences in fish, in which wild-caught fish from high predation areas show more risk-prone behaviour. The existence of such stock differences in laboratory-reared fish, as well as the behavioural differences found among families of coastal cod, suggest that there may be a heritable component to this variable behaviour. Since condition factor and weight were lower in migratory fish and in fish that emerged to explore, it is not possible to rule out an effect of size and condition on the observed differences in behaviour, especially between stocks. Similarly the higher levels of cortisol in fish of migratory origin compared with control fish of coastal origin, raises the possibility that differences in stress responsiveness may also have contributed to differences in the propensity of individuals to take risks. Of course, these different explanations are not necessarily mutually exclusive. For example, variation in cortisol responsiveness (Fevolden et al. 1999) and condition factor (Bonnet et al. 1999) may have a genetic component and thus potentially underpin the inherited differences in risk-taking between families or stocks.

Population differences in risk taking, such as those described here, have a number of implications. For example, the vulnerability of fish populations to fishing may be affected by population differences in risk-taking and consequently stock assessment models may benefit from incorporating such information (Frank and Brickman 2000). Furthermore, in aquaculture, fish production may be improved by the selection of stocks exhibiting a greater propensity to risk-take. Fish that avoid risk seem to have an especially hard time, especially if timidity is associated with a tendency to avoid fights (Huntingford and Adams 2005) and consequently selection of individuals from a risk-taking population may improve both survival and welfare. The effect of variable risk-taking behaviour on survival in farmed cod merits further study.

Chapter 7

General Discussion

7.1 Summary of Objectives and Findings

The original objectives of this research were not those outlined in this thesis, but instead were to provide a detailed description of cannibalism in larval cod, including a direct assessment of the developmental and environmental factors contributing to the onset and incidence of cannibalism in this species. However, in order to carry out a more detailed examination of cannibalism in larval cod than that described here, the Home Office requested that an ethogram be produced, describing the behaviour of cod during a cannibalistic attack. The aim was to find reliable behavioural predictors of the occurrence of a cannibalistic attack, so that experiments could be terminated after the predictor had been observed but before the attack proper. Unfortunately, although cannibalism appeared to be rife in production tanks, it was extremely difficult to capture cannibalistic attacks on camera. Consequently, studies were undertaken that addressed some questions related to the original objectives of the research, but that did not involve cannibalism *per se*.

7.11 Ontogenetic trends in the development of larval cod head morphology and the effect of prey size on development (chapters 3 and 4)

The objectives of the studies outlined in chapters 3 and 4 were to examine patterns of change in larval cod head morphology, including the extent to which these patterns of change varied within a cohort, and to examine the effect of prey type on the development of head morphology. Both studies sought to examine morphological development in larval cod reared under standard culture conditions and using common commercial feeds, and had the broader objective of elucidating developments in trophic morphology that could potentially relate to the development of cannibalism in this species.

Cod reared from hatch to 78 dph were found to exhibit clear patterns of change in various measures of head morphology Most of the recorded measures exhibited comparable patterns of growth, increasing in relative size from week 4 onwards and then falling from week 9. However, jaw width followed an opposite trend, decreasing in relative size from week 4 and increasing again from week 9. Each period of change in head morphology coincided with points at which the larval diet was changed and may have been caused by these changes in diet. Individuals varied strikingly in the extent to which they were influenced by the

underlying processes, such that, by the end of the larval/early juvenile period, there was marked individual variation in head morphology.

In a further two separate, but related, experiments, fish reared on different prey types were shown to develop marked differences in certain aspects of head morphology. Specifically, fish reared on smaller prey items developed more fragile heads than fish reared on larger prey items. Furthermore, fish reared on smaller prey items developed larger eyes, relative to the size of the jaw, than fish fed the larger prey items. This latter difference resulted solely from the development of larger eyes and smaller jaws in fish fed the smaller prey items and not from any changes in the development of fish fed the larger prey items. Analysis of dead fish indicated that at least some of the observed differences in the head morphology of fish fed the different diets resulted from the differential growth of body parts in response to the nutritional content or size of individual feeds, rather than on different patterns of morph-specific mortality in fish on the different diets. Furthermore, analysis of a small number of cannibalistic larvae indicated that fish fed the larger prey items (i.e. the enriched *Artemia* commonly used in aquaculture) developed morphology comparable with that of the cannibalistic morphs.

In the studies outlined in chapters 3 and 4, there exists an inconsistency in the pattern of development of head morphology in cod fed similar diets. In the study outlined in chapter 3, fish fed Algamac enriched *Artemia* (from 27 dph) went through a phase when they developed larger eyes and smaller jaws between weeks 5 and 9 (29 to 57-58 dph). In contrast, in the study outlined in chapter 4 neither eye diameter or jaw width changed significantly in size in fish fed Algamac and Selco enriched *Artemia* (from 28 dph) over a similar period. One possible explanation for this inconsistency is that fish development in chapter 4 was further influenced by the addition of Selco enriched *Artemia* to the diet. However, Algamac and Selco enrichments supplement *Artemia* with similar types and levels of fatty acids (Tamaru et al. 1999) and therefore it seems unlikely that this difference in the diet was responsible for the inconsistency. At present, it is not clear what caused the observed differences in the development of head morphology in the two studies.

7.12 Aggression in larval cod (chapter 5)

The objective of the study outlined in chapter 5 was to describe aggressive interactions in larval cod and to examine the extent to which these interactions represented an early form of cannibalism, as opposed to a battle for resources. In an attempt to elucidate the nature of aggression, the study also sought to examine the effect of the availability of feed on the incidence of aggressive attacks in larval cod.

Aggressive attacks were characterised by nips to conspecifics and became prevalent around 45 dph. Fish were found to be significantly less motivated to attack during periods of feeding than prior to feeding, irrespective of the overall densities of prey provided. Furthermore, attacks were preferentially directed at the tail of victims, to victims of a smaller or similar size than the attacker and to victims that exhibited abnormal body posture. Together, these results provided strong evidence that some of the aggression I observed reflected an attempt to consume part of the victim and were thus indicative of cannibalism later in development. The overall level of prey provided did not influence the incidence of aggression, although analysis was confounded by a decline in levels of aggression at increasing fish densities. In addition to nipping, fish exhibited a large amount of burst swimming behaviour (termed ‘darts’) in which they appeared to respond to a real or perceived threat of attack. These darts were not affected by the presence or absence of food, but were more common in fish fed the higher prey densities, possibly as a result of increased fish condition or size.

7.13 Variable risk taking in cod: effects of stock and family (chapter 6)

This study arose opportunistically out of another project in the Division and offered the opportunity of working with larger cod, in another country and in a Research Institute rather than a University. The objectives of the study outlined in chapter 6 were to characterise behavioural variation in the risk taking behaviour of one-year old cod and to determine the whether the propensity to take risk varied between cod of different stock or family origin. The study also sought to relate any observed behavioural differences in risk taking to plasma cortisol levels.

Fish were characterised (by discriminant function analysis of the movements of a sub sample of directly observed fish) as risk avoiders if they failed to emerge from undercover or

emerged only to attempt to escape from the arena, or as risk takers if they emerged to explore the open arena. Fish of North-eastern Arctic (migratory) stock origin took significantly more risks than fish of coastal origin. Furthermore, although there were no significant differences in risk-taking between families of migratory stock origin, a weakly significant difference did exist between families of coastal stock origin. Features of behaviour during exploration (e.g. time to emerge from undercover) did not vary significantly between stocks or between families of migratory stock origin, but did vary significantly between families of coastal stock origin. Cortisol levels did not vary between risk avoiders or risk takers, but were significantly higher in control fish of migratory origin compared to control fish of coastal origin. The existence of differences in risk-taking among families within the coastal stock suggests that some of the variation in this trait may have been inherited. The weight and condition of fish was significantly smaller in fish that emerged to escape and, consequently, differences in these factors may have contributed to the observed behavioural differences between fish of different stock or family origin.

7.2 Implications

7.21 Implications for aggression and cannibalism in cultured cod

As discussed in chapter 1, large numbers of larval and juvenile cod perish in the aquaculture environment and cannibalism appears to be the primary cause of these losses (Howell 1984; Folkvord 1989). In light of the increasing interest in the culture of Atlantic cod, there is a burgeoning requirement for greater elucidation of the processes underlying the development of cannibalism in this species, which in turn can contribute to the development of rearing protocols that mitigate this behaviour. Many of the implications of the aforementioned studies relate to this subject.

The results of the study outlined in chapter 4 indicated that feeding cod different prey types could produce differences in cod head morphology and that cod fed enriched *Artemia* could develop morphology comparable with that of cannibalistic morphs. Irrespective of the underlying mechanisms (selective mortality or developmental plasticity), these results suggest that feeding cod with *Artemia*, and especially enriched and consequently large *Artemia*, may be partly responsible for the high levels of cannibalism that occur during the culture of larval and juvenile cod (see Future Studies). In terms of reducing levels of cannibalism, it may

therefore be beneficial to maintain fish on a smaller feed, such as rotifers. The results of both experiments indicate that feeding rotifers to cod for an extended period would not compromise survival rates, although the effect of such a regime on growth and condition is less clear (see Future Studies).

The results of the study outlined in chapter 5 indicated that aggression was particularly prevalent in larval and juvenile cod. Since injuries sustained as a result of nips to the body may lead to infection and disease (Greaves and Tuene 2001), it is likely that many of the deaths observed in young cod result not just from cannibalism, but as a result of an aggressive attack. Mitigation of this behaviour may therefore be as important for cod aquaculture as mitigation of cannibalism in this species. In light of the fact that fish were rarely motivated to attack conspecifics in the presence of food, a reduction in levels of aggression may be achieved by more frequent feeding of fish. Furthermore, since aggressive attacks appeared to reflect incipient cannibalism, frequent feeding may also lead to a reduction in levels of cannibalism later in development.

In addition to aggressive behaviour, cod were frequently observed burst swimming (‘darting’), apparently in response to a perceived threat of attack. Burst swimming has been shown to use up large amounts of energy in other fish species (Krohn and Boisclair 1994), and as such should also be mitigated in cultured cod. Darts were more common in fish fed the higher prey densities, possibly as a result of the increased condition and/or body size of these individuals. In order to reduce levels of both aggression and darting it may therefore be necessary to increase the frequency of feeding while at the same time reducing the overall provision of prey.

7.22 Implications for stock selection of cod for aquaculture

The results of the study outlined in chapter 6 indicate that risk-taking behaviour in cod has a genetic component and, as a result, cod of different stock and/or family origin vary in the extent to which they will explore a potentially dangerous environment. Population differences in risk-taking behaviour could potentially affect the vulnerability of cod populations to fishing (Frank and Brickman 2000) and consequently stock assessment models may benefit from incorporating such information. In cod aquaculture, population and/or family differences in risk-taking behaviour could have important implications for stock selection. For example,

since fish that avoid risk seem to have an especially hard time, especially if timidity is associated with a tendency to avoid fights (Huntingford and Adams 2005), selection of individuals from a risk-taking population/family may improve fish production.

7.3 Future Studies

That the original objectives of the research were not met means that a number of still interesting questions regarding cannibalism in cod remain. For example, there is much speculation, but little information, regarding the onset of cannibalism (with regard to age/size) or the incidence of cannibalism at various stages of development. Similarly, little is known about the developmental pathways that lead to this behaviour, for example, whether cannibalism reflects a facultative response to features of the environment or is inherited. It is also not clear to what extent the onset and incidence of cannibalism is influenced by environmental factors such as the availability of alternative prey or the availability of refuges. Some of these questions were assessed with regard to aggression in the present thesis and as such, the results outlined here do provide some indication of what may mitigate cannibalistic behaviour in cod. However, it would be useful to confirm whether factors such as, for example, prey availability do indeed reduce levels of cannibalism in larval cod.

Perhaps the most obvious objective of any study leading on from this thesis would be to describe the morphology of cannibalistic cod in a larger number of individuals than the six examined here. It would then be possible to confirm the potential for *Artemia*-fed cod to develop morphology comparable with that of cannibalistic cod. Assuming such a study validates the results outlined in this thesis, it would then be useful to assess the potential for fish to be fed rotifers rather than *Artemia* and specifically to assess whether growth rates and body condition could be maintained in fish fed this way.

The hypothesis that levels of aggression in cod may be reduced by more frequent feeding, merits examination. Such a study might also involve analysis of the effect of feeding frequency on the incidence of cannibalism. In the experiment outlined here, cod were fed live prey twice a day, as they commonly are in commercial aquaculture and consequently any study should examine the effect of increasing the number of feeds beyond this figure. It seems likely that the optimal feeding frequency will be that which ensures a constant presence of feed in the tank.

In light of the high incidence of darting behaviour observed in larval cod, this behaviour also merits a more detailed study than that undertaken here. For example, it would be useful to identify the stimulus that results in a dart and to confirm whether this behaviour is indeed a response to a real or perceived threat. It would also be useful to examine why increased levels of feed lead to an increase in the incidence of this behaviour (e.g. size or condition) and to establish what other environmental factors influence this type of swimming. Such information could then be used to reduce the incidence of darting behaviour in culture cod.

Finally, it would be useful to examine the effect of risk-taking behaviour on levels of survival in cultured cod. Such information could then inform decisions regarding the selection of stocks and/or families of cod for culture. It would also be useful to examine whether levels of risk-taking in individual cod are consistent across different situations and correlate with other types of behaviour, such as aggression. Such information has important evolutionary implications since correlations between behaviours may result in sub optimal behaviour in certain contexts (Bell and Stamps 2004).

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