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The influence of stocking density and acoustic conditioning on the behavior and growth of Atlantic cod, Gadus morhua

Daniel Ward

University of New Hampshire, Durham

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THE INFLUENCE OF STOCKING DENSITY AND ACOUSTIC CONDITIONING ON THE BEHAVIOR AND GROWTH OF ATLANTIC COD, *GADUS MORHUA*

BY

DANIEL WARD

B.S., University of Rhode Island, Kingston R.I. 2005

THESIS

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This thesis has been examined and approved.

Thesis Director, Dr. Winsor H. Watson III
Professor of Zoology

Dr. W. Huntting Howell
Professor of Zoology

Dr. David L. Berlinsky
Associate Professor of Zoology

12/10/10
Date
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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS..................................................................................iii

LIST OF FIGURES.......................................................................................v

LIST OF TABLES..........................................................................................vi

ABSTRACT.....................................................................................................vii

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1. ACOUSTIC CONDITIONING OF ATLANTIC COD, <em>GADUS MORHUA</em>, TO INCREASE FEEDING EFFICIENCY</td>
<td>9</td>
</tr>
<tr>
<td>2. DENSITY DEPENDANT BEHAVIORS OF ATLANTIC COD, <em>GADUS MORHUA</em>, IN A NEAR SHORE NET-PEN</td>
<td>42</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>76</td>
</tr>
<tr>
<td>FUTURE STUDIES</td>
<td>78</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>80</td>
</tr>
<tr>
<td>APPENDIX A: IACUC APPROVAL LETTERS</td>
<td>81</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

CHAPTER I

1.1 Schematic of the experimental cages under the UNH Marine Research Pier...........16
1.2 The feeding schedule used for the three experimental cages.............................20
1.3 Temperature profile during the experiment; 3/18/2009 – 6/2/2009........................23
1.4 The presence of fish in the area where feed was delivered 5 minutes prior to feeding.................................................................25
1.5 The behavior of cod 1 minute prior to feeding................................................27
1.6 The number of fish from each treatment that were in the area where feed was delivered before, during and after feeding.................................28
1.7 Behavior of fish while food was being delivered..............................................30
1.8 Stomach content analyses ..................................................................................32
1.9 Growth in length, weight and overall percent change(weight and length) throughout the course of the study .................................................................34

CHAPTER II

2.1 Research site and net pen dimensions. .................................................................48
2.2 The distribution of fish in the net pen when stocked at a low density of 5 kg/m$^3$.....55
2.3 The distribution of fish in the net pen when stocked at a middle density of 10 kg/m...56
2.4 The distribution of fish in the net pen when stocked at a high density of 25 kg/m$^3$...57
2.5 The relationship between fish density and depth distribution based on video data...58
2.6 A comparison of fish distribution data obtained from both video cameras and the HTI telemetry system .................................................................60
2.7 Video and telemetry data comparison. .................................................................61
2.8 Percent of time spent in each third of the water column within the net-pen, based on telemetry data.................................................................63
2.9 Depth profiles for a 24 hr time period from a single fish, at all three densities .......64
2.10 Daily Vertical Movement in Reference to a 2m Threshold ..................................66
2.11 Swimming activity of cod held at three different densities ..............................68
2.12 The relationship between average daily swimming speed and time of day .........69
LIST OF TABLES

CHAPTER I
1.1 Changes in FCR, condition (K), and daily growth rate during the study .......... 36

CHAPTER II
2.1 Stocking densities and water temperatures ........................................... 51
ABSTRACT

THE INFLUENCE OF STOCKING DENSITY AND ACOUSTIC CONDITIONING ON THE BEHAVIOR AND GROWTH OF ATLANTIC COD, GADUS MORHUA

BY

Daniel Ward

University of New Hampshire, December, 2010

The goal of experiment one (Chapter One) was to determine if stocking density had an influence of the swimming behavior and spatial distribution of adult cod that were being raised in an aquaculture cage at the University of New Hampshire’s Coastal Marine Laboratory. Acoustic telemetry and underwater video were used to quantify the behavior and distribution of cod that were stocked at four densities (5, 10, 25 and 45 kg/m$^3$). At the lowest density (5 kg/m$^3$) cod remained deep in the cage and spent 64.3 ± 0.8% of their time below 1.80m (of a 2.70m cage). This contrasts to the middle density (10 kg/m$^3$) and the high density (25 kg/m$^3$) treatments, during which fish spent 48.7 ± 1.2% and 46.8 ± 0.8% of the time below 1.80m, respectively. One of the objectives was to determine if adult cod will school if raised at a high density, therefore reducing milling activity. At no time did the cod school in the experimental cage, regardless of raising the density to 45 kg/m$^3$. The objective of experiment two (Chapter Two) was to determine if conditioning cod with an acoustic stimulus presented during feeding would increase their growth rate. Conditioned fish became active as soon as the sound stimulus was presented and spent
less time milling at the surface prior to feeding, as compared to the group which was fed at the same time twice daily, but without a conditioning sound stimulus. Length and weight measurements for each group were not different at the end of the experiment, though the acoustically conditioned group had the lowest food conversion ratio (FCR, 4.17), and a higher daily growth rate (0.11 g/d) than the group with the randomly changing daily feeding times. The results from these two studies further develop our knowledge of cod behavior in an aquaculture setting. Understanding how acoustic conditioning can reduce wasted feed, will allow farmers to reduce waste and increase growth rates. By better understanding how density impacts cod behavior, cages can be better designed for cod in particular, and density can be further considered in farm management to better optimize fish farming.
INTRODUCTION

Aquaculture

World capture fisheries have been in a stable state throughout much of the past decade and it has been estimated that the maximum capture fisheries potential from global waters has been attained. Moreover, as many as fifty percent of the world's commercial fish stocks are thought to be fully exploited (FAO, 2008). In response to the increased need for seafood, global aquaculture production is rapidly expanding. For example, from 2004-2006 aquaculture grew at an annual rate of 11% in value, and 6.1% in volume. In fact, aquaculture production has grown so rapidly in some countries that it is poised to overtake traditional capture fisheries for food fish production. While aquaculture is currently growing faster than any other animal-producing sector (8.9% annually), currently most of the growth (89%) is found in Asian countries, especially China. (www.fao.org, 2010).

The groundfishing industry in the northeast United States was once considered to be one of the most productive fisheries in the world. Overfishing, however, has caused a steady decline in catch, resulting in devastating consequences for fishing communities throughout New England (NMFS, 2002). With US wild groundfish stocks in decline, the US must increase its aquaculture production, or risk increasing the already $8 billion annual trade deficit in seafood (FAO, 2008). Over 70% of seafood consumption in the US is currently imported, and at least 40% of that is farm-raised (http://www.fas.usda.org, 2007). Moreover, the US population continues to grow, and the per-capita consumption
of seafood is expected to increase more than 30% in the next 20 years as farm-raised products become cheaper (http://www.fas.usda.org, 2007).

While the aquaculture industry must expand in order to meet rising demand, there are numerous obstacles to gaining widespread support. For example, aquaculture can have negative effects on aquatic ecosystems. In Norwegian salmonid aquaculture, there are widespread sea lice problems, and constant treatment results in environmental damage and sea lice resistant to chemical eradication (Berg and Horsberg, 2008). Another issue facing the culture of carnivorous species is the heavy reliance on capture fisheries as a protein source, and this reliance can deplete other fishery resources. Salmon and trout aquaculture rely 100% on commercial feeds that get a majority of their protein from fish meal and fish oil (Naylor et al, 2000; Deutsch et al, 2006). Habitat destruction, waste disposal, and escapes from fish farms are also potential problems that result from aquaculture (Moe et al, 2007). It is clear that more research is needed to allow aquaculture to expand to meet rising global protein demands in a way that is both environmentally sound and economically feasible.

**Atlantic cod aquaculture**

Wide spread declines in wild cod stocks have brought about renewed interest in gadoid aquaculture. Several demonstration sites in the United States have been federally funded, though there is still a very small Atlantic cod aquaculture industry in the US. The University of New Hampshire (UNH), has had an offshore demonstration site growing various marine fishes (summer flounder, haddock, halibut, steelhead trout, and cod) since 1998. The first cod were transferred offshore at 45g, and had a 97% survival rate over 17
months (Chambers and Howell, 2006). In Sorrento ME, GreatBay Aquaculture LLC has
been growing Atlantic cod since 2008. The company has 8 cages, stocked with 100,000
cod each (per comm. George Nardi). The farms in New Hampshire and Maine constitute
the entire US cod aquaculture industry, with the UNH site having just finished their
second harvest (per. comm. Michael Chambers).

Internationally, there has been much more interest in Atlantic cod aquaculture. Atlantic
Canada, Iceland, the United Kingdom, and Norway all currently have cod
hatchery and grow-out programs (Rosenlund and Skretting, 2006; www.fao.org, 2010). Norway
is by far the leader in Atlantic cod production, and after years of applied research
into best practices, production is growing rapidly (www.fao.org, 2010). In 2007, Norway
alone produced 11,000 metric tons of cage-raised Atlantic cod
(www.ssb.no/english/subjects/10/05/nosfiskeoppdretten, 2010).

Many advances have been made to increase the profitability of cod aquaculture in
recent years. For example, manipulation of photoperiod to delay sexual maturation has
been shown to improve growth (Bjornsson et al, 2007), and differences in growth rates
have been reported for fish raised in flow-through, in comparison to recirculating,
systems (Bjornsson and Olafsdottir, 2006). Nevertheless, while progress is being made,
there are still many aspects of cod aquaculture that need to be investigated (Chambers
and Howell, 2006). The current project is focused on optimizing cod aquaculture by
determining: 1) the optimal stocking density for cod and; 2) the influence of acoustic
conditioning on feeding behavior and growth.
Changes in juvenile cod behavior have been attributed to both stocking density, as well as size disparity (Uglem et al, 2009). Juvenile cod were found to be able to be stocked at >40 kg/m³ with no reduction in growth rate, provided the water quality is high (Bjornsson and Olafsdottir, 2006). However, overcrowding can result in a decrease in specific growth rate. Though growth may slow at higher densities, raising fish at higher densities may be a better use of the water, though it remains to be seen if the conservation of growth rate remains as the cod continue to grow (Lambert and Dutil, 2001).

While salmon will school at any age, cod have been found to school only as juveniles at a high density (Rillahan et al, 2009). It remains to be investigated whether schooling can reduce the number of escapes, and at what density adult cod will begin schooling behavior. Unlike salmon, cod have the tendency to pull at the net, and find ways to escape the confines of the ocean net pen. Many more cod than salmon escape from net pens (10-20 times more), and this is largely attributed to their “milling” behavior, which lends itself to finding ways to escape (Moe et al, 2007). Cod are not uniformly distributed within the net pen, i.e. they prefer certain regions. As densities increase, social interactions, hierarchies, and territorial borders will develop (Li and Brocksen, 1977; Jobling, 1985) thereby forcing greater cage utilization as cod move higher into the water column.

While all early indications suggest this to be the case, at least for cod, we do not know what factors influence their positional preferences. One of the objectives of the current research is to investigate the density at which adult cod will school, and the impact this has on swimming behavior. The benefits of cod schooling may lead to reduction in escapes, though the impact on swimming speed and cage utilization is not
known. Another aim of the current work, is to look at cage utilization by adult cod when stocked at different densities in order to investigate changes in behavior, and to make comparisons between low and high stocking densities.

**Cod hearing**

Fish are able to sense acoustic changes in their environment through two means; their lateral line and their acousticolateralis system. The ear and lateral line do have functional overlap; the ear can detect sound frequencies within 50-2000Hz in certain species (Popper and Carlson, 1998), while the lateral line responds to differences between the motion of the fish and motion of the surrounding water (Montgomery *et al*, 1995); usually at much lower frequencies. The main difference in sensitivity between the two systems is the distance from the fish from which the sound is generated. The lateral line detects signals that originate within one or two body lengths from the fish, while the ear can detect signals generated from a much greater distance (Kalmijn, 1989).

Atlantic cod have advanced hearing abilities compared to many other species of teleost fishes, because their hearing is assisted through the presence of a swimbladder. The swimbladder acts as an amplifier of the kinetic component of the sound, and in many cases the swimbladder may also act like a resonator and change the quality of the sound (Tavolga, 1971; Sand and Enger, 1973). The resonating capabilities of the swimbladder allow cod to hear through both particle displacement and as changes in the acoustic pressure wave (Chapman and Hawkins, 1973). Since cod lack the Weberian ossicles necessary to transfer the sound from the bladder to the inner ear more effectively, sound is transferred to the hearing organs directly through the body tissues (Brawn, 1961; Sand
Acoustic conditioning to control fish behavior

The stimuli most often investigated to control fish behavior are sound, light, chemicals, temperature, and pressure. Since sound travels at such a high rate of speed, attenuates slowly, and is very directional, it has been shown to be effective in controlling fish behavior (Popper and Carlson, 1998). There are many reports of using sound to repel fish around power plants and hydroelectric dams (Popper and Carlson, 1998; Ploskey et al, 2000). Traditional fishermen have long known that sounding a bell when feeding fish in a pond will entrain the fish to stay in the immediate area (Lines and Frost, 1999).

Abbott (1972) trained a group of rainbow trout to respond to 150Hz and 300Hz tones by generating the tones in association with feeding and the fish quickly learned to come to a specific feeding site. In an attempt to control costs and migration of hatchery-reared fry out of a stocking area, Japanese researchers investigated sound conditioning to feed. A 300Hz signal was emitted in conjunction with a feeding event, and the fish were conditioned over a period of 19 days. They found that the recapture rate was many times higher with sound-trained fish, as compared to fish that were only released from the hatchery (Okei and Tajima, 1999).

Biotellemetry

Biotellemetry has been primarily used with wild fish to study their movement patterns. However, some scientists have also applied it in aquaculture settings. Juell and Westerberg (1993) developed a system in which they deployed 4 hydrophones
surrounding a salmon sea cage to detect acoustic tags implanted in the fish. The system was able to detect both the horizontal and vertical positions of fish, which in turn allowed estimates of swimming speed, direction and depth. This was the first time that such a system was deployed in an aquaculture setting to document the behavior of farmed fish. The authors outlined the limitations of such a positioning system (wrong positions based on incorrect time measurements) as well as the benefits (being able to show a breakdown in diel rhythms based on density) (Juell and Westerberg, 1993).

Several authors have since investigated both fish physiology and behavior in aquaculture net pens with similar systems. Chandroo et al. (2005) studied energetic response to fish transport, Bridger et al. (2001) monitored site fidelity of released steelhead trout, and Claireau et al. (1995) monitored distribution and energetics of cod with changing water conditions. Most recently Rillahan et al. (2009) investigated the behavior of cod grown in an offshore, submerged, net pen.

In this thesis I used biotelemetry to investigate the behavior of Atlantic cod that were being raised in relatively small inshore net pens. In Chapter One I summarize experiments designed to determine if it is possible to condition fish to a sound stimulus associated with feeding. I further examined the influence of conditioning on feeding and growth of these fish, in comparison to treatments where fish were either fed at random times or fed without a sound stimulus associated with the food. In Chapter Two I present telemetry data that were obtained from cod stocked at three different densities. The goal was to determine if stocking density influenced their swimming behavior and distribution within the net pen.
OBJECTIVES

Conditioning (Chapter One)

Oiestad et al. (1985) showed that cod, contained in extensive aquaculture ponds, could be conditioned to feed when a sound stimulus was played. The present experiment sought to investigate if conditioning fish in an aquaculture setting can improve growth rates through greater feeding efficiency.

Density (Chapter Two)

Determining optimum stocking density is one of the most important factors to consider when planning a net-pen aquaculture operation. Rillahan et al. (2009) demonstrated that juvenile cod will school at high stocking densities, in the current experiment I investigated if adult cod display the same behavior at high densities. I sought to determine the effects stocking density on the schooling behavior and cage utilization of adult Atlantic cod in a small near-shore net-pen.
CHAPTER 1

ACOUSTIC CONDITIONING OF ATLANTIC COD, GADUS MORHUA, TO INCREASE FEEDING EFFICIENCY

Abstract

The purpose of this study was to determine if Atlantic cod (Gadus morhua) could be conditioned to associate a sound signal with feeding and, if so, determine if this would lead to an increase in feeding efficiency. Three cages (28 m$^3$), containing 1000 cod each, were used for three different treatments. The fish in Cage 1 were fed at the same times, twice daily, and a 150 Hz tone was played for one minute prior to each feeding (conditioned group). The fish in Cage 2 were fed twice daily at the same times each day, but with no acoustic stimulus (control group). Finally, the fish in Cage 3 were fed twice daily but the times were changed randomly each week (random group). After two weeks of conditioning, the fish in Cage 1 exhibited increased aggregation near the feeding tube when the tone was played prior to feeding. Aggregation was quantified by counting the number of fish in each frame of a video taken underwater in the vicinity of the feeding tube. Shortly after the tone was played the number of fish increased from $1.09 \pm 0.25$ to $3.56 \pm 1.10$ (n=69 frames), in the acoustically conditioning group. The fish in Cage 2 appeared to anticipate feeding as well, despite not hearing a tone, with the number of fish increasing slightly from $4.05 \pm 0.65$ to $4.32 \pm 0.72$ (n=78) just prior to feeding. Finally, very few fish were visible near the food source just prior to feeding in Cage 3 and the
change was minimal, from $0.03 \pm 0.03$ to $0.00 \pm 0.00$ (n=60). Over the eight week experimental period all the fish grew in length, though the final lengths were not different. The overall change in weight was lowest for the random group (88.8% increase), compared to the conditioned group (103.9%) and the control fish (109.5%). While feed conversion ratios (FCR’s) for all three groups were artificially high (>4), due to the 5% BW daily feed ration, the daily growth rates were best for the two groups that had the same daily feeding times (0.11 g/d). These data indicate that cod can be conditioned to anticipate food delivery and, by doing so, the method could improve the efficiency of aquaculture applications by yielding good fish growth with less food costs.
Introduction

The rapid decline in Atlantic cod stocks has facilitated the emergence of a new industry in gadoid aquaculture in Europe and North America (Rosenlund and Skretting, 2006). It was thought that the Atlantic cod industry would develop quickly in both Norway and the United States, much as the Atlantic salmon industry, which currently produces 1.5 million tons per year (FAO, 2006). Although cod aquaculture has lagged in the US and Canada, in 2005 Norway’s cod aquaculture industry produced 5,500 tons, and it was projected to produce as much as 150,000-200,000 tons by 2010 (Standal and Utne, 2007).

Mass rearing of cod began in Norway in 1983. Eggs and larvae were first stocked into a salt pond and fed on naturally occurring zooplankton. The resultant juveniles were acoustically trained to come to feeding stations in the pond where pelletized feed was delivered (Oiestad et al., 1985; 1987). The gadoid farming industry has quickly developed in Norway, Canada, Iceland, and the U.K., and it is beginning to increase in the US as well (Rosenlund and Skretting, 2006). The University of New Hampshire began an offshore aquaculture demonstration project and has been farming marine fish at the farm since 1998 (ooa.unh.edu). While the project has demonstrated that offshore cod aquaculture is technologically feasible (Chambers and Howell, 2006), the next step is to make advancements so that it becomes profitable.

Food is the most expensive reoccurring cost of any aquaculture operation. Therefore, any improvements that can be made to increase the efficiency of feeding, or the conversion of food to fish protein, will improve profits. When determining a feeding
strategy many variables must be taken into account, such as temperature, fish size, activity demands, and social interactions, among others. Cod fed to satiation twice a week had lower growth rates than those fed three or five times per week, though at high densities cod fed five times per week showed better growth than cod fed three times per week (Lambert and Dutil, 2001). Feeding to satiation every second day results in lower liver size, and improved protein retention, although growth rates are the same as cod fed once per day (Hemre et al., 1989; Rosenlund et al., 2004). It has been shown that the maximum number of meals per day is best limited to 2-3 (Boujard and Leatherland, 1992). Clearly, the optimal feeding schedule and method of food delivery has not been determined and more research into this area is needed.

If is both important to determine how often and how much to feed fish, as well as how best to feed fish so that food is not wasted. Feed can be administered by hand, automatically, or through self-feeders, though each method has drawbacks. Hand feeding is labor intensive, and even when feeding to apparent satiation based on the surface activity of fish, this can be misleading and will, in turn, lead to over or under feeding (Ang and Petrell, 1998; Noble et al., 2007). Auto-feeders rely on growth rate tables and feed manufacturer specifications to determine the amount of feed to provide per feeding event, often miscalculating the actual nutritional needs of the fish, which leads to wasted feed or competition for limited feed (Noble et al., 2007). Self-feeding has the advantages of good feed conversion ratios (FCRs) and lower labor costs (Paspatis et al., 1999), and has been shown to be effective at ensuring efficient feed intake. However, self-feeders can also cause competition at the feeding site (Noble et al., 2007), or reduced feed intake due to lighting conditions around the feeder (Yamamoto et al., 2002). Studies with cod
have shown no significant difference between feeding regimes on growth, feed efficiency or feed intake (Yamamoto et al., 2002). Similar results were obtained with sea bass, showing no difference in growth rates when fed either with a self-feeder, by hand (twice daily), or by auto-feeders; though when fed through auto-feeders the sea bass were always overfed (Paspatis et al., 1999).

It can be very difficult to determine the correct amount of feed to administer at each feeding time. Many farmers tend to rely on surface activity or real-time video to indicate satiation, though monitoring surface activity has been found to be an unreliable method of monitoring group satiation (Ang and Petrell, 1998). In a study investigating feeding behavior of yellowtail, salmon, and trout, satiation reached within 15-25 minutes of feeding, and in all species ingestion rate declined as the meal progressed. Fast pellet delivery rates can cause wasted feed if administered too fast, though feed rates that are too low can cause competition and undernourished fish (Talbot et al., 1999).

One possible way to improve feeding efficiency may be to condition fish so that they could anticipate feeding and thus be ready to consume a high percentage of the food provided at each feeding. When Atlantic cod were first ranched in Norway in 1985, sound was used to condition the cod to stay within a small area of the pond and then sound was used to help harvest them (Oiestad et al., 1987). In the Norwegian study, cod were conditioned to stay within the feeding area in a fjord by playing a 150Hz tone in conjunction with feeding whole herring and capelin. This led to effective conditioning within a period of weeks. Furthermore, when sampled, almost all of the conditioned cod had only the feed given by the researchers in their stomachs. This demonstrated that
conditioning cod to feed immediately after an audio signal was played has the potential to increase feeding efficiency (Bjornsson, 2002).

Cardiac conditioning experiments with juvenile cod have helped to determine that they hear best in the range of 60-310Hz (Chapman and Hawkins, 1973). The ability to detect the direction of a sound source (Schuijf and Buwalda, 1974; Schuijf, 1974) appears to be due to the otolith organ's response to the kinetic component of a sound, and not the lateral line (Sand and Enger, 1973; Sand and Hawkins, 1973). Because of this, choice experiments utilizing food rewards have shown that cod will almost immediately turn towards the source of a sound, regardless of whether their lateral line is intact or not (Schuijf, 1975; Schuijf and Buwalda, 1975).

Feeding fish at the same time every day may induce food anticipatory activity (FAA), characterized by an increase in activity several hours prior to feeding time (Boujard and Leatherland, 1992; Reebs and Lauge, 2000; Purser and Chen, 2001). In this study, I was interested in not only the effect of consistent daily feedings on the behavioral response in fish, but also if applying an acoustic stimulus would elicit a greater FAA response. While the classic definition of FAA is an increase in activity levels several hours before each daily meal, during my preliminary studies I noticed that the greatest difference in the behavior of the three groups of fish in this study occurred during the 5-10 minutes just prior to feeding. Therefore, in this particular study, I used the number of fish that moved to the location where food was going to be delivered, five minutes prior to feeding, as the index of FAA.
The overall goals of this study were: a) to determine if cod could be conditioned with a sound stimulus and anticipate the presentation of feed; and b) to determine if conditioned fish fed more efficiently and grew faster than fish fed at the same time each day, or fish fed at random times each day.

**Materials and Methods**

All experiments were carried out at the University of New Hampshire Marine Research Pier in Newcastle, NH in 3 small (28 m³) net pens (Fig. 1.1). A fourth pen of the same size was used to hold the fish that were to be used in subsequent replicates. All three experimental cages, as well as the holding cage, were equipped with auto-feeders (Aquatic Eco-Systems, Apopka, FL).
Figure 1.1. Schematic of the experimental cages under the UNH Marine Research Pier. (A) 28 m$^3$ experimental net pen. (B) Auto feeder designed to drop feed to the cage below regardless of tidal height. (C) Building that housed all the recording and sound generating equipment.
All three experimental cages had two underwater cameras installed facing toward the center of the cage at depths of 0.70 and 2.09 m. A DVR was set to record the inputs from the cameras for 5 min preceding feeding, until 5 min after the 60-90 sec long feeding period ended. The camera was located 70 cm below the surface of the water, facing towards the center where the feed would fall into the cage from the auto-feeders above. The field of view of the camera was approximately 1-3 meters from where the camera was mounted pointing into the center of the cage, and approximately 1-2 meters wide, depending on water clarity and ambient light levels. Food was delivered at the same rate for 60-90 seconds during each feeding, starting with 60 sec during the initial part of each experiment and increasing to 90 sec as the fish grew.

The holding cage was initially stocked with 12,000 juvenile Atlantic cod (average weight ~2.5 g) obtained from GreatBay Aquaculture, Portsmouth, NH, in October of 2008. In early March 2009, 3,000 cod were removed from the holding cage, and stocked equally into each of three experimental cages (1,000 fish in each). When transferring the fish, 100 cod from each cage were sampled for total length and wet weight. The remaining 9,000 Atlantic cod remained in the holding cage, to be utilized for future replicates. The holding cage was equipped with temperature loggers (Onset Corp., Bourne, MA) attached at depths of 1, 2, and 3 m that logged temperature data every 5 min throughout the study.
Experimental protocol

a) Cage 1: Consistent daily feeding schedule with conditioning sound stimulus

The fish in Cage 1 were fed 5% of their body weight (BW) daily via an auto-feeder. Feeding was split into two events spaced 8 hrs apart, both of which occurred during daylight hours (8AM and 4 PM). An underwater transducer (Lubell Labs, Columbus, OH) and hydrophone (Aquarian Audio, Anacortes, WA) were installed in the front of the cage, directly in front of where the auto-feeder dropped in the feed.

Previous studies have determined that it takes between 2-3 weeks to condition fish to associate a sound stimulus with a feeding event (Okei and Tajima, 1999; Bjornsson, 2002) and that 150 Hz is the optimal sound frequency for conditioning (Oiestad et al., 1987; Bjornsson, 2002). This is, in part, because 150 Hz is within the optimal hearing range for cod (Chapman and Hawkins, 1973). In this study the cod were fed for 60-90 seconds, twice daily. The 150 Hz tone was played for 1 min preceding the feeding event until feeding started.

Prior to the start of the feeding trials, the acoustic attenuation of the conditioning signal was evaluated between cages in order to ensure that there would be no cross-conditioning between groups. I put a hydrophone sequentially in each cage while giving the 150Hz tone, and monitored the sound level in each cage. I could not hear the sound with the hydrophone in the other two cages while it was being played in the conditioning cage. In order to be sure the sound was not having an effect on either of the other two cages which were not being conditioned, I also spaced an empty cage between the experimental cages (adding distance between cages), as well as making sure to feed the
other non-conditioned cages prior to feeding the conditioning cage (and applying the stimulus).

b) Cage 2: *Fish fed on constant schedule, but with no conditioning stimulus.*

The fish in Cage 2 were fed twice daily, at the same times every day. It served as a control to simulate natural aquaculture operations, and to determine if feeding efficiency is increased merely through food anticipatory activity (FAA), or if acoustic conditioning is necessary in order for fish to anticipate feeding. The auto-feeder was programmed to feed twice daily, at the same times throughout the experiment. The daily feed ration was calibrated every two weeks to be 5% of average body weight, and separated into 2 feedings, spaced 8 hours apart (one at 7AM, and the other at 3PM). Feeding always occurred when there was ample light, and at times different than those of the other two cages.

c) Cage 3: *Random daily feeding schedule*

The fish in Cage 3 were fed the same 5% BW ration as the fish in the other two cages, separated into two daily feedings, eight hours apart. However, the feeding times were changed every week to avoid the development of food anticipatory activity (FAA).
Figure 1.2. The feeding schedule used for the three experimental cages. Feeding times (in black) were kept the same at 8 AM and 4 PM for the acoustically conditioned group (Cage 1), and 7 AM and 3 PM for the group with the same daily feeding time (Cage 2, control). The feeding times were changed randomly every week for the third group (Cage 3), although the feedings were still spaced 8 hours apart (for example, 9 AM and 5 PM at the beginning). Video was recorded for 5 minutes prior, until 5 minutes after, feeding time (in grey).
Data analyses

Video data from a 2 week period in the middle of the 12 week trial were used for analysis of FAA and feeding activity. Still frames from videos were taken 5 min prior to feeding, 1 min prior to feeding, at the time feeding commenced, and 1 min after feed began to enter the cage (Fig 1.2). For each frame that was captured, all the fish visible were counted and logged. At each time three stills were analyzed: one at the time, one 15 sec before, and one 15 sec after the time. Data from these three images were then averaged for each time period.

Every 14 days, 50 cod from each cage were randomly selected for total length and wet weight measurements, and then 25 were sacrificed for stomach contents analysis. Sampling took place 1 hr post feeding and all dissections and stomach content analyses were performed within 24 hrs. The contents were first identified as either feed or other biological matter that the fish consumed from inside the cage, and then individual gut contents were weighed.

Feed conversion ratio (FCR) was calculated as:

\[ FCR = \frac{\text{weight of food}}{\text{final fish wt} - \text{initial fish wt}} \]

Fulton’s condition factor (K), was calculated as:

\[ \text{Fulton's K} = 100 \times \frac{\text{wt}}{\ln (\text{wt})^3} \]

The above procedure was continued for 12 weeks from 3/9/2009 to 6/3/2009. During the trial, length and weight measurements were taken every other week for an
eight week period from 3/9/2009 to 5/6/2009, while stomach contents analyses were started on 3/9/2009, and continued through 6/3/2009 (the end of the 12 week period). After 12 weeks, all of the cod from the 3 experimental cages were moved into a secondary holding cage.
Figure 1.3. Temperature profile during the experiment; 3/18/2009 – 6/2/2009.
The above experimental protocol was replicated from June-August 2009, with a fresh group of 3,000 fish distributed evenly into the three experimental cages. The experimental design was for replicate three to occur from September-November 2009. However, due to high mortality in both the experimental cages and the holding cage in August, most likely due to high water temperatures (>18°C) (Fig. 1.3), we were not able to finish the second or third trials satisfactorily. Therefore, only the data from trial one are presented. Since only data from one trial are presented, statistical tests were not used to determine if there were statistically significant differences in the behavior and growth of the fish in each of the three groups. However, changes in the behavior of the fish within each group, over time, were quantified using various statistical tests.

Results

Fish in all three treatments were rarely observed in the upper portions of the net pen. However, as feeding time approached, cod in the Control Cage that were fed at the same time each day, began to move to the region near the feeding tube. Five minutes prior to feeding there were $4.05 \pm 0.65$ (SEM here and throughout, $n=78$ frames analyzed) fish observed in each frame of the video, compared to $1.09 \pm 0.25$ in Cage 1 (conditioned) and $0.03 \pm 0.03$ in Cage 3 (Random)(Fig. 1.4). The number of fish near the surface 5 minutes prior to feeding was consistently greater in the conditioned group, as well as the control group, as compared to the randomly fed group. The food anticipatory activity (FAA) expressed by the cod in the Control cage was only observed for this one group, and was not seen for the others.
Figure 1.4. The presence of fish in the area where feed was delivered 5 minutes prior to feeding. Images were captured from a video camera that was positioned at a depth of 70cm and faced towards the center of the cage. A. Acoustic conditioning group, displaying minimal activity near the surface prior to the time when the 150 Hz tone was played. B. Group that had the same feeding time everyday expressing evidence of FAA. C. Random feeding time group, showing no activity in the feeding area 5 minutes prior to feed entering the cage. The areas viewed by the cameras was approximately 1x1m².
One min prior to feeding time, when the acoustic signal was played, there was an significant increase in the number of fish visible in the field of view for the acoustically conditioned group of fish (from $1.09 \pm 0.25$ to $3.56 \pm 1.10$, n=69; $p=0.0016$, Wilcoxon matched-pairs signed-ranks test; Figs. 1.5 & 1.6), though not for the other two groups. In the cage which had the same feeding time every day, there was not a significant increase in the number of fish visible ($4.05 \pm 0.65$ to $4.32 \pm 0.72$, n=78; $p=0.4442$, Wilcoxon matched-pairs signed-ranks test), in part because the fish were already in the area. In Cage 3 (Random) the average number of fish visible actually decreased to zero.
Figure 1.5. The behavior of cod 1 minute prior to feeding. A. Acoustic conditioning group, showing increased activity near the surface when the 150 Hz tone was played. B. The distribution of fish from the Control group, showing some increased activity, though lower in the water column. C. Fish in the Random group, that did not rise to the surface in anticipation of being fed, or in response to an acoustic stimulus.
Figure 1.6. The number of fish from each treatment that were in the area where feed was delivered before, during and after feeding. Data were obtained by counting the number of fish in a single video frame. Both conditioning fish and feeding them at the same time each day increased the number of fish that rose to the surface to feed both before and during each feeding event.
When feeding began, consistently more of the conditioned fish and control fish were near the area of feed delivery in comparison to the randomly fed fish (Fig. 1.7). One minute after feeding stopped, all three groups showed distinctly different behaviors. While the acoustically trained group (Cage 1) had only 2.74 ± 0.70 fish visible per frame, fish in the control group were still near the surface (5.24 ± 0.65 visible), and there were still very few of the Cage 3 fish near the surface (0.32 ± 0.17 visible) (Fig. 1.6).
Figure 1.7. Behavior of fish while food was being delivered. A. Acoustically conditioned group, aggressively attacking the feed as soon as it hit the water, high in the water column. B. Control group, also feeding high in the water column, though not as many fish were visible as the acoustically conditioned group. C. Random feeding group, showing very little feeding activity high in the water column and some fish feeding lower in the frame.
The group that was acoustically conditioned ate the highest number of pellets per 25 fish sampled for the first 4 weeks, and then there was an unexplained drop towards the end of the experiment (Fig. 1.8). At the week 4 sampling (5/6/2009), 100% of the fish sampled had pellets in their stomachs. After this point the number of pellets eaten by the acoustically conditioned group decreased, though the number of pellets eaten by the other two groups remained stable.

The number of fish with pellets in their stomach dropped toward the end of the experiment for all three groups, though more for the acoustically conditioned group. One factor that might influence the amount of feed consumed was the amount of other material fish were eating. The most abundant alternative food source was a naturally occurring population of copepods.
Figure 1.8. Stomach content analyses. A subset of the fish in each treatment were dissected 1 hr after feeding and the number of pellets in their stomachs, and the total weight of the pellets, were determined. A. Percent of fish sampled found with pellets in their stomachs. B. Average pellet weight for each group. C. Percent of fish sampled found with copepods (foraged food) in their stomachs. D. Average weight of copepods. Overall, throughout the trial period, the acoustically conditioned group always had both lower rates of copepods in their guts, as well as lower average copepod stomach content weight.
The length change for all the fish was limited during the first 4 weeks of the study (Fig. 1.9), most likely because the water temperatures were low (Fig. 1.3). The control group had the greatest change in weight (24.2%), as well as the greatest change in length overall (31.4%) (Fig 1.9).

All three groups started with very similar starting weights (Fig. 1.9) and during the first 4 weeks there were much greater changes in weight as compared to the small changes in lengths. For example, the acoustically trained group increased by 54.3%, from a starting weight of 6.35 ± 0.12g to 7.67 ± 0.16g during this period. In contrast, the group fed the same time every day increased their weight by 71.8%, and the group with the feeding times changed weekly increased in weight by 39.9%. The largest increase in weight overall was found in the control fish (109.5%), followed by the conditioned fish (103.4%) and randomly fed fish (88.8%).
Figure 1.9. Growth in length, weight and overall percent change (weight and length) throughout the course of the study. A. The conditioned group had the greatest starting length and ending length, but the lowest overall change (19.95%) in length. The control fish exhibited the greatest overall change in length (31.2%) over the 8 week study. B. The conditioned fish weighed the most at the end of the study, while the control group exhibited the greatest increase in weight (109.5% overall). C. The control fish exhibited the greatest increase in length and weight during this study.
Feed conversion ratios (FCRs) for all groups were quite high (Table 1.1), demonstrating that the 5% BW daily ration was above what the fish could process, and the excess feed probably fell through the cage. FCR for the conditioned group was the lowest at 4.17, indicating that conditioning allowed the fish to feed more efficiently than the other two groups. The group which was fed the same time everyday had a lower FCR (4.20) than the group with the randomly changing feeding times (4.77), indicating that the same daily feeding time also had a positive effect on feeding efficiency. All three groups had similar starting condition (K), though the greatest improvement in condition was within the acoustically conditioned group. This group had the largest change in weight in relation to change in length over the 12 week period, and therefore had the greatest increase in K.
<table>
<thead>
<tr>
<th>Trial 1</th>
<th>FCR</th>
<th>K (start)</th>
<th>K (end)</th>
<th>Growth Rate (g/day)</th>
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</thead>
<tbody>
<tr>
<td>Cage 1- conditioned</td>
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<td>0.73</td>
<td>0.86</td>
<td>0.11</td>
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<tr>
<td>Cage 2- control</td>
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<td>0.88</td>
<td>0.11</td>
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<tr>
<td>Cage 3- random</td>
<td>4.77</td>
<td>0.77</td>
<td>0.84</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table 1.1. Changes in FCR, condition (K), and daily growth rate during the study. Overall Feed Conversion Ratios (FCRs) were very high, most likely due to the 5% body weight daily feed ration. The greatest increase in condition (K) was seen in the group that was acoustically conditioned, while the group with the largest length and weight increase (same feeding times) decreased in condition.
Discussion

One of the most striking findings of this study was that when cod were trained to associate a sound stimulus with the presentation of food, they were able to anticipate arrival of the food and therefore feed more efficiently. As a result, their condition factor and growth rate was higher and FCR lower, compared to fish fed at a randomly changing feeding time. It is also interesting to note that changing the feeding time every week (which may occur on aquaculture farms), had a dramatic effect on the feeding behavior and growth of the fish. While the trial was too short to notice significant changes in weight or length, and no replicates trials were made because of rapidly increasing water temperatures, I believe that this project has demonstrated the importance of both a temporally regimented feeding regime and the potential utility of acoustic conditioning to enhance the growth of farmed fish.

Initial cod acoustic conditioning experiments were designed to aggregate and harvest juvenile cod (Oiestad et al, 1987). These experiments showed that cod could be trained in a period of weeks, and I was able to show the same response in the current project. When fed at the same time, twice daily, in conjunction with a 150 Hz tone, the cod were trained within 2 weeks. Video analysis from 5 minutes prior to feeding time (4 minutes prior to the acoustic stimulus) showed little to no activity from the acoustically conditioned group. This shows that the fish were not merely rising to the top displaying FAA, as was the case with the fish fed at the same times every day. The acoustically conditioned fish stayed lower in the water column for a longer period of time, and would then rise up only when “called” by the acoustic signal. This may have an advantage in
commercial farms where they would presumably be less vulnerable to predators, and be less exposed to bright sunlight and warm surface waters (Claireaux et al., 1995; Ferno et al., 1995; Oppdal et al. 2001; Hobsen et al., 2007; Korsoen et al., 2009). The group that displayed the most FAA (the group which was fed the same time everyday) spent the greatest amount of time near the surface of the cage. This not only put them at risk for attack from predators, but also caused them to display higher swimming speeds, and not the characteristic milling behavior when they are not feeding (Moe et al., 2007).

Acoustic conditioning allowed that group of fish to consume the food higher in the water column than the other two groups, which in a large scale system would allow more contact time between the fish and the feed falling through the cage. Conditioned fish had both the highest rate of feed consumption and the highest individual pellet stomach content weights (Fig. 1.8) indicating that this group of fish were ready to feed when the feed was administered, and consumed the feed higher in the water column. The other two groups of fish were not as prepared for the feed to enter the cage, and therefore had lower pellet incidence rates and pellet weights.

Changing the feeding time randomly every week had a detrimental effect on feeding activity. Fish were less prepared for a feeding event, and therefore their feeding behavior and growth were different that the other two treatments. Fish in the randomly changing feeding times group were not near the surface prior to a feeding event, and thus fed lower in the water column, resulting in less time to catch the feed falling through the cage. The acoustically trained group had a more pronounced response to the feed falling into the cage, and they fed much higher in the water column as well. The advantage of knowing when the feed was going to enter the cage resulted in more feed ingested for
both cages 1 and 3 (0.27 ± 0.05g, 0.30 ± 0.08g, per fish respectively) as compared to the group which had their feeding time changed each week (0.24 ± 0.05g).

When the stomach contents were analyzed, it was surprising to see the amount of naturally occurring food (most commonly copepods) found in the stomachs of fish fed at randomly changing feeding times. This group not only had the highest rate of copepods found in their stomachs and greatest average copepod stomach content weight, but also had the lowest rates of pellets found in their stomachs and average wet pellet weights. Either they ate copepods, and thus were not as hungry when feed was delivered, or, because they did not capture sufficient pellets during feeding, they had to supplement their diet with other sources of food. This demonstrates the importance of keeping the feeding time the same in aquaculture farms. Past research at the OOA farm at UNH also demonstrated a large percentage of the fish consuming naturally occurring food in the cage (mussels) (Rillahan, 2008).

Growth rates for juvenile farm raised cod are always higher in the spring and summer when the temperatures are warmer (Hawkins et al., 1985; Bjornsson and Steinarsson, 2002). In the current experiment there was little growth when the temperatures were still quite cold at the beginning of the experiment (Fig. 1.3), but growth rate increased as the water temperatures increased. The increase in weight was highest during the final weeks in all treatments, and a longer trial with more replicates may have been able to show a stronger trend towards increased feeding efficiency with conditioning. Overall length increased more for the acoustically conditioned group, though a statistically significant increase may have been seen during warmer months when growth was faster.
The acoustically conditioned group had the largest increase in condition factor (K) over the entire trial period. Because this is a measure of weight per unit length, it may be the best indicator of feeding efficiency in such a short trial. The overall (8 week) feed conversion ratio (FCR) was lower (4.17) than other groups. FCRs of all groups, however, were high, possibly because of over-feeding or loss of food through the cage. Taken together, these data suggest that conditioning could improve farmed cod condition and feeding efficiency, leading to a better product at a reduced cost.

Different feeding methods have been shown to produce different growth rates in cod, as well as other species (Clark et al., 1995; Ang and Petrell, 1998; Talbot et al., 1999). In the current study I was able to show that conditioning cod causes more fish to approach the feeding site, and feed higher in the water column which may, in the long term, produce higher growth rates. In contrast, fish that were not acoustically conditioned, and those fed at different times, were more variable in size (larger standard deviations). Thus from a farm management perspective, acoustic conditioning may require less sorting, and more consistent growth rates throughout the population.

This study yielded several unexplainable results. One was the sudden drop in number of fish with pellets in their stomachs, and weight of the fish in all groups, especially the conditioned group, one month into the experiment. This could have been due to increasing water temperatures, increases in the number of people around the cages in the summer, or more potential predators around the cage in summer months; causing the fish to not rise to the surface to feed. It is likely that changes in copepod incidence in fish guts was due to changing seasonal abundance rather than changes in the feeding preferences of the fish.
Future research into the topic of acoustic conditioning to increase feeding efficiency should include similar experiments with variable number of feedings per day, as well as variable feeding rates. This would allow the researchers to document the impact being ready to feed at the top of the water column has on the group of fish feeding. In the next experiment it would also be useful to quantify wasted feed to be able to compare the exact amount eaten as compared to feed wasted. The current project was not investigating acoustic conditioning as a remedy for competition for food, because the 5% BW feeding amount was excessive. Future research should investigate feeding only 1% BW and compare growth. Finally, future experiments on this topic should be conducted over a longer period of time so that any changes in condition and growth due to experimental manipulations would be more obvious.
CHAPTER 2

THE INFLUENCE OF STOCKING DENSITY ON THE SWIMMING BEHAVIOR OF ADULT ATLANTIC COD, GADUS MORHUA, IN A NEAR SHORE NET-PEN

Abstract

Adult Atlantic cod (Gadus morhua) were stocked at four densities (5, 10, 25, and 45 kg/m³) in 46 m³ net pens located under a pier in Newcastle, NH. Acoustic tags were placed into the abdominal cavity of 5 fish per density so that their swimming behavior could be monitored continuously throughout the study. Positional data was obtained from each fish at ~2-5 sec intervals, for 4-30 days, and an array of hydrophones made it possible to calculate their position in 3D. A group of three underwater cameras made it possible, during the day, to obtain additional data about the distribution of fish in the cage. At the lowest density, cod consistently spent the majority (64.3 ± 0.08%) of their time in the bottom third of the 1.80m deep net-pen. As density increased, the fish moved higher into the water column, and this most evident at night, at all densities. There was a statistically significant correlation between swimming speed and density ($R^2=0.834$, $p=0.011$), with fish stocked at the highest densities swimming more slowly ($0.20 ± 0.0$ bl/sec). At all densities the swimming speed was higher during the day than at night. These data indicate that stocking density has a large impact on the distribution and swimming activity of cod in a net pen and this information could be useful for optimizing cod farming operations.
Introduction

The groundfishing industry in the Northeast United States was once considered one of the most productive fisheries in the world. However, overfishing has caused a steady decline in catch, resulting in devastating consequences for fishing communities throughout New England (NMFS, 2002). While US wild groundfish stocks are in decline, the per-capita consumption of seafood is expected to increase more than 30% in the next 20 years as farm-raised products become less expensive (http://www.fas.usda.org, 2007). Over 70% of seafood consumption in the US is currently imported, and at least 40% of that is farm-raised (http://www.fas.usda.org, 2007). The aquaculture industry in the US must expand to meet this growing demand, and Atlantic cod (Gadus morhua) is one species that seems to have potential.

While Atlantic cod is expected to become the dominant species for gadoid aquaculture, significant R&D obstacles need to be overcome to ensure success, and thus increase investor support (Rosenlund and Skretting, 2006). Currently cod are raised in either cages engineered for salmon farming, or in multi-species cages such as the Sea-Station. The design of these cages can leave underutilized space that can reduce farming efficiency (Rillahan et al., 2009). The most effective feeding methods are also still under investigation. For example, if the feed is administered by hand, automatically, or through self-feeders, there can be differences on growth, feed efficiency or feed intake (Yamamoto et al., 2002).

One of the most challenging aspects of net pen farming is finding the optimal stocking density for each new species. Although profits may be maximized when fish are
raised at the greatest possible density, there is a delicate balance between potential profits resulting from stocking fish at a high density and the resulting stressful conditions that can lead to adverse effects to the fish. For example, several studies have shown that as density increases, growth rate decreases (Vijayan and Leatherland, 1988; Lambert and Dutil, 2001; Trenzado et al., 2006), but the cause is unclear. One hypothesis is that growth rate decreases at high densities because crowding causes the fish to exert more energy avoiding one another in the cage. Rainbow trout reared at high densities (100 kg/m³), however, did not have greater metabolic rates than trout raised at much lower densities (25 kg/m³) (Lefrancois et al., 2001). Boujard et al. (2002) suggested that it is not the high density itself that is causing the lower growth rate; rather it is the ability to feed efficiently that is impaired at high densities. For adult cod, in particular, rearing at a high density (40 kg/m³) results in a decrease in specific growth rate of as much as 42% (Lambert and Dutil, 2001). It remains to be seen if the slow growth stems from higher energetic costs, or from lack of feed intake as a result of overcrowding. Juvenile cod have been successfully stocked at >40 kg/m³ with no reduction in growth rate, provided the water quality is high (Bjornsson and Olafsdottir, 2006). One of the major goals of this study was to determine how changes in stocking density influenced cod swimming activity in a net pen, using both telemetry and video methods to quantify their behavior.

Altering the density of fish in a net pen can influence various aspects of fish behavior. For example, in a study of cod swimming behavior in an offshore net-pen, Rillahan (2008) found that juvenile cod began to express schooling behavior when the density exceeded ~32 kg/m³. He further reported that when the juvenile cod schooled, they expressed much less of a daily pattern of activity, and yet this was likely to have
only a small impact on their overall daily metabolism. Compared to salmon, 10-20 times more cod escape from net pens annually. This is largely due to the fact that they do not school, but rather express a form of “milling” behavior that lends itself to finding ways to escape (Moe et al., 2007). While salmon will school at any age, cod have been found to school only at high densities while in the juvenile life stage (Rillahan, 2008). A second goal of this study was to determine if adult cod will also school if stocked at a high enough density.

Stocking density can also influence the distribution of fish in a net pen. Rillahan et al. (2009) showed that cod tended to reside in the bottom third of a 3000m³ Sea Station net pen at all times when at low density (3.2-4.8 kg/m³). Although the impact of higher densities on the distribution of cod was not examined in that study, in general as densities increase, social interactions, hierarchies, and territoriality develop (Li and Brocksen, 1977; Jobling, 1985), so cod would likely have move higher into the water column, resulting in greater cage volume utilization. Stocking density can also influence diel activity patterns (Claireaux et al., 1995; Lokkeborg, 1998), and at higher densities, due to crowding, this pattern of activity may change (Rillahan et al., 2009). A third goal of this study was to determine the impact of stocking density on the distribution of cod in a net pen, as well as their tendency to express a diel pattern of swimming activity.

While many scientists have used acoustic telemetry for monitoring the behavior of wild fish populations (Steig et al., 1999; Steig et al., 2005; Chittenden et al., 2009), few have integrated the technology into an aquaculture application. In this study I used the video and ultrasonic telemetry methods developed by Rillahan et al. (2009) to investigate the swimming activity of cod in a small, inshore net-pen. While video is a
good tool for gaining insight into the behavior of fish, its main drawback is the inability to see what is happening at night. In contrast, acoustic telemetry has the obvious benefit of remotely monitoring and recording the behavior of individuals at all light levels, and the ability to quantify the swimming behavior and distribution of fish throughout the day and night. In this investigation both video methods and acoustically telemetry were used to monitor fish activity and both approaches yielded very similar information; demonstrating that telemetry data from select fish can provide information that is representative of the population. Moreover, while the telemetry system used (Hydroacoustic Technologies Inc, HTI; Steig, 1977) has been used extensively in freshwater, and on at least one occasion in a marine setting (Rillahan, 2008) this is the first time this form of 3D tracking has been deployed in a small, inshore net pen to monitor the behavior of fish with a very high resolution.

Materials and Methods

Study site and monitoring instrumentation

Two net pens (46m$^2$), attached to a floating dock at the UNH Marine Research Pier in Newcastle, NH pier, were used for the study (Fig. 2.1A). Supports for the cameras and HTI telemetry system were attached to the walkway surrounding one of the net pens (Fig. 2.1B). The cabling was then attached to the pier, and run into a shed on the pier, which housed all the equipment needed for the project (laptop, HTI receiver, DVR, monitor, etc.).
Three black and white, low light, video cameras (Model PC88WR; Supercircuits; Austin, TX) were mounted 0.70, 1.39, and 2.09 m below the water surface just outside the experimental net pen. From these depths it was possible to accurately capture the entire vertical profile of the cage. Cameras were housed in custom-made housings constructed of PVC, acrylic glass, and waterproof connectors (SubConn, Burwell, NE). They were connected to waterproof cables (McMaster-Carr, Chicago, IL) that extended to the shed that housed the electronic monitoring systems. Visual data were recorded using a digital video recorder (DVR) (Supercircuits; Austin, TX), programmed to record continuously throughout daylight hours. In order to correlate swimming activity with temperature and light intensity, light and temperature loggers (Onset Corporation; Bourne, MA) were attached to the experimental cage, at 1 m intervals. The loggers were set to sample at 20 min intervals for the duration of the study period.

The acoustic telemetry system (Hydroacoustic Technologies Inc; HTI; Seattle, USA) consisted of four hydrophones connected to an integrated receiver (Model 291) by 250’ cables. The hydrophones were attached to rigid supports arranged so that the net pen was in the middle of the hydrophone array where the resolution was best (Fig.2.1 B). Data were collected for subsequent analyses using a PC laptop (Dell Inspiron 600m) connected to the HTI 291 Receiver.
Figure 2.1. Research site and net pen dimensions. A. UNH marine research pier, Newcastle NH, illustrating the instrument building, automatic feeders and cages below the pier. B. Hydrophone locations, attached to supports, in reference to a large cage. All four hydrophones used for positioning were located outside the cage. C. Dimensions of one of the two net-pens used in this study.
Fish, implantation of tags and experimental protocol

The adult cod used in this experiment were taken from the 2006 cohort of fish raised by the Atlantic Marine Aquaculture Center (AMAC; University of New Hampshire, Durham, NH; ooa.unh.edu). In August 2008, some of these fish were harvested live and transported to one of the inshore holding pens. During this time I sampled the fish and transferred some to a second cage (46m³) in which the study occurred (Fig. 2.1 B, C). At the start of the study, because of the high surface temperatures (>18°C) and handling stress, there was high mortality. Therefore the project started with the lowest density possible, and densities were subsequently increased. Fish size at the start of the experiment was 1204.3 ± 34.8g average wet weight, and 48.4 ± 0.4cm average total length. Growth was minimal throughout the 9 months of the study.

Implantation of acoustic tags

In order to track the fish, an acoustic tag was surgically inserted into each cod, and set to “ping” at a specific interval (2.00-2.25 sec between pings). Previous work has shown that in order to prolong tag life, as well as generate data that is accurate, tag periods should be no longer than 10 sec (Rillahan et al., 2009). Because of the small size of the net pens in this experiment, which caused fish to turn more frequently than in the previously used large Sea Station net pen, a pulse interval of ~ 2 sec was used (pulse duration 1.0 msec, frequency 307kHz). It should be noted that each pinger was programmed to have a slightly different pulse interval to discriminate between individual fish.
During each trial five fish were removed from the holding pen, anesthetized using MS222 (concentration: 50 ppm), and implanted with an acoustic tag (HTI model 795A fish tag, 307 kHz, 7 x 23 mm, 2.3g in water) internally, as described by Cote et al. (1999). Tagged fish were also be marked with an external T-bar Floy tag to facilitate identification during video analysis. Fish were held in seawater filled containers (1 m³) until they were fully recovered from the anesthesia, and then placed back into the experimental net pen. Previous studies have demonstrated that neither growth rates nor swimming speeds of implanted fish are altered by this procedure (Cote et al., 1999; Rillahan et al., 2009) and our preliminary studies indicated that cod behave normally following implantation. Data collection was delayed for 24 hours to ensure that the fish were completely recovered from the surgery.

Four stocking densities were established (Table 2.1): low (~5 kg/m³), medium (~10 kg/m³), high (~25 kg/m³), and very high (~45 kg/m³). Densities were achieved by transferring an appropriate number of cod from the holding cage to the experimental cage. As the fish were transferred a random sample of 25 cod were measured for total length and wet weight. These measurements were used to calculate approximate biomass contained within the experimental cage. Length measurements also were used to determine swimming speed (in body lengths/sec (bl/sec)), during post processing of biotelemetry data. Video was recorded during all daylight hours for each trial and subsequently analyzed to determine: 1) distribution within the net pen; 2) swimming speed; and 3) the extent to which telemetry data corresponded with visual observations.
<table>
<thead>
<tr>
<th>Density</th>
<th>Dates</th>
<th>Total number of fish</th>
<th>Number of fish tagged</th>
<th>Average daily temperature (°C)</th>
<th>Final Density (kg/m³)</th>
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Table 2.1. Stocking densities and water temperatures. Acoustic tracking occurred from July 2008 through May 2009, and temperature profiles varied depending on seasonal changes. At each density five fish were implanted with acoustic tags for telemetry and the behavior of all the fish was recorded using three underwater cameras.
After tracking five cod at a given density for ~3 weeks, the density was either increased or decreased by adding or removing fish from the experimental pen. Another set of five cod were then implanted with another set of five ultrasonic transmitters, and they were tracked for a minimum of 3 weeks. The above procedure was repeated with each density. All positional, video, light, and temperature data were periodically downloaded for analyses and stored on a computer.

After trials were completed for all four densities, shorter replicate trials were carried out from March-May of 2009. The low (~5 kg/m³), middle (~10 kg/m³), and high (~25 kg/m³), densities were replicated in the same cage for a period of 1 week each. Because the highest density (45 kg/m³) was not replicated, data from this trial were only used to determine if adult cod would school if held at such a high density. Also the second replicate of medium density (10 kg/m³) trial was not used in subsequent analysis because, due to tag failure, only two fish were successfully tracked for the duration of the trial.

Data analysis

The HTI system is not designed to discriminate between acoustic data during the data collection stage. Rather, it is necessary to process the data subsequently, to remove artifacts. First, the program Marktag was used to separate true signals from background acoustic noise, and then the HTI software Acoustictag, was used to calculate positions in 3 dimensions. Afterward an algorithm was applied to the entire data set to remove any points that were not physically possible given the boundaries of the cage (points that fell outside the cage). Following this, additional calculations, such as determination of
swimming speeds, depths, etc., were carried out using Microsoft Excel and MATLAB after exporting data from a Microsoft Access database. Many of the MATLAB scripts used to calculate swimming speed were developed in conjunction with Martin Fore, a PhD student at NTNU in Trondheim, Norway. Swimming speed was averaged in 10 minute bins for each fish across each density for subsequent diel and temporal analysis.

Video analyses were used to quantify the vertical distribution of fish in the net pen and these data were then used to verify the telemetry data. In addition, video data were used to determine if cod were expressing any schooling behavior. There were three video cameras and each was positioned to view a horizontal slice of the net pen that was equivalent to one third of the total depth. Ten random times during each daily video were chosen, and the number of fish that appeared on the screen, from each camera (depth) were counted. Counts were not obtained within 2 hours of feeding. This procedure was completed for each video, for each trial. Using the average number of fish visible in each portion of the cage (top (<0.90m), middle (0.90-1.80m), and bottom (>1.80m)) made it possible to compare the distribution of fish based on video observations to the distribution based on the telemetry data. This comparison was used to assess if the sentinel fish tagged for telemetry data were in fact representative of the population as a whole.

Telemetry data were also used to calculate average swimming speeds and calculate the distribution of fish in the net pen. Data from each fish was averaged over a 10 min time period throughout each sampling period. These data points were then used to compare changes in depth and swimming speed as they compared to changes in density,
temperature, light (and diel rhythms), and the tendency to display schooling behaviors at different densities.

**Results**

**Distribution of Fish in the Net Pen: Video vs. Telemetry Data**

Data obtained using both video and telemetry techniques indicated that cod held at a low stocking density cod tended to stay as deep in the net pen as possible, as well as in the darker, shadowed part of the cage (Fig. 2.2). While this was true for all densities, as the density of fish in the net pen increased the cod spent more time both in the middle and upper layers of the water column (Figs. 2.3, 2.4), and utilized the entire width of the cage. At the high density, although the overall number of fish in the cage increased (and therefore the number of total fish observed with video at any one time increased), the relative number of fish located higher in the water column also increased. Regardless of the intensity of light, fluctuations in water temperature, or density within the cage, the bottom third of the cage was always the area most frequently occupied by the cod in each trial (Fig. 2.5). Overall the low density treatment (5 kg/m³) resulted in the fewest fish in the upper part of the water column (p<0.001, non-parametric ANOVA, Kruskal-Wallis post test).
Figure 2.2. The distribution of fish in the net pen when stocked at a low density of 5 kg/m³. The left panel shows single video frames taken from depths of 0.70m, 1.39m, and 2.09m. Very few fish were ever observed in the upper portion of the net pen. The right panel, top, is a 3D representation of the positions of a single fish, during a one hr time period. Below it are the same viewed from the top of the cage. In both plots cooler (blue) colors denote lower depths, and warmer colors denote depths higher in the water column (range: 0.00-2.70m). Note in top perspective, that fish stayed toward one side, which was under pier and therefore darker, as well as shielded from people walking by, or birds flying above.
Figure 2.3. The distribution of fish in the net pen when it was stocked at a middle density of 10 kg/m³. The left panel shows single video frames taken from depths of 0.70m, 1.39m, and 2.09m. Note that the number of fish is greater in the middle and upper frames at this density, in comparison to the lower density (Fig. 2.2). The right panel, top, is a 3D representation of the positions of a single fish, during a one hr time period. Below it are the same data displayed from a different perspective (viewed from the top of the cage). Again, note how fish avoid the half of the cage that has the most light.
Figure 2.4. The distribution of fish in the net pen when it was stocked at a high density of 25 kg/m$^3$. The left panel shows single video frames taken from depths of 0.70m, 1.39m, and 2.09m. Note that at this density many more fish are now visible higher in the water column, and more fish are visible in the field of view of the lowest camera because the net pen is becoming much more crowded. The right panel, top, is a 3D representation of the positions of a single fish, during a one hr time period. Below it are the same data displayed from a different perspective (viewed from the top of the cage).
Figure 2.5. The relationship between fish density and depth distribution based on video data. The analysis demonstrates a clear preference to spend the most time near the bottom of the cage for fish at all densities. The highest number of fish at any time, for any density, was always in the lowest third of the cage.
One of the major advantages of using biotelemetry is that it is possible to study the behavior of fish at night as well as in the day. However, a major drawback of telemetry is that one can only study the behavior of a small percentage of the total fish in a population. Therefore, in order to both validate the telemetry method and confirm that the behavior of the five fish that were being tracked were representative of the population, I compared video and telemetry data (Fig. 2.6). The camera placement in the cage effectively split the cage into top (<0.90m), middle (0.90-1.80m), and bottom (>1.80m) areas, and these areas were used for comparison to the telemetry data in the same three areas, during the same time periods. As Figure 2.6 illustrates, both techniques yielded similar results, reinforcing the finding that, at all densities, fish tended to spend the most time near the bottom of the net pen. The number of fish per frame for the lowest density was always significantly less in (p<0.001, Kruskal-Wallis test) in the top (≤0.90m) and middle (0.90-1.80m) sections, than in videos from the bottom of the cage. The number of fish visible per frame was also significantly higher in the lowest portion of the cage (≥1.80m) (p<0.001, ANOVA) at the middle and high densities. These data also clearly show that the telemetry data obtained from the five sentinel fish yielded results that were almost identical to the results obtained with video from the whole population (Fig. 2.7).
Figure 2.6. A comparison of fish distribution data obtained from both video cameras and the HTI telemetry system. Graphs A-C: Average number of fish per frame for each density obtained from the video data. Graphs D-F: Percent of the time that the sentinel fish tagged for telemetry analysis were found to be in a given third of the cage, corresponding to the areas viewed by each of the three cameras. All data were obtained during daylight hours, on the same day.
Figure 2.7. Video and telemetry data comparison. A comparison between the percentage of the time spent in a given area of the cage obtained from video data and the percentage of the time spent in the same areas of the cage based on telemetry data. While only five fish were tagged and tracked using biotelemetry, the strong correlation between video and telemetry data demonstrates that the behaviors expressed by the tagged sentinel fish were representative of the population as a whole.
Day vs. Night Activity

At a high density cod spent the majority of their time (84.4 ± 0.1%) in the bottom and middle of the cage (Fig. 2.8). The fish tended to spend more time within the bottom third of the cage (<0.90m) during the day (46.8 ± 1.3%) as compared to the night (45.5 ± 0.8%), but this difference was not statistically significant (unpaired t-test, p=0.0865, t=1.799, df=21) (Fig. 2.9). At a high density the fish also spent significantly more time in the top third (>1.80m) of the net pen at night (16.6 ± 0.6%) as compared to during the day (14.5 ± 0.9%) (unpaired t-test, p=0.0469, t=2.111, df=21). However, even though there was a tendency for the fish in all treatments to spend less time near the surface in the day, in comparison to the lower two densities, when cod were stocked at a high density they spent more time near the top of the net pen during the day.

When fish were stocked at a low density their distribution was different than when they were stocked at a high density. There were no statistically significant differences between the amount of time they spent in the bottom or middle portions of the net pen during the day in comparison to the night (bottom third; unpaired t-test, p=0.7452, t=0.3293, df=21; middle third: unpaired t-test, p=0.3429, t=0.9703, df=21; Fig. 2.8). The amount of time spent above 1.80m was lowest at this density compared to the two higher densities (p<0.001, Kruskal-Wallis test). Thus, overall, there was a strong tendency for fish to avoid bright light, by moving deeper and into shaded areas of the net pen during the day. However, at the higher densities, due to crowding, fish spread out more and were distributed more uniformly throughout the cage during both the day and night. I compared the changes in depth to temperature changes throughout the trials, and there was found to be no correlation between temperature and depth (R²=0.018).
Figure 2.8. Percent of time spent in each third of the water column within the net-pen, based on telemetry data. Preference for a given area during the day or night was determined by calculating the total time spent in each third of the net pen during either the day (7AM-5PM), or night, (5PM-7AM) hours. The higher the density, the more fish moved higher in the water column both during the day and night. Overall the low density treatment (5 kg/m³) had the fewest fish in the upper part of the water column (p<0.001, Kruskal-Wallis test).
Figure 2.9. A-C. Depth profiles for a 24 hr time period from a single fish, at all three densities, A=Low, B= Middle, C= High. Lines denote 1m and 2m depth. D. Schematic of cage showing 1m and 2m depths thresholds. Note at low density the fish never cross the 1m threshold at any point during 24hrs. At the middle and high densities the fish spend more time above both the 1m and 2m thresholds; with more time spent higher in the water column as the density increases.
Vertical excursions in the water column

The tendency for fish to make vertical excursions differed between densities. While cod tended to spend the majority of the time in the bottom 0.90m of the cage, there were frequent vertical excursions throughout the day. At the lowest density fish made an average of 47.3 ± 0.6 vertical excursions above a two meter reference depth per hour (Fig 2.10), with most vertical excursions occurring during the day (49.2 ± 0.6 per hour) compared to at night (45.7 ± 0.8 per hour) (unpaired t-test, p=0.0023, t=3.439, df=22). As the density increased, there were fewer and fewer excursions over two meters, with the fewest at the high density at night (24.4 ± 0.5 per hour) (Fig. 2.12). This is consistent with the amount of time fish spent swimming higher in the water column at night at the higher densities. Likewise, as the density increased, the amount of time spent below two meters decreased linearly, therefore the number of excursions below the two meter threshold also decreased (Fig. 2.10).
Figure 2.10: Daily Vertical Movement in Reference to a 2m Threshold

A. Average number of hourly vertical excursions above 1m. B. Average number of hourly excursions over 2m. More vertical movement from the Low density fish, as well as the Middle density fish as compared to the High Density group. The group of fish at the Low density made more vertical excursions over the two meter threshold both per hour, and per day. Because the 2m threshold is at the bottom of the cage, this signifies that at the higher densities the fish were staying higher in the water column and not moving down to cross the 2m threshold as often.
Swimming speed

The average daily swimming speed was significantly higher (one-way ANOVA with Tukey-Kramer Multiple Comparisons Test, p<0.01) for all densities during the day, as compared to night (Fig. 2.11). At the high density the average daily swimming speeds during both the day (0.207 ± 0.0 BL/sec) and night (0.197 ± 0.0 BL/sec) were significantly lower than either the swimming speeds at either time of day, for both middle or lower density treatments (one-way ANOVA with Tukey-Kramer Multiple Comparisons Test, p<0.05). In fact, as density increased, the average swimming speed was found to simultaneously decrease (R²= 0.834, Fig. 2.12). The fish at 25 kg/m³ were much more densely packed into the net-pen, and therefore were only able to swim at a reduced speed throughout the day. Swimming speed was also compared to temperature, and there was a very poor correlation (R²=0.399). Therefore, even though replicates of each treatment took place at different times of year, data from the replicates were pooled when comparing the impact of density on various behavioral patterns.
Figure 2.11. Swimming activity of cod held at three different densities. A. Average daily swimming speed, in body lengths (bl) per sec, for each density. The fish stocked at the highest density had a significantly lower mean daily swimming speed compared to the other two densities (one-way ANOVA with Tukey-Kramer Multiple Comparisons Test, \( p<0.05 \)). B. Comparison of average swimming speeds for each treatment in the day vs. the night. At all densities fish swam significantly faster in the day as compared to the night. The mean swimming speed at the high density was significantly lower during both the day and night when compared to either the middle or lower densities (one-way ANOVA with Tukey-Kramer Multiple Comparisons Test, \( p<0.01 \)).
Figure 2.12. The relationship between average daily swimming speed and time of day. Grey areas denote night (5PM-7AM) and white areas day. Data points are derived from three different density trials, with two replicates for each density (n=6). Fish stocked at the highest density (~25 kg/m$^3$) had the lowest swimming speed throughout the 24 hr daily cycle.
Discussion

In the current study, I quantified the influence of stocking density on several different behaviors of adult Atlantic cod in a small, inshore net pen. I found that stocking density influenced utilization of the space within the net pen, as well as daily rhythms of activity and swimming speeds. Therefore, by carefully managing the stocking density in cod aquaculture operations it may be possible to optimize growth, animal welfare, and profits by using densities that yield the maximum weight of fish per net-pen, while minimizing stress to the fish.

Telemetry vs. video

Although biotelemetry has been used in fisheries applications since the 1950’s (Arnold and Dewar, 2001), its adaptation to aquaculture research has been slow. One limitation of using telemetry for marine aquaculture operations is that most telemetry systems are designed to maximize the area of coverage, or distance that tags will transmit, rather than small scale resolution (Juell and Westerberg, 1993; Rillahan et al., 2009). The telemetry system used in this study was originally designed for use in freshwater, and therefore, due to the high frequency of the signals emitted by the tags (307.2 kHz), the signals do not travel far in the marine environment. However, this is not an issue in a net pen operation, and, in fact, the tags and the associated 3 dimensional tracking system are ideal for tracking fish in either a large (Rillahan et al., 2009) or small inshore net pen like the one used in this study.
Because this is one of the first investigations to use this system in such a limited area, I first needed to “calibrate” it by comparing telemetry and video data. As illustrated in Fig. 2.6, both video and telemetry distributional data yielded nearly identical results, confirming that the telemetry system provided data that were at least as accurate as video data, and that as few as five sentinel fish can provide information that is representative of the behavior of the population as a whole. While telemetry is a more expensive way to track fish, it takes less time to analyze the data obtained. Moreover, as pointed out throughout this paper, a major advantage of telemetry is the ability to monitor the behavior of fish throughout the net pen, during the day or night. Therefore, at present, I believe it yields the most accurate and complete data about fish behavior in an aquaculture setting. Hopefully, with time, telemetry systems can be refined so that they are customized for use in an aquaculture setting and are less expensive and easier to use.

The influence of stocking density on cage utilization, daily rhythms and swimming activity

Numerous variables effect the distribution of fish in a net-pen, including light (Oppedal et al., 2001; Korsoen et al., 2009), depth (Stensholt et al., 2001), temperature (Claireaux et al., 1995), and predator avoidance behavior (Ferno et al., 1995). I found that at low densities fish spent the majority of the time below 1.80m (64.3 ± 0.8%), as compared to the middle or the high densities where the fish only spent 48.7±1.2% and 46.8±0.8% of the time below 1.80m, respectively. In addition, at the low and middle densities the fish tended to prefer the shaded side of the cage. There are many reasons
why the fish preferred the bottom third of the cage, which are discussed below, and appear to be related to light levels. However, as the density was increased, due to crowding, some of the fish were forced to move into areas of the net pen that were not preferred, and thus they were higher in the water column and more widely distributed in the cage.

The natural tendency of the Atlantic cod is to occupy habitats that are deep and dark (Hobson et al., 2007), which in this instance corresponded to the bottom and shaded portion of the net pen. There are many factors that might cause cod to prefer the bottom portion of the net pen. They could be avoiding the light, as studies with salmon have found (Juell and Fosseidengen, 2004), seeking colder temperatures, remaining at the same depth to avoid having to fill or empty their swim bladders, or avoiding predators at the surface (Ferno et al., 1995). In the small net pens used in this experiment the thermal gradient from top to bottom was very small and the depth change was too small to have a big influence on buoyancy (see Fig 2.1, in Methods). While it is possible that they could have been displaying anti-predatory behavior, this seems unlikely because the same pattern of cage utilization has been documented for cod in a larger net-pen (Rillahan et al., 2008). In that cage, fish rarely moved above the middle of the cage, except to feed. The fact that cod preferred the shady side of the net pen and rose up in the water column at night, suggest that light levels had a large influence on their distribution in the net pen. Therefore, my working hypothesis is that cod preferred the deepest areas of net pens because of their natural tendency to inhabit dark, benthic habitats, in their natural environment.
Increasing the density of fish in the net pens caused a change in both their overall distribution and their tendency to change depths during the day and night. First, as density increased fish spent a much greater proportion of their time up in the water column, away from the bottom third of the net pen. This change in behavior is likely the result of crowding, causing the fish to spread out and occupy a greater proportion of the available space, despite a preference for the bottom third of the net pen. Interestingly, at middle and high densities there was also a change in their daily behavior rhythms; at night they would move toward the surface and during the day they would be lower in the net pen (Fig. 2.8). This same pattern has been documented in wild cod, which make vertical migrations towards the surface at night to feed (Aglen et al., 1999).

If nightly vertical migrations are an innate feeding behavior, it should be conserved even at the lowest density, but that did not appear to be the case. The fact that at lower densities the fish lost their diel rhythm, suggests that the vertical movement at night might have more to do with the fact that, at high densities in the dark it becomes more difficult to avoid contact with other fish. Therefore, they spread out and occupy a wider area of the net pen. This hypothesis is also supported by the finding that, at high densities, the swimming speeds of cod slowed down in comparison to fish stocked at the middle and low densities. Moreover, Atlantic salmon in net-pens also reduce their swimming activity and spread out vertically in the water column at night (Ferno et al., 1995; Oppedal et al., 2001; Korsoen et al., 2009). This decrease in swimming speed could, in an aquaculture setting, lead to a reduced metabolism and thus increased rates of growth.
Schooling behavior

One of the goals of this study was to determine if adult cod would express schooling behavior when stocked at a high density (>35 kg/m$^3$). This was motivated by an earlier study that reported schooling in juvenile cod when the density was increased (Rillahan et al., 2008). From an operational perspective, it might be useful to stock cod at higher densities to encourage schooling and thus, perhaps, reduce escapement. For example, salmon escape from net pens much less than cod, and it has been hypothesized it is because salmon school throughout their life, and therefore have less propensity to explore the net for holes, or to create them (Moe et al., 2008). At the lower densities (5 kg/m$^3$, 10 kg/m$^3$, 25 kg/m$^3$), I never saw an instance of synchronous swimming, or any type of schooling behavior. Even when I experimentally increased the density to 45 kg/m$^3$, in order to surpass the density reached by Rillahan et al. (2008), I never observed schooling behavior. This lack of schooling behavior by adult cod could be attributed to an ontogenetic loss of group behavior, perhaps because they are much less susceptible to predators or because their mode of feeding does not rely on schooling behavior.

What is the optimal stocking density and cage design for cod aquaculture?

For many fish there is an inverse relationship between growth rate and stocking density (Lambert and Dutil, 2001). An optimal stocking density should be low enough to allow for good growth, yet high enough to maximize the harvest per cubic meter. Therefore cage utilization must be investigated for each farmed species to optimize stocking densities and yield. Several factors should be kept in mind when considering
cage design for cod. First, because cod prefer to occupy the bottom of the cage, cages should be designed to be wide and shallow. Second, because adult cod do not school, even at a high density, it is probably not necessary to use a cylindrical pen, like those often used for salmon. Third, efforts should be made to stock cod at a high density so that they will reduce their swimming speeds, and thus conserve energy and enhance growth (Clark et al., 1995; Claireaux et al., 1995). Finally, because of the swim bladder and their tendency to stay near the bottom, especially during the day, food should be delivered low in the cage.
CONCLUSIONS

Current aquaculture operations in the United States are often not economically viable, and offshore aquaculture in particular has had difficulty gaining traction. In order for aquaculture operations to be profitable the fish need to be raised at the greatest density that will not impair growth or impact normal behavior. In the current study I compared the behavior of adult Atlantic cod at four densities, and found that behavior was indeed changed by increasing stocking densities. At the lowest density cod spent more of their time in the bottom third of the cage than fish that were stocked at intermediate or high densities. Stocking density also had an impact on the swimming speeds of fish. At the high density, the average swimming speed of cod during the day and night were significantly lower than the swimming speeds of fish stocked at the middle and lower densities. Therefore, at higher densities fish occupy a greater portion of the cage and swim at a slower speed.

Conditioning fish to feed may increase feeding efficiency, as well as having utility as a harvesting tool. In the present study, when cod were conditioned to feed when presented with a 150 Hz tone, their feeding behavior was markedly different than those fish that were not conditioned. The conditioned fish spent less time milling at the surface prior to feeding compared to the groups that were fed at the same time twice daily or at randomly changing feeding times. This reduced time spent at the surface means less time exposed to predators, and possibly lowers stress levels, as well as a greater opportunity for feeding as the feed falls through the cage.
Length and weight measurements for each group were not statistically different at the end of the experiment. However, the acoustically conditioned group had the lowest FCR, the largest increase in K, and a higher daily growth rate than the group with the randomly changing daily feeding times. This indicates that while the length and weight data remain inconclusive, acoustic conditioning ‘better prepared’ the fish to feed, and minimized their time spent higher in the water column, out of their preferred area at the bottom of the cage. Furthermore, especially with fish raised offshore, raising fish that will come to a certain location when they hear a tone might facilitate harvest as well.
FUTURE DIRECTIONS

Density

Future studies should investigate the effect of densities higher than 25 kg/m³. In this study, fish held at higher densities showed reduced swimming speed and increased use of the entire water column within the cage. Thus even higher densities may cause fish to utilize the entire cage, and if this could be done without inducing density-dependent stress, it could increase profits.

Lower escape rates of salmon, compared to cod, have been attributed to their propensity to school. In prior studies (Rillihan et al., 2009), cod were found to school when at high densities (>30 kg/m³), though that was not found in the current study. Perhaps as higher densities are investigated, the threshold in which adult cod will school will be found. If this density can be achieved, without causing undue stress to the fish, it could increase cage utilization, lower escape rates, and lower swimming speeds, thereby increasing growth.

Conditioning

Unfortunately in the current study I encountered extremely high temperatures in the second trial that caused mass mortalities, and subsequently caused the project to be cut short. Missing trials and replicates should be completed at cooler temperatures. Nevertheless, the trial that was completed indicated that conditioning not only prepared the fish better to feed, but also could result in lower feed conversion ratios. In future
experiments, researchers could endeavor to quantify uneaten feed so more accurate FCRs could be calculated.

One of the challenges of cage culture is live harvest, as well as partial harvest, technology. Acoustically conditioning fish to aggregate, or enter harvest cages, could facilitate these processes, result in lower labor costs and in increased safety.
LIST OF REFERENCES


85


University of New Hampshire

Research Integrity Services, Office of Sponsored Research
Service Building, 51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

16-Feb-2009

Watson, Winsor H
Biological Sciences, Rudman Hall
Durham, NH 03824

IACUC #: 081203
Project: Acoustic Conditioning of Juvenile Atlantic Cod
Category: C
Approval Date: 17-Dec-2008

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments: The IACUC made the following comment(s) on this protocol:

1. While the researcher clarified what he is doing in #4 of the experimental design, the IACUC will remove the term "re-homogenized" as it is problematic for an IACUC member.
2. In the SOP information addressing fish feed, the IACUC added to the end, "and will be replaced as needed."

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File
    Ward, Daniel
University of New Hampshire
Research Integrity Services, Office of Sponsored Research
Service Building, 51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

09-Apr-2009

Watson, Winsor H
Biological Sciences, Rudman Hall
Durham, NH 03824

IACUC #: 090202
Project: Density Dependent Behaviors in Atlantic Cod
Category: C
Approval Date: 25-Feb-2009

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

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For the IACUC,

Jessica Bolker, Ph.D.
Chair

cc: File
Ward, Dan